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Early priming of the immune system: Identifying predictive markers of innate immunity and calcium signalling for the development of asthma

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Table of contents

1 Introduction

1.1 Childhood asthma

Asthma is a complex chronic pulmonary disease with four main pathogenetic properties: airway hyperresponsiveness (AHR), bronchial obstruction, airway inflammation and airway remodelling. It is the result of a complex interaction involving the respiratory tract, inflammatory cells, cytokines and other mediators [1]. A variety of influences from (epi-)genetic background to environmental exposure as well as respiratory tract infections have been shown to influence the pathogenesis of asthma [2-4]. While asthma affects patients of every age, its pathogenetic origins most likely lie in the early stages of life [5]. Subsequently, asthma is considered to be the most common chronic disease in childhood. Prevalence is continually increasing and varies between 5-20% in 13- to 14-year-old children in different parts of the world [6-8]. In younger patients, especially preschool children, making a reliable asthma diagnosis has proven to be a challenge, though not impossible, as obtaining objective lung function measurements is difficult and airway inflammation as an important criterion has been poorly studied in preschool children [9]. As the most frequent asthma-related symptom in paediatric patients is wheezing, a high-pitched whistling sound during expiration, wheeze - and not asthma - is the term mainly used to describe the illness in this group [10, 11]. The prevalence of wheeze is high as almost every third child will experience wheeze at least once until school age, with recurrent wheeze affecting roughly 8% to 20% of children [12-15].

However, wheeze as a pulmonary symptom is not automatically equivalent to an actual diagnosis of asthma, while not all asthma patients necessarily show wheezing [16]. This makes for a challenging process of correctly differentiating and then treating these groups which are very heterogenous in clinical presentation, progress and response to therapy [6, 17, 18].

Current treatment guidelines, such as the 2018 GINA (global initiative for asthma) guidelines, mainly propose age-adjusted treatment steps according to the level of disease severity as well as symptom control, with varying priorities on different medications such as steroids and β_2-agonists, only just beginning to implement more individualized approaches [19-24]. This might be one reason for the varying response

to pharmacological therapy, accounting for patients with mild asthma who still have substantial residual disease and some patients with severe asthma who are classified as non-responders [25-27]. Non-responders are patients suffering from severe or uncontrolled asthma even under medication, a group that is especially relevant in preschool children under four years of age [28]. Not only do these children suffer a greater loss of quality of life, but some additionally propose a possibly increased risk of developing COPD (chronic obstructive pulmonary disease), a disease with features similar to asthma but characterized by irreversible airflow obstruction, in the third or fourth decade of their life [29]. As asthma cannot be cured at the moment, effective therapy will be the key to limit this substantial risk, relieve health care costs and, most of all, to increase the patients' quality of life [30-32].

In summary, childhood asthma, despite all efforts, continues to impose a considerable burden on paediatric patients as well as on health care systems [33, 34]. Disease prevention as well as the access to adequate therapy remain limited for many children as long as especially the underlying pathogenetic mechanisms of asthma are incompletely understood.

A recent, promising theory about asthma pathogenesis is the view of asthma as a complex disease. This term describes a condition in which a mild genetic hit can have a major impact on the clinical presentation when acting together with environmental factors, integrating the two main known pathogenetic pathways as well as the emerging clinical view of asthma as a syndrome [35]. This new understanding lays the foundation for a more precise classification of patients into clinical phenotypes as well as biological endotypes, which will be the first step on the way to more individually tailored care for patients with childhood asthma, supported by biomarkers[1] used to improve diagnostic processes and disease monitoring [26, 36]. As a long term goal, especially patients suffering from severe or poorly controlled asthma would benefit from more effective therapies and maybe even possible cures, developed and delivered to precisely defined patient subgroups with the help of biomarkers [37-39].

1 Biomarkers are indicators of physiological or pathogenetic processes, or even of a response to a therapeutic intervention, that can be objectively measured and evaluated.

1.2 Phenotyping

1.2.1 Clinical phenotypes

In clinical practice, phenotypes define groups of patients presenting a similar combination of symptoms. There are several ways to define those phenotypes, two of the most commonly used being a clinical and an epidemiological approach [40]. For preschool children, the most frequently used approach is based on clinical criteria and takes the patient's history, diagnostic techniques and treatment responses into account. Following this system, children with preschool wheeze are classified in two subgroups and then treated accordingly: Either multi-triggered wheeze (defined as wheeze being caused by at least two of the following six criteria: cold, effort, dust, animal contact, grass pollen, or others) or episodic viral wheeze, in which wheeze typically occurs during distinct episodes of respiratory viral infection, mainly with rhinoviridae [13, 17, 41-44].

However, data about the prevalence and, even more important, about the reliability of these phenotypes have only been published recently. For example, van Wonderen et al. showed in a cohort study that stable multitrigger and episodic viral wheeze are relatively uncommon as about 80% of the children changed phenotypes in the period of 24 months [45]. In accordance, there have also been reports about significantly varying prevalence between wheeze and asthma, which renders the current use of wheeze as a proxy symptom for asthma problematic [16]. Additionally, the clinical approach is based on the paediatrician's subjective perception of child and wheeze in a particular, not necessarily representative moment. Thus, perception and labelling of wheeze symptoms often varies among paediatricians and other clinicians, diminishing its reliability considerably [37, 40, 46].

These limitations cause a serious problem in separating preschool children with self-limiting episodic viral wheeze from children who will continue to experience symptoms and eventually receive an asthma diagnosis. However, this differentiation is crucial because one does neither want to submit a child to unnecessary therapy not withhold necessary therapy from a child that needs it as early as possible in order to limit long-term complications and alleviate overall morbidity [14, 47].

In children older than six years, the most common clinical phenotype distinction is mainly based on IgE, an immunoglobulin used to measure allergic sensitization. This results in a group of patients classified as allergic asthmatics, who present a high

level of IgE (and in particular a positive specific IgE for the most common aeroallergens in combination with characteristic clinical symptoms) as well as pulmonary symptoms like wheezing, coughing, or an impairment of lung function. As there is specific therapy available in the form of anti-IgE-antibodies, the classification as allergic asthma has a direct impact on treatment. Meanwhile, a low level and/or negative specific IgE without any allergy-related clinical symptoms is what defines the non-allergic phenotype [48].

However, the roles of atopy and IgE as a potential biomarker are under re-evaluation especially in younger patients, as atopy takes time to manifest and a clear causal connection to asthma pathogenesis has never been proven, suggesting an influence of other factors like viral infections [38, 49, 50]. Subsequently, recent studies about the use of IgE in prediction of response to omalizumab (an anti-IgE antibody) left the role of IgE as a specific biomarker increasingly unclear [50]. More promising results were obtained when using the count of blood eosinophils, which is usually elevated in asthma [51].

Even so, these results are still not satisfying, suggesting that the dichotomy of allergic versus non-allergic asthma (and in parallel terms, multitrigger wheeze and episodic viral wheeze) does not do the disease's heterogeneity justice. Instead, there has been a change towards looking for other methods to define phenotypes, ultimately combined in the approach of endotyping that will be explained later on, in the hope of a better prediction of treatment responses and outcome as well as the development of individualized therapeutic options [36].

1.2.2 Epidemiological phenotypes

Another approach to phenotyping is based on epidemiology and driven by data stemming from birth cohorts. Mainly latent class analysis (LCA) is used to define groups with similar features in a larger heterogeneous group of subjects. As it traditionally refers to longitudinal data, it focuses on longitudinal time patterns. This way of phenotyping results in longitudinal time patterns and the subsequent phenotypes are defined as *early transient*, *persistent* or *late-onset* wheeze as illustrated below [17, 18].

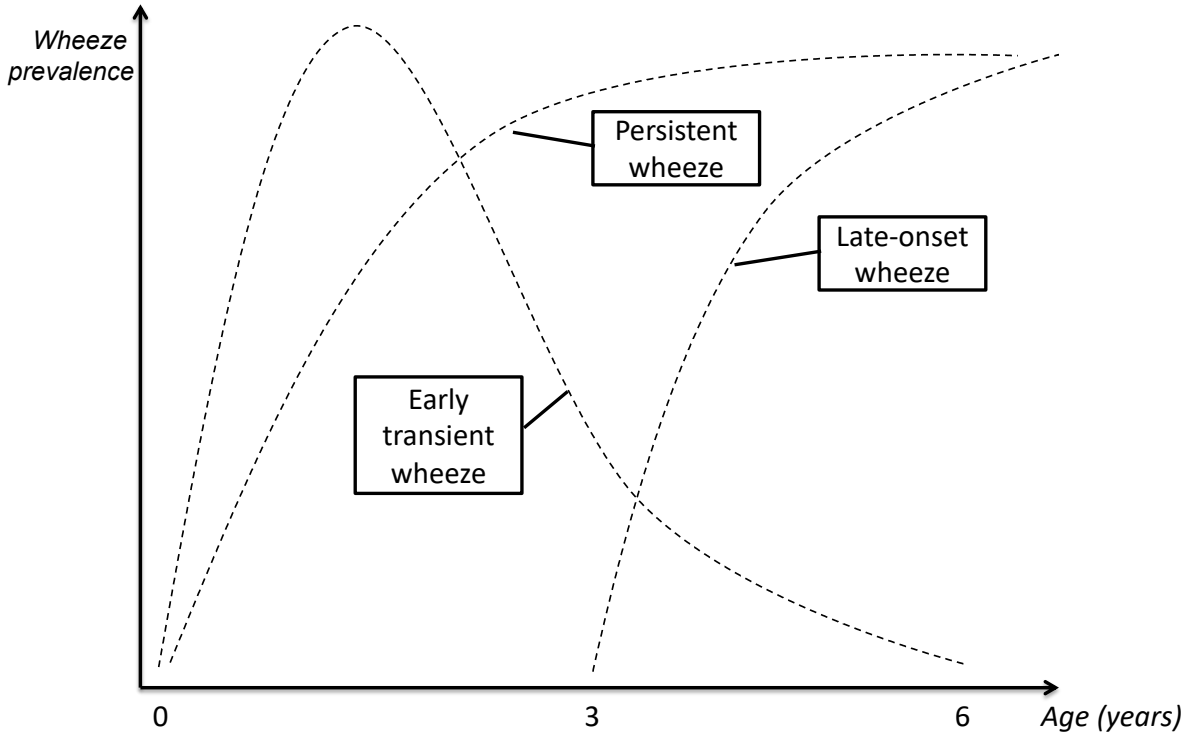

Figure 1: Course of longitudinally defined phenotypes. Modified after [52].

However, as it can only be performed in retrospective, this method is most commonly used for research purposes. For example, *early transient* wheeze is defined as the patient having started to wheeze before three years of age but stopped around three years, which can only be said in retrospect, and is thus not applicable for clinical management of a patient [13, 38].

Recent efforts to combine both the clinical and the epidemiological approach in order to gain benefits from both have found some cases in which the clinical phenotypes match the LCA phenotypes, as for example in *multitrigger* wheeze and *persistent* wheeze, respectively. While this is promising, in other cases they differ greatly, further stressing the need to combine more methods for additional precision [17, 18].

In an effort to include the change in clinical picture over time, which is common especially in children, a recent study by Gardner et al. tried a new approach to increase both reliability and stability in connecting LCA phenotypes defined at different time points through transition probabilities. Parallel to the above, they found evidence supporting some but not all clinical phenotypes [53].

A possible way to increase their accordance and subsequently, to define more precise and thus more reliable phenotypes could be gaining more in-depth insights into the underlying pathogenetic immune mechanisms and taking them into account when merging the clinical and epidemiological approach to phenotyping.

1.3 Immunological mechanisms in the pathogenesis of asthma

An understanding of the complex underlying mechanisms of the immune system is needed prior to integrating immunological insights in the definition of asthma phenotypes. The following chapter will comprise a short summary of known mechanisms important for asthma pathogenesis in innate and adaptive immunity.

1.3.1 Adaptive immunity

In general, adaptive immunity can be divided in T-cell immunity and B-cell immunity. B-cells are mainly responsible for the humoral part of adaptive immunity as they produce highly specific antibodies. T-cells are further classified into $CD8_+$-T-cells with cytotoxic function and $CD4_+$-T-cells with their multiple subsets (see figure 3 below). Any kind of adaptive immune response requires the T-cell receptor (TCR) to recognize antigen fragments presented via major histocompatibility complex (MHC) I or II, depending on the cell type, on antigen presenting cells (APC). This crucial pathway is regulated through expression patterns of pattern recognition receptors (PRR) on epithelial cells (EC) and APCs. This seems to play a role not only in allergic sensitization but also in ongoing asthma [54, 55].

MHCI is expressed on all nucleated cells in the body, while the coreceptor necessary for antigen presentation, CD8, is only expressed on $CD8_+$ T-cells. Via MHCI, cytoplasmatic proteins are presented, which in physiological circumstances induces self-tolerance from natural killer cells. In case of intracellular, viral infection highly specific, cytotoxic $CD8_+$ T-cells are activated to eliminate the pathogen.

Antiviral immunity in particular has long been of interest for asthma pathogenesis, as epidemiologic studies have shown respiratory viral infection to be an independent risk factor. The exact pathophysiological mechanisms through which viral infection influences asthma development are still to be elucidated, but an altered immune response to viral infection seems to be an important factor. For example, respiratory syncytical virus, a very common infection in small children, is thought to overstimulate a Th_2-type response, which in turn is associated with allergy and asthma development as explained in the following [56, 57]. Antiviral immunity is also a good example of how asthma is a disease featuring the adaptive as well as the innate immune system. There are many intersections through which both systems

communicate and influence each other, physiologically maintaining a fine balance between inducing and resolving inflammation [26, 58].

In contrast, MHCII is only expressed on myeloic cells with the capacity to present antigens, like dentritic cells (DC), macrophages and also B-cells as well as ECs. On MHCII, fragments of extracellular antigens that have been taken up and processed by antigen-presenting cells are presented to naïve $CD4_+$ T-cells, also called T-helper cells, in the lymph nodes draining e.g. the lungs [59, 60].

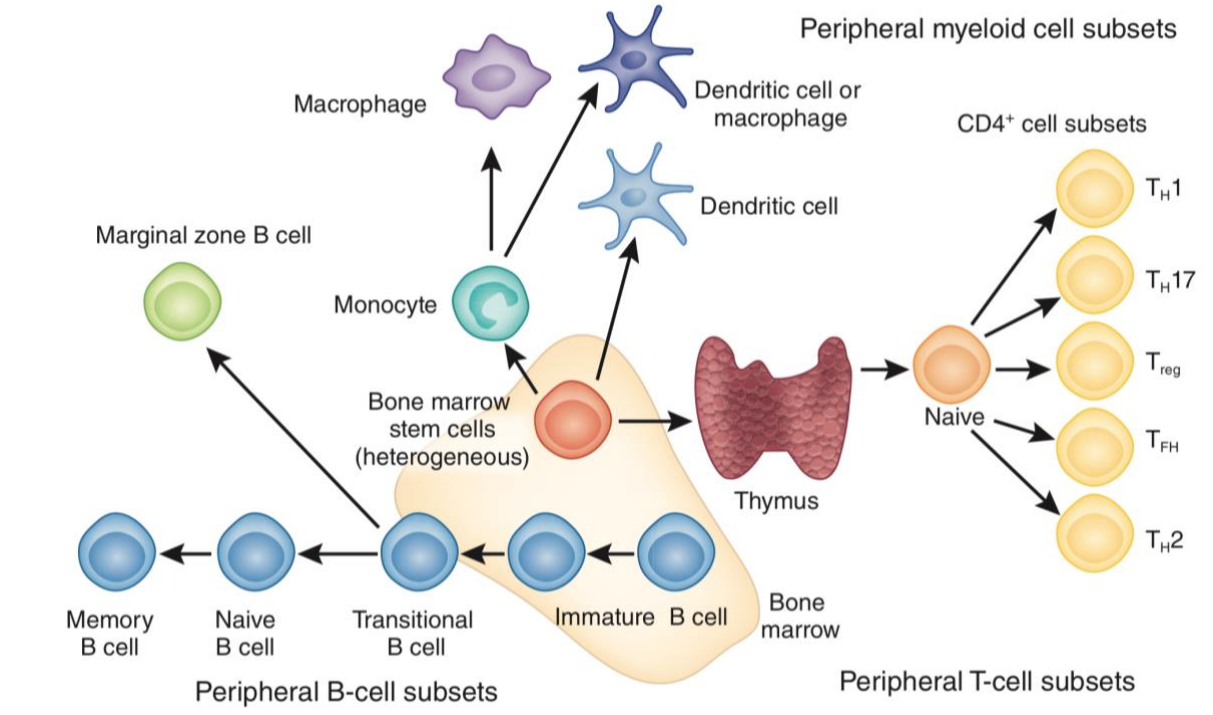

Figure 2: Overview of differentiation pathways from progenitor cells for cells of the immune system. Peripheral T-cell subsets also include Th19-cells and Th22-cells (not shown), from [61].

In the past, the main role of adaptive immunity for asthma has often been limited to the role of the T-helper-Cells (Th-cells). Th_1-type cells mainly produce IL-2 and IFN-γ, which, in high doses, have suppressive capacities on activated B-cells, which is important for terminating an immune response. Additionally, they can activate macrophages, resulting in cytotoxicity, which makes them an effective weapon against for example acute viral infection.
Th_2-type cells, more prominent in chronic inflammation, produce interleukins (IL) IL-4, IL-5 and IL-13, helping B-cell dependent humoral immunity in the production of immunoglobulins (Ig) like IgE. IgE is an antibody strongly linked to allergy as it can

lead to histamine release from mast cells when cross-linked to the $F_{c\varepsilon}$-receptor on mast cells. Reinforcing the relation to allergy, Th_2-cells induce mucosal mast cell proliferation (mainly via IL-4) as well as proliferation of eosinophils (mainly via IL-5), both cell types activated by IgE. Physiologically, these mechanisms are at least partly suppressed by Th_1-type cells [59].
In previous asthma research, the "Th_2-hypothesis" links the typical symptoms of allergic asthma to an imbalance between a diminished Th_1-type answer and a heightened Th_2-type response, leading to IgE production, histamine signalling and then airway inflammation [54]. This Th_2-shift is especially prominent in the neonatal immune system, a time thought to be crucial for asthma development [62]. Additionally, Th_2-type cytokines have been found to be increased in asthmatic children. APCs might at least partly at fault for this disequilibrium as they are responsible for the presentation of allergens in mucosal surfaces and start the immune response leading to allergy in the genetically susceptible individual [59, 63].

As the view of asthma evolves, the Th_2-hypothesis has lost influence in favour of an immunological response that is as heterogenous as the disease with many different contributing pathogenetic mechanisms [64]. For example, the involvement of other Th-cell subtypes has been shown, such as Th_{17}-cells, which are said to drive neutrophilic and macrophage inflammation in non-allergic asthma [54, 65].
IL-17, the cytokine produced by this Th-cell subset, counteracts the anti-inflammatory properties of another Th-cell subset, the Th-regulatory cells (Treg) [66]. Lluis et al. showed this reciprocal regulation in a cord blood study and proposed that early immune maturation of Th_{17}-cells depends for example on genetic predisposition among other factors [67]. In studies investigating protective factors, maternal farming has been found to positively influence both Treg count and function [68]. Consistently, Tregs have been identified to have an overall immuno-supressive function. However, they have also been shown to be enriched in children with allergic asthma, which could indicate a counter-regulatory process [2].

1.3.2 Innate immunity

As it becomes increasingly clear that childhood asthma has its roots in the earliest stages of life, the role of innate immunity has shifted into focus. The reasoning behind this is that as adaptive immunity takes time to mature to its full capacity, the

innate immune system is very prominent especially at the beginning of life and thus in the period seen as critical for asthma development [5].

Innate immunity is the human body's first line of defense against pathogens such as fungi, bacteria and viruses as well as a variety of altered cells like apoptotic, degenerated and virally infected cells. Its task is to recognize these structures as antigens (i.e. any structure that eventually leads the immune system to produce antibodies) and, in case of pathogens, to signal the adaptive immune system an infection has occurred.

Its cell types, as illustrated below, can be divided into primarily antigen-presenting cells like dendritic cells and primary killer cells, like macrophages, neutrophilic granulocytes and natural killer cells. In contrast to the adaptive system, there is no memory function in innate immunity.

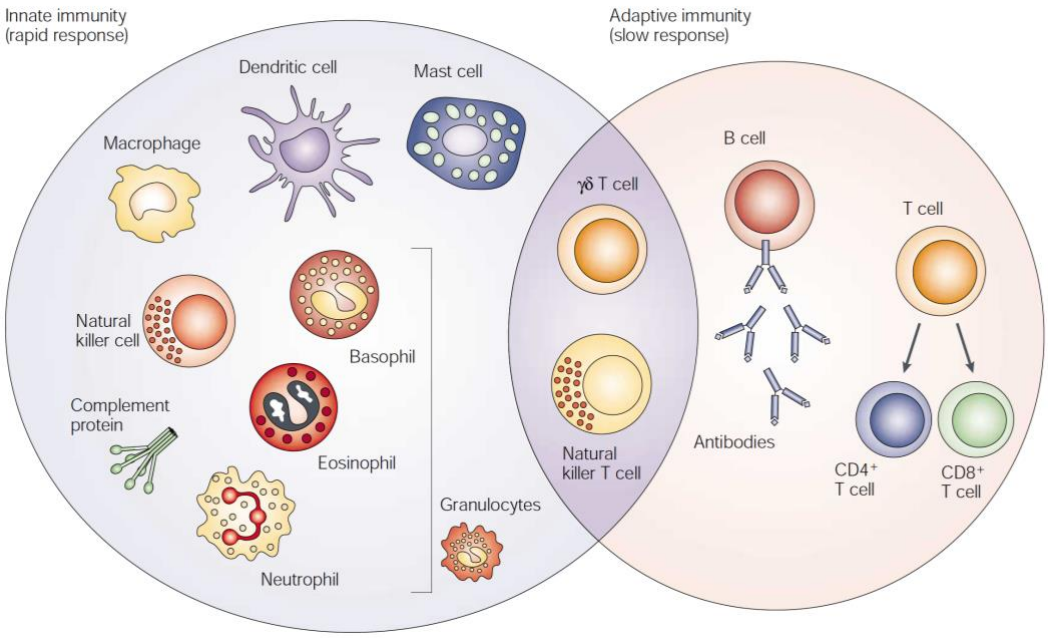

Figure 3: Cell types of innate and adaptive immunity. Each system consists of a cellular part and a humoral part (complement protein or antibodies, respectively). At the interface of adaptive and innate system, see natural killer cells and γδ T-cells, both cytotoxic lymphocytes. From [69].

The pathogen receptors of the innate immune system are called pattern recognition receptors (PRR) and recognize pathogen associated molecular patterns (PAMPs), conserved molecular motifs shared by a class of microbes, which are relatively unspecific. They are highly conserved in evolution and commonly divided into four subgroups: Toll-Like receptors (TLR), C-Type Lectin receptors (CLR), RIG-I-like receptors (RLR) and Node-like receptors (NLR).

Upon ligand binding, a complex signalling cascade leads to the typical features of innate response: generation of ROS (reactive oxygen species), activation of the arachidonic acid cascade, an increase of intracellular calcium, and the activation of the mitogen-activated protein kinase (MAPK)-pathway, which influences transcription factors such as e.g. NFκB (nuclear factor 'kappa-light-chain-enhancer' of activated B-cells) and NFAT (nuclear factor of activated T-cells). Both transcription factors are found in almost all immune cells, in the innate as well as in the adaptive system. Functioning and activation of NFkB and NFAT are largely dependent on Ca^{2+} related mechanisms. Their targets for enhanced transcription include signalling proteins, cell surface proteins, other transscription factors and also cytokines like IL1β, IL-18 and IL-8 [70-72].

Additionally, PRRs also recognize endogenous damage-associated molecular patterns (DAMPs), which occur e.g. when tissue is damaged by ongoing inflammation. Physiologically, macrophages eliminate them in order to limit immune activation, preventing excess inflammation [26].

Many of the innate immune system's cell types - including monocytes, lung-specific macrophages, natural killer cells (NK), dendritic cells, neutrophils, mast cells and eosinophils have been shown to be enriched in asthma patients [73].

In particular, dendritic cells are vitally important innate immunity cells as they do not only express the most PRRs, but they also orchestrate immune response following their activation. They, like other immune cells, produce different mediators, like IL1β and histamine, which is an important cytokine in allergic reactions. Through these mediators, they can also influence the adaptive system (and vice versa), for example in the pathogenesis of allergy [73, 74]. Recently, another cell subtype, innate lymphoid cells, has also been proposed to be involved in allergic sensitization and inflammation, linking innate to adaptive immunity [75, 76].

Furthermore, innate immunity is also represented by epithelial cells in the airways, which act as sentinels and express innate receptors in the same manner as professional antigen presenting cells like DCs [55, 73].

Undoubtedly, airway inflammation and subsequent airway remodelling are main features of asthma and most likely contribute to its other known features such as airway hyperreagibility [1]. Changes in the regulation of the innate immune system are likely to contribute to the pathogenesis of asthma in many ways. For example,

Boeck et al. showed a link between innate antiviral immunity and asthma manifestation through distinct differences for the expression of innate immunity antiviral receptors such as LY75, as well as other genes related to calcium signalling, in children with allergic as well as non-allergic asthma [77]. Other studies investigating the connection of innate immunity and childhood asthma show increased neutrophilic inflammation levels for children with non-allergic asthma and decreased innate immunity-related gene expression for children with allergic asthma [78]. As the lungs constitute an important immunity barrier to the outside world, an altered antiviral immunity early in life, and thus in the vulnerable period, might lead to excess inflammation and subsequent airway remodelling, finally facilitating the development of asthma [5, 62].

1.4 Endotyping

The effort to integrate immunological insights into the definition of asthma phenotypes has led to the definition of the term *endotype*, describing a specific pattern of pathogenetic mechanisms and/or treatment responses leading to a specific clinical presentation of asthma [26, 79]. Adding another level of complexity, different endotypes could present as the same phenotype [80].

Parallel to the growing acceptance for the existence of endotypes, another paradigm shift to looking at asthma as a syndrome rather than as a multi-faceted disease might allow for more individualised therapy. This definition of an “asthma-syndrome” consists of wheeze phenotypes, each of them with their own distinct endotypes, including specific immunological patterns and environmental factors [2, 40, 54, 81]. With this new key hypothesis, the vast amount of clinical and research data can be organized in order to explain the heterogeneity of asthma [26, 37]. These data include various birth cohorts which have tried to illuminate the influences, in particular early in life, that contribute to the development of a specific asthma endotype. Ongoing efforts to harmonize these date will allow joint analyses as well as comparisons between different cohorts, and thus contribute to more detailed definitions of wheeze phenotypes [82]. Through these and other efforts, certain endotypes have already been established, with the next paragraphs detailing a few of them that are of special interest for this work.

For example, a defunctional antiviral immunity of diminished interferon type 1 production involving epithelial airway cells might be responsible for higher susceptibility to exacerbation in some asthma patients [73, 83]. Also, polymorphisms in IL-18, a cytokine potently inducing production of IgE as well as IFN-γ (leading to neutrophilia) and IL-13 (leading to airway remodelling), have been linked to certain asthma presentations [84].

Another proposed set of endotypes focuses on inflammation-resolving mechanisms. For example, the levels of anti-inflammatory IL-10 as well as the eicosanoid lipoxin A4 have been found to be lowered in severe asthma [85-87]. Eicosanoids are lipid mediators derived from arachidonic acid. The arachidonic acid metabolism in general is an interesting interface to study the balance of pro- and antiinflammatory stimuli. Additionally increasing this pathway's relevancy for asthma is its relation to glucocorticoids whose anti-inflammatory capacities are mediated by the induction of lipocortin-1, which blocks phospholipase A_2, inhibiting the synthesis of all eicosanoids [60].

The Th_2-hypothesis mentioned above fits into the concept of endotypes as Th_2-high endotype in allergic asthma with a specific Th_2-gene expression. However, as already mentioned above, IgE alone does not appear to be a reliable biomarker, whereas the Th_2-inducing IL-25 and basal eosinophilia are correlated with the response to asthma therapy in general, or steroids, respectively [54, 88, 89].

On the plane of non-allergic asthma, Th_{17}-cells driving neutrophilic inflammation might be responsible for the notorious steroid resistance in these patients, strongly linking this potential endotype to innate immunity [54, 90].

Up to this point, innate immunity had been thought to play a minor role in chronic inflammatory processes like asthma as it was limited to acute inflammation [26]. Recently, inflammation mediated by innate immunity through DCs and macrophages has come into focus and its part has proved to be major. For example, the reduced ability of macrophages to find and remove defunct cells under oxidative stress results in activation of PRR through a spill of DAMPs, leading to auto-inflammatory processes in both innate and adaptive immunity [91]. These revelations could explain a considerable percentage of steroid insensitivity as well as why steroid sensitivity decreases when asthma severity increases, as the innate system per se is resistant to steroids [92].

This brief overview of already proposed endotypes shows that investigating underlying mechanisms, especially in innate immunity, is a good starting point to gain more insight into asthma pathogenesis to define new, precise and consistent asthma endotypes, especially in non-Th2-related asthma [81].

1.5 Immunological pathways in this work[2]

In order to contribute to endotype definition and further understanding of asthma pathogenesis, this work will investigate pathways that, in previous studies mainly from our research group, have already shown to be differentially regulated in older children with asthma, or have been connected to asthma pathogenesis by others. Thus, the candidate genes chosen for this work contain receptors of the innate immune system as well as genes associated to calcium signalling, with the main question being whether their expression is already altered at birth.
The following paragraphs will introduce and describe the candidate genes, mainly chosen based on previous results from our group reported by Boeck et al. showing differential gene expression in school age children in genes related to calcium signalling (namely, *ATP2A3*, *CALM2*, *ITPR2*, *ORAI1*, *ORMDL3*, *S100A9*, and *STIM2* among others) as well as genes associated with innate immunity (namely *CD209*, *FPR2* and *LY75* among others) [77].

1.5.1 Receptors related to calcium signalling

The severity of asthma has long been coupled to a local increase in polycations such as calcium. Subsequently, perturbations in the mechanisms controlling the intracellular and extracellular concentration of calcium are of great interest in understanding the development of asthma. During (airway) inflammation or infection and the following activation of immune cells, the concentration of extracellular calcium ($[Ca^{2+}]_e$) is high as calcium serves as a danger signal [93]. It then leads to increased intracellular levels of calcium ($[Ca^{2+}]_i$), amplyfing inflammation by triggering pro-inflammatory signaling cascades. Also, a high $[Ca^{2+}]_i$ primes airway smooth muscle cells to react to pathophysiological stimuli with a lower threshold, which in turn contributes to airway hyperreagibility and airway remodelling [94-97].
Physiologically, calcium is an important and versatile second messenger involved in signal transduction and apoptosis in many cells of the human body, also in the cells

[2] For a better overview of the candidate genes and their functions, see a graphical illustration ("gene map") in the appendix (9.5).

of the innate immune system. It is stored in intracellular organelles such as the ER, which is responsible for keeping the cytoplasmatic levels at a constant 50-100 nM while the extracellular concentration of calcium is at approximately 1mM [96, 98]. Its function as a second messenger relies on subtle changes and oscillations in the cytoplasmic levels, evoked by many different mechanisms involving either cyclic adenosine monophosphate (cAMP) or, more important, inositoltriphosphate (IP_3) as second messengers [99, 100].

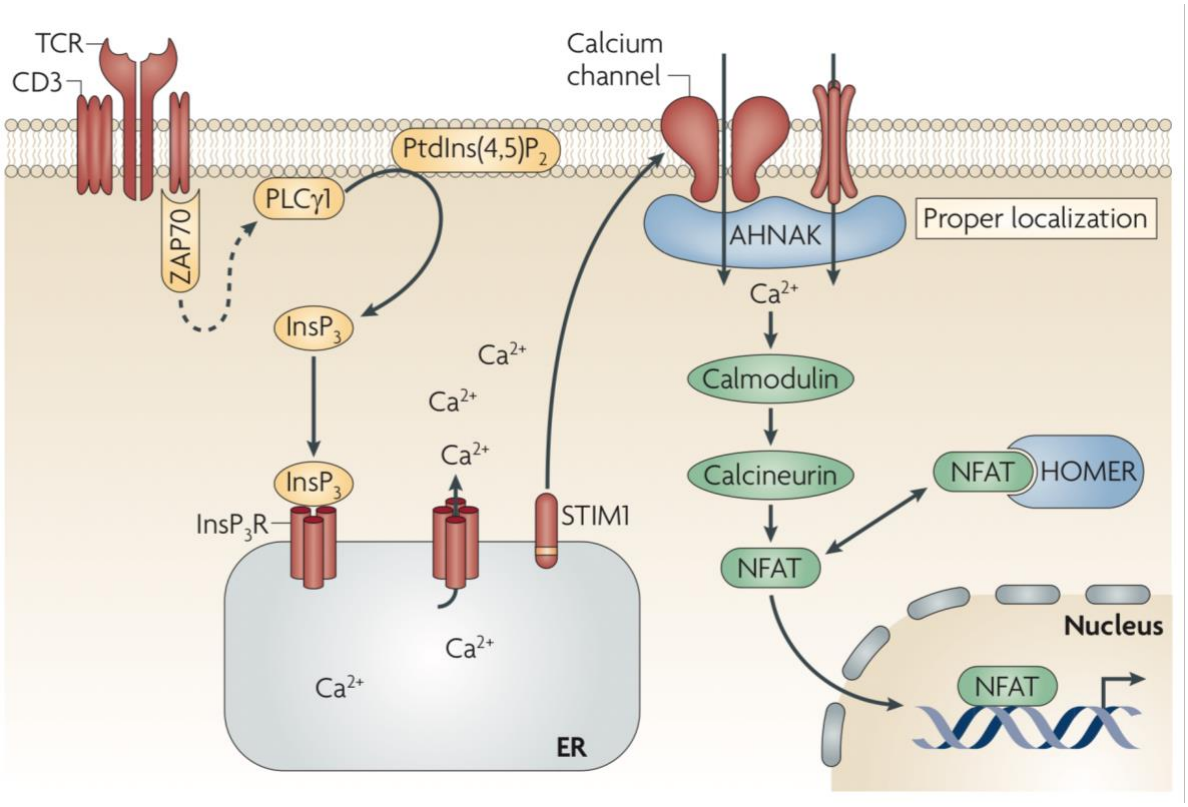

Figure 4: Immunologically relevant pathways of calcium signalling in the cell. On the left, calcium release to the cytoplasm triggered by the TCR (T-cell-Receptor), on the right side of the membrane store-operated calcium entry (SOCE) through STIM1 (STIM2: not shown, but with equivalent function). In the lower left part, effects of intracellular calcium on transcription factors. $InsP_3$: inositol triphosphate. $InsP_3$-R: receptor for inositol triphosphate, equivalent to ITPR2. The calcium channel depicted is exemplary and could e.g. contain ORAI1. From [101].

IP_3 is generated by the enzyme phospholipase C (PLC), which hydrolyses phosphatidylinositole-4,5-bisphosphonate (PIP_2) to IP_3 and diacylglycerole (DAG). PLC is activated upon stimulation of immunoreceptors like the TCR, the B-cell Receptor, Fc-receptor (responsible for phagocytosis and subsequent antigen presentation in macrophages) and mast cell receptor $F_{ce}R$ (responsible for the release of mast cell mediators such as histamine) [98, 102].

IP_3, which can also be released via cAMP, then binds to its receptor, ITPR2, a calcium channel located in the ER and thus, calcium is released from the ER to the cytoplasm [103, 104]. ITPR2 is predominantly expressed in mast cells and often

described as *signal integrator*, as it is most sensitive to ATP and IP_3 but also ROS, and reacts to a low $[Ca^{2+}]_i$ with increased activity [105]. CAMKII and calmodulin terminate calcium influx via protein interaction, together with a negative intrinsic feed-back through a high $[Ca^{2+}]_i$ [106].

Calmodulin 2 (CALM2) is a key protein for Ca^{2+} signal transduction as it serves as a primary receptor for elevated calcium levels and in turn activates CAMKII (calmodulin-dependent kinase II), a kinase selectively expressed in macrophages [98, 107]. It is activated by IP_3, a mechanism regulated by STIM2 [108, 109] and ultimately signals to NFAT [110]. A proasthmatic effect of CAMKII through elevated generation of ROS and activation of the NLRP3 inflammasome as well as the NFκB pathway has recently been proposed especially for allergic asthma [111, 112].
Store-operated calcium entry (SOCE) is a common mechanism also used by cells of the immune system to refill ER calcium stores and evoke Ca^{2+}-signals, regulating a wide range of effector mechanisms including gene expression and cytokine release [113]. Stromal interaction molecules (STIM) 1 and 2 both detect depletion of the ER's calcium stores and inflict changes on the intracellular $[Ca^{2+}]_i$. While they share the same function, they differ in sensitivity as STIM2 detects depletion with a higher sensitivity, i.e. earlier than STIM1. After depletion has been detected, STIM is translocated to the plasma membrane and interacts with ORAI1, the pore subunit of a store-operated calcium channel (SOCC) called CRAC [114-116]. CRAC is responsible for SOCE in T-Lymphocytes and mast cells, regulating activation or degranulation, respectively [102]. Additionally, STIM2 also influences non-store operated changes in calcium levels via CALM2 [109]. It is responsible for stabilizing the basal Ca^{2+} concentration via calcium oscillation and does so independently of STIM1, sometimes even antagonizing its effects [102, 117].
Once activated by binding of calcium to ORAI1, the CRAC channel opens and allows calcium entry through the plasma membrane [104]. In SOCE, ORAI1 is responsible for the amplitude of the calcium influx. Ultimately signalling to NFAT, it is very important for normal T-Cell-functioning [102, 118, 119]. Both ORAI1 and the CRAC channel are inactivated in a calcium-dependent manner through a cooperation of CALM2 with STIM1, effectively terminating SOCE [114, 120].
The resting cytosolic calcium levels are then restored by ATP2A3, a sarco-endoplasmatic reticulum Ca^{2+} - ATPase (SERCA) by pumping calcium from the

cytoplasm back to the ER [107, 121]. If ATP2A3 expression is diminished, a higher grade of airway remodeling, characteristic for asthma, has been observed [122]. SERCAs such as ATP2A3 are controlled and inhibited by ORMDL3 [121]. In various studies, ORMDL3 and SNPs related to its expression have been directly associated with childhood asthma [123]. Also, ORMDL3 modifies T-lymphocyte activation by modulating SOCE after ligand binding to a TCR, as an increased expression of ORMDL3 leads to reduced NFAT signalling [118]. Apart from this influence on adaptive immunity, ORMDL3 is also involved in the mediation of cellular stress through e.g. unfolded protein response (UFR). An increase in intercellular calcium levels has been shown to disturb the ER's correct folding of proteins, resulting in UFR [124].

Elevated levels of intracellular calcium, for example via the IP_3- pathway, can also lead to the augmented presence of proteins called calgranulins in the outer membrane and to their release from phagocytes: S100A8 is such a calcium-binding protein mostly found in complex with S100A9 (then known as calprotectin) [125, 126]. They are abundantly expressed in inflammatory context, mainly in neutrophils and activated macrophages, where they promote inflammation by acting as DAMPs and through their chemotactic function for neutrophils [126-128]. Calcium binding results in signalling via MyD88, modulated by PGE2 and cAMP as well as IL-10, thus enhancing the effect of TLR signalling. This creates a positive feed-back loop as both S100A8 and S100A9 are induced by IL1β. S100A9 is also involved in airway remodelling via the induction of IL-17 [127-129]. Another property of S100A8 and S100A9 is their influence on the cell redox state mediated by ROS, which has for example been shown to mediate IgE-related mast cell signalling in allergic asthma as well as generally promoting inflammatory signalling through both the inflammasome and NFκB pathway [111, 112, 127, 129].

Calprotectin is also involved in the metabolism of arachidonic acid, liberating arachidonic acid from the cell membrane through phospholipase A_2, which is induced by pro-inflammatory cytokines like IL-6 and IL1β [128, 130]. Especially in mast cells and DCs, arachidonic acid is metabolized into leukotrienes (LT) and prostaglandines (PG), both promoting inflammation. Physiologically, there is a fine balance between the broncho-dilative PGs and strictly broncho-constrictive LTs, which is disrupted in asthma. Polymorphisms in the enzyme lipoxygenase which is responsible for the synthesis of leukotrienes have been related to asthma [131]. Leukotrienes are potent

mediators for smooth airway muscle contraction and pulmonary inflammation in asthma. More importantly, they are also said to modulate T-cell response in the direction of Th_2-type inflammation [132].
However, the arachidonic acid metabolites also include anti-inflammatory lipoxins, e.g. lipoxin A4, mediating a resolution of inflammation. Their concentrations have been found to be low in case of severe asthma [133]. Overall, a dysbalance of pro-inflammatory leukotrienes and anti-inflammatory lipoxins seems to be a possible pathogenetic mechanism in severe asthma [134].
Lipoxins are recognized e.g. by the G-protein-coupled receptor FPR2, a mechanism through which FPR2 seems to protect from asthma exacerbations [133, 135]. FPR2 is an example of how calcium signalling and innate immunity (in the form of pattern recognition receptors) interact. Similar points of interaction are of special interest in understanding the pathogenesis and interrelations of airway hyperreagibility and airway inflammation, as they seem to stem from imbalances in calcium signalling or innate immunity, respectively.

1.5.2 Receptors related to innate immunity

The main properties of FPR2 lie in innate immunity as it is expressed in neutrophils and monocytes. Its functions are similar to a PRR, recognizing a vast number of bacterial signalling peptides and formylpeptide, inducing the migration of DCs from the perivascular to the peribronchial tissues in allergic airway inflammation [136, 137]. Upon ligand binding, FPR2 leads to ROS generation, an increase in the intracellular concentration of calcium via PLC activation and IP_3, and to activation of the MAPK-pathway, influencing SOCE as well as transcription factors such as NFAT and NFκB [137].

Another point of interaction, this time between innate and adaptive immune system, can be found in the NLRP3- inflammasome. It consists of three subunits: NLRP3, Caspase-1 and ASC, which serves as an adaptor for the previous two. Caspase-1 is the effector subunit which cleaves pro-IL1β and pro-IL-18, transscribed under the influence of NFκB, into their mature forms. Its activation is triggered and regulated by various factors, cAMP and a high $[Ca^{2+}]_i$ being very important [138-141].
The inflammasome can also directly be influenced and activated by ROS. Some of the mechanisms influencing the cell redox state have already been mentioned above, another are RIG-I-like-receptors DHX58 and DDX58 and their shared adaptor protein

IPS1, all a part of the body's viral defences through the recognition of viral structures like DNA [142, 143]. IPS1 is responsible for tight regulation of the RLRs' effect upon ligand binding. Once activated, IPS1 signals to the inhibitors preventing NFκB from translocation to the nucleus and thus from actively influencing transcription, lifting this inhibition and allowing NFkB to translocate [142, 144]. Its other effector functions include the production of antiviral IFN1 that has been linked to a high susceptibility for asthma exacerbation (see chapter 1.4).

The PRR LY75, which belongs to the CLR family, recognizes cell death material from virally infected or apoptotic cells and also acts as a mannose receptor. It is expressed in both immature and mature DCs, but changes its location from intracellular to surface when the DC is activated. Upon ligand binding, the antigen is taken up and enters the MHC-presentation pathways. In a steady state, DCs continuously process antigens for both the MHCI and the MHCII pathway, contributing to peripheral T-Cell tolerance, targeting CD4+, CD8+ and T_{reg} cells [145, 146]. LY75 expression is elevated in the lungs of patients suffering from allergic asthma, induced e.g. via the inflammasome product IL1β, potentially disrupting the balance between the induction of tolerance or immunity [147].

Another CLR with a function similar to LY75 is CD209, which is upregulated via IL-13 (an important cytokine in Th_2-type immunity), to be found on immature DCs and macrophages, also in the lungs [58, 148]. Recognition of carbohydrates or Der-p, the main antigen of house dust mite, leads to endocytosis and antigen presentation via MHC II [149]. Additionally, the arachidonic acid cascade, which has been described above, is activated, especially in mast cells and DCs [150, 151].

Interestingly, both CD209 and LY75 seem to be dependent on calcium for ligand binding and downstream signalling, making them another point of interaction between calcium signalling and innate immunity [152].

CD209, like LY75, has tolerance-inducing capacities, e.g. by acetylation of NFκB if an antigen is recognized without an additional inflammatory stimulus. In general, CLRs are said to modulate the immune answer after TLR activation [153, 154]. However, ligand binding leads to diminished expression of the receptor, a mechanism that, if constantly activated, has been proposed to increase the risk of life-long allergy and allergic asthma [58, 155].

1.6 Prediction of wheeze outcomes

All of the receptors and proteins that have been chosen for further investigation in this work are part of keeping a fragile balance between tolerance and immunity as well as pro- and anti-inflammatory signalling. However, it is crucial for the immune system to learn how to keep this balance early in life, and a disruption facilitates the development of diseases like asthma.

Appropriately, it is common for asthma to develop during early childhood, often even during the first few years of life. For the 30-40% of preschool children that experience wheezing, there are two possibilities: progression to childhood asthma or remission. It has been shown that in retrospect, 75% of children with persistent asthma had started to wheeze before age three, with their wheeze continuing first into school age and later into adulthood [13, 156]. In addition, studies have shown that the degree of severity tends to be firmly established early in life [157]. It is safe to say this group would largely benefit from early, consistent therapy.

On the other hand, less than 50% of those patients who have experienced early childhood wheeze continue to do so [6, 158]. Also, according to current knowledge, it seems likely that patients who do remit do not develop another late-onset wheeze later in their lives [38]. Clearly separating these patients from those who will go on to develop childhood asthma is at least equally important as defining reliable asthma phenotypes: It would spare many children unnecessary treatments that inevitably have unwelcome side effects like for example steroids do, and in turn relieve the economic burden asthma represents for society.

This overarching goal can be summarized as the improvement of the prediction of wheeze outcomes, with possible outcomes including remission as well as the different pheno- and endotypes of childhood asthma.

Currently, there are several tools for diagnosing wheeze and asthma early in life, such as the ISAAC *(International study of asthma and allergy in childhood)* criteria [159] or the modified version of the asthma predictive index (mAPI) as an effort to improve the prediction value of the original API [160, 161]. Despite this attempt to improve the prediction value of the API, its usefulness in clinical practice remains the subject of controversial debate [162].

In the name of improving the prediction of wheeze outcomes as well as the clinical scoring systems needed for this prediction, many influences in the development of

asthma and the course of the disease have been discovered, both risk factors (such as virally induced wheezing episodes in early childhood [42] or a family history of atopy [163]) and protective factors such as farm exposure, whether prenatally or during childhood [164, 165].

However, despite extensive research, currently very few diagnostic tools include the respective underlying immune mechanisms and the resulting endo- and phenotypes by for example adding biomarkers to the picture. This would certainly increase the predictive value of a score, as the important role of immunological pathways and their regulation in the pathogenesis of asthma is undisputed [28].

Consequentially, this work seeks to identify possible new biomarkers in calcium and innate signalling, as including them in clinical scoring systems will lead to a more precise diagnosis and then to individualised phenotypes, enabling a personalized, effective therapy and thus relieving the burden of childhood asthma [2, 3, 166].

2 Aim of the study

Asthma starts to develop in early childhood, albeit two scenarios are possible for children with preschool wheeze: remission or progression to childhood asthma. Despite great efforts in research, the pathogenesis of asthma is still not well understood. Especially in-depth insights about the role of immune regulation in infancy are still missing, wasting the opportunity for early therapeutic interventions in children at risk for developing asthma. In order to optimize therapeutic strategies, it is crucial to identify children at risk for asthma development as early as possible.
In addition, currently available therapeutic strategies are significantly lacking in two ways: They leave a considerable group of children under-treated with residual disease and inadequate symptom control. In contrast, another group of patients is over-treated for a wheeze that would eventually remit even without any intervention. Those groups would largely benefit from improved prediction of wheeze phenotypes as well as the classification of asthma into endotypes defined by their pathophysiology in order to administer individualized, effective therapy.
Genome wide association studies (GWAS) have identified over 100 candidate genes and pathways linked to asthma development [26, 167]. The candidate genes chosen for this work had already raised interest through differential expression in other asthma cohorts investigated by our group. As it is becoming increasingly clear that prenatal events can already crucially shape the immune system, this study assessed immunological pathways at the earliest point possible, meaning directly at birth in cord blood mononuclear cells (CBMCs) [38, 166].
The aim of this study is to identify immune mechanisms underlying the pathogenesis of asthma that are already altered at birth in order to determine possible candidates for new biomarkers to predict and prevent the development of asthma in preschool children. Therefore, the following questions were assessed in this thesis:

- To study whether genes of the calcium signalling pathway and selected innate receptors are expressed in cord blood immune regulation of neonates.
- To investigate if the expression of the chosen genes at birth differs between children with subsequent different wheeze patterns and healthy controls.
- To specifically study whether a pattern in gene expression variation can be found between children with multitrigger wheeze and other wheeze patterns as well as healthy controls.

- To specifically study whether a pattern in gene expression variation can be found between children with virally induced wheeze and other wheeze patterns as well as healthy controls.

3 Materials and methods

3.1 Materials

3.1.1 Reagents and chemicals

0.5M EDTA (pH 8.0)	Sigma-Aldrich, St.Louis, USA
100bp DNA ladder (500µg/ml)	New England BioLabs, Ipswich, USA
Agarose LE	Biozym Scientific, Oldendorf, Germany
Boric Acid	Sigma-Aldrich, St.Louis, USA
Bromphenol blue	Roth, Karlsruhe, Germany
Ethidiumbromide (10mg/ml)	Biorad, Hercules, USA
Glycerol	Sigma-Aldrich, St.Louis, USA
H_2O bidest	H.Kerndl GmbH, Weißenfeld, Germany
Primers	Life technologies, Invitrogen, Carlsbad, USA
Trizma Base	Sigma-Aldrich, St.Louis, USA
Xylene cyanol	Merck, Darmstadt, Germany

3.1.2 Solutions and buffers

5X TBE buffer	54g trizma base
	27.5g boric acid
	20ml 0.5M EDTA (pH 8.0)
	1l H_2O bidest.
DNA ladder	10µl 100bp DNA ladder
	80µl 0.5x TBE-Buffer
	10µl loading dye diluted solution
Ethidiumbromide [500µg/ml]	100µl ethidiumbromide
	1.9 ml H20
Loading dye stock solution	0.25g bromphenol blue
	0.25g xylene cyanol
	30% glycerol
	70ml dH2O
Loading dye diluted solution	5ml loading dye stock solution
	13.5ml glycerol
	31.5ml dH2O

3.1.3 Reagent kits

QuantiTect Reverse Transcription Kit	Qiagen, Hilden, Germany
Sso Advanced Universal SYBR Green Supermix	Biorad, Hercules, USA

3.1.4 Consumables

96-Well White Shell PCR Plates	BD Biosciences, Heidelberg, Germany
Biosphere® filter tips 0.1-20µl	Sarstedt, Heidelberg, Germany
Biosphere® filter tips 100-1000µl	Sarstedt, Heidelberg, Germany
Biosphere® filter tips 2-100µl	Sarstedt, Heidelberg, Germany
Microseal “B” Seal	Biorad, Hercules, USA

3.1.5 Laboratory equipment

CFX 96 Touch ™ Real-Time PCR Detection System	Biorad, Hercules, USA
Electrophoresis power supply	VWR International, Radnor, USA
Gel iX Imager	Intas Science Images Instruments, Göttingen, Germany
Nanodrop 2000	Thermo Scientific, Waltham, USA
Owl D3-14 wide gel electrophoresis system	Thermo Scientific, Waltham, USA
PeqStar Thermocycler	Peqlab, Erlangen, Germany
Perfect Spin P	Peqlab, Erlangen, Germany
Research plus pipette 10-100µl	Eppendorf, Hamburg, Germany
Research plus pipette 0.5-10µl	Eppendorf, Hamburg, Germany
Research plus pipette 0.1-2.5µl	Eppendorf, Hamburg, Germany
Research plus pipette 2-20µl	Eppendorf, Hamburg, Germany

3.1.6 Software

Biorad CFX Manager 2.1	Biorad, Hercules, USA
Endnote X9	ISI ResearchSoft, Berkeley, USA
Ensembl genome browser	http://www.ensembl.org
National Center for Biotechnology Information	http://ncbi.nlm.nih.gov
Vector NTI Advance 11.5	Invitrogen, Carlsbad, USA
SPSS Statistics 23.0	IBM, Armonk, New York, USA
R	General Public License under the Free Software Foundation

3.1.7 Primer sequences

The primer sequences used for this work can be found in the appendix (9.4).

3.2 Study population[3]

3.2.1 PAULINA

As a part of the birth cohort PAULINA (Pediatric Alliance For Unselected Longitudinal Investigation of Neonates for Allergy), umbilical cord blood was obtained from healthy neonates (n=118), although due to sample availability or non-participation in the follow up, the number available for single analyses varied, born in the Munich metropolitan area, Germany [168]. Midwives and delivery room nurses recruited subjects from October 2004 to September 2007 during the last trimester of pregnancy at the university's women's hospital (Frauenklinik Maistraße, Munich). Inclusion criteria comprised healthy neonates (assessed via APGAR score and clinical evaluation) and mothers with uncomplicated pregnancies. Exclusion criteria included preterm deliveries, multiple gestations, maternal infections and/or use of antibiotics during the last trimester, perinatal infections and chronic maternal diseases. Questionnaires were used to assess parental health and socioeconomic status as possible covariates.

The birth cohort comprises peripheral blood samples (1ml EDTA and 4,9ml serum) from atopic mothers (n=48) and non-atopic mothers (n=70) as well as cord blood samples from their children (see below). Atopy was defined as a doctor's diagnosis of

3 All questionnaires mentioned in the following paragraphs can be found in the appendix (9.6).

asthma and/or eczema and/or hay fever. Additionally, maternal total and specific IgE were measured via radio-allergo-sorbent test (RAST), with a positive specific IgE defined as ≥0.35 IU/mL to one or more common allergens from a panel of 20 allergens (Mediwiss Analytic, Moers, Germany). Approval was obtained from the human ethics committee of the Bavarian Ethical Board, LMU Munich, Germany. For detailed study information, see [168].

3.2.2 PAULCHEN

In the PAULCHEN Study (Prospective Cord Blood Study in Rural Southern Germany), 82 mothers were approached before delivery. The cohort included farming (n=22) and non-farming (n=60) mothers. Subjects were recruited from September 2005 until December 2008 in the obstetric clinic of Bad Tölz, Germany [68]. Farming was defined as the mother living or regularly working on a farm in the last five years and/or during pregnancy. Non-farming mothers lived in the same rural area, without having direct contact to a farm environment. Inclusion and exclusion criteria as well as potential covariates were equivalent to the previously described PAULINA-Study. Approval was obtained from the human ethics committee of the Bavarian Ethical Board, LMU Munich, Germany. For detailed study information, see [68].

3.2.3 Outcome at age three and age six

As the recruited children reached age 3 and then age 6, follow-ups on the study populations were performed. All data were collected by detailed questionnaires filled in by the parents, including epidemiologic parameters (e.g. socio-economic status and parental smoking). The children's possible outcomes included atopic dermatitis, food allergy, wheeze (clinical symptoms and doctor's diagnosis) and sensitization to allergens, measured by specific IgE in a part of the study population. A positive allergen test was defined by at least one positive specific IgE to one of twenty common inhaled or food allergens. The 6-year follow-up questionnaire additionally included outcomes of allergic comorbidities such as rhinoconjuctivitis.

3.3 Laboratory methods

The main focus of this work was to investigate gene expression via qRT-PCR in RNA from CBMCs, and to compare gene expression levels between different subsets of wheeze in the follow-ups of the birth cohorts described, which were recruited from

2004 onwards. Thus, naturally some of the laboratory methods were not conducted by the author herself but by other members of the laboratory.
For the sake of completeness, all laboratory methods involved in this study will be described in the following paragraphs. However, every step that was not conducted by the author herself will be marked with an asterix (*****).

3.3.1 Study inclusion procedure and criteria

3.3.1.1 Collection of cord blood (*)

Parents gave informed written consent about participation in the study. In case of uncomplicated, on-term delivery, cord blood was drawn from the umbilical vein of healthy neonates. A volume of 30 ml, separated in 10 ml tubes coated with liquemin as anticoagulant was obtained in most cases.

3.3.1.2 Isolation and culture of cord blood mononuclear cells (CBMCs) (*)

Cord blood samples were processed within a maximum of 24 hours after withdrawal. CBMCs were isolated by density-gradient centrifugation with Ficoll-Hypaque (Amersham Bioscience, Uppsala, Sweden) after dilution in phosphate buffer saline (PBS, Gibco, Karlsruhe, Germany). Cells were centrifuged for 30 min at 20°C with 1400 rpm, then resuspended in RPMI 1640 (Gibco, Carlsbad, USA) until a dilution to $5x10_6$ cells/ml in 10% human serum (Sigma Aldrich, Steinheim, Germany) was reached. CBMCs were then cultured unstimulated or stimulated with lipid A (LpA, 0.1µg/ml) or phytohemagglutinin (PHA, 5µg/ml) for 72h.
Lipid A is the biologically active component of LPS, an endotoxin of gram-negative bacteria, stimulating innate immune cells.
Phytohemagglutinin is a potent mitogen, stimulating mainly T-cells.

3.3.2 RNA extraction (*)

One millilitre TRIzol (Invitrogen, Carlsbad, Germany) was added to the harvested and centrifuged CBMCs. Samples were then frozen at -80°C. After thawing, 0.2ml chloroform was added and the samples were centrifuged at 4°C and 1200 rpm for 15 minutes. The supernatants of the RNA precipitation were discarded and 0,5 ml 100% Isopropanol as well as 1µl Glycogen were added. After another centrifugation 75% Ethanol was added and samples were centrifuged again for 5min. Samples were then dried for 10-30min at 42°C and, after resuspending in RNAse-free water, incubated at +60°C. RNA was stored at -80°C.

3.3.3 cDNA extraction[4]

Concentration of the extracted RNA was measured via photometry (nanodrop 2000, Thermo Scientific, Waltham, USA). 1µg RNA was transferred to 20µl cDNA with a Quantitect-kit (Quiagen, Hilden, Germany), including a wipe out of genomic DNA. cDNA was stored at -20°C.

3.3.3.1 Primer design

DNA primers used for PCR are chemically synthesized oligonucleotides of about 20bp. They are needed as starting point for the DNA-synthesizing enzyme, polymerase. Primers for the genes explored in this work were designed with “Vector NTI Advanced10” (Invitrogen, Carlsbad, USA) and were then ordered at Invitrogen. Sequences were drawn from the genome database “Ensembl”, a joint project of the European Bioinformatics Institute (EBI), the European Molecular Biology Laboratory (EMBL) and the Wellcome Trust Sanger Institute (WTSI).

The following rules were applied to find pairs of forward (fw) and reverse (rv) primers:

1. Primer length should be between 18 and 27 base pairs.
2. The resulting PCR product should be about 200 base pairs long.
3. The 3’ end of each primer should start with guanine (G) or cytosine (C).
4. The content of guanine (G), cytosine (C) should be between 40% and 60%.
5. The melting temperature of each primer should be between 54°C and 65°C, with the difference in temperature between the forward and the reverse primer being no bigger than 0.5°C.
6. Primers have to be behind the start codon (ATG), as transcription to mRNA starts only after this sequence.
7. The energy by which the primers form dimers or hairpins should be no more than +/- 2kcal/mol, because otherwise the primers will not anneal themselves to the cDNA in a sufficiently specific way.
8. The distance between the forward and the reverse primer should be as wide as possible, e.g. covering multiple exons, to limit the possibility of unspecific amplification.

The delivered primers were first diluted with DEPC-water to a stock of 1mM. Then, with an intermediate dilution to 0.1mM, the final dilution of 1µM was used for PCR, containing both forward and reverse primer. The final dilution was stored at +4°C.

4 For this, the author was partly supported by the laboratory’s technician.

All primers were tested in a two-step scheme. First, the primer was tested with cDNA from test samples, with a focus on whether any amplification was detected at all. Also, the melting curve was analysed.
In the second step, the primer was tested on its specificity for cDNA by performing a test-qRTPCR with DNA and RNA. If no amplification occurred, the primer was considered as usable for qRT-PCR.
In case amplification occurred when testing with DNA, an exception was made if the melting curves of DNA product and cDNA product were clearly distinguishable through different melting temperatures. Additionally, contamination of the isolated RNA and thus the cDNA with DNA was considered as highly unlikely.

3.3.4 Polymerase chain reaction (PCR)

3.3.4.1 Principle of the Polymerase chain reaction

PCR (short for polymerase chain reaction) is a method imitating physiological processes used to specifically amplify even small fragments of DNA.
Basically, the double helix formation of the DNA is heat denaturized. The two complimentary single strand DNA molecules are annealed after the specific primers bound to their complimentary sequence. Then, a heat-stable polymerase resynthesizes a complementary strand to each of those single strands, effectively duplicating the original fragment.

A typical PCR thus consists of three steps being repeated in multiple cycles:

1. Denaturation: the already existing double strand(s) are melted into single strands at 95°C before each cycle.
2. Annealing: at 62.5°C, hybridization of primers at the 5’-3’-ends of the single strands that are to be amplified within 30 seconds. Choosing the right temperature for this step is crucial to avoid non-specific PCR products like e.g. primer dimers. All temperatures mentioned in this paragraph are specific for this work.
3. Elongation: at 72°C, the aforementioned heat-stable taq-polymerase starts to elongate the primers. The desoxyribonucleotid-triphosphates needed for this

step are part of the mastermix, added in abundance. Elongation stops once the polymerase reaches the end of the single strand, or the temperature raises once cycle restarts with denaturation.

In theory, the DNA is amplified exponentially, as shown by this equation:

$$N_n = N_0 \times 2^n$$

N_n : amount of cDNA after the n_{th} cycle

N_0 : original amount of cDNA at the start of the PCR

n : number of cycles

However, this equation assumes that the taq-polymerase is working with 100% efficiency. As this efficiency is not reached in practice, the analysis of PCR data includes an efficiency correction.

3.3.4.2 Quantitative real-time PCR (qRT-PCR)

In this work, gene expression in stimulated and unstimulated CBMCs was studied at mRNA level. To allow the assessment of mRNA, it was transcribed to cDNA. The cDNA was then used for qRT-PCR.

In addition to traditional PCR, quantitative real-time PCR permits conclusions to be drawn about the quantity of the original product that was to be amplified. Quantification is obtained by fluorescence measurements, as the fluorescence increases proportionally with the amount of PCR product.

In this case, Sso Advanced Universal SYBR Green Supermix (Bio-Rad, Hercules, USA) was used as fluorescent dye for qRT-PCR. It intercalates in (c)DNA and then starts to fluoresce.

The cycle of threshold (C_t) describes the cycle during which a fluorescent signal above a background signal, thus coming from the PCR product, is detected. Consequentially, the C_t value is linked to the original amount of cDNA: The lower the C_t value, the higher the initial concentration cDNA. In regard to cDNA, or respectively mRNA, a lower C_t value equals higher gene expression. For objective analysis, all C_t values are referred to the C_t value of the housekeeping gene with stable and ubiquitous expression. In this work, 18S was used as housekeeping gene like. However, SYBR Green detects all kinds of double stranded cDNA, also primer dimers or unspecific PCR products, thus necessitating a melting curve analysis.

3.3.4.3 Preparatory calculations

For PCR, a mastermix, a cDNA-Mix and an NTC-Mix (for the non-template controls) were prepared.

In all calculations, *n* indicates the number of wells.

- For the mastermix: (*n* + 2 pipetting error) x 5µl SYBR Green, equivalent to 5 µl mastermix per well
- For the cDNA-Mix per condition: (*n* + 1 pipetting error) x (0.12 µl cDNA + 1.68 µl DEPC), equivalent to 1.8µl per well
- For the NTC-Mix: (*n* + 1 pipetting error) x (1.8 µl DEPC + 5 µl SYBR Green), equivalent to 6.8µl per well

In addition, 3.2µl of final primer dilution were added to each well, resulting in a total volume of 10µl per well.

3.3.4.4 Pipetting scheme

As can be seen in this scheme, a 96-well-plate (Bio-Rad, Hercules, USA) was used.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S100A8 M	S100A9 M	ORMDL3 M	IPS1 M	ATP2A3 M	ITPR2 M	ORAI1 M	STIM2 M	LY75 M	CD209 M	FPR2 M	CALM2 M
B	S100A8 M	S100A9 M	ORMDL3 M	IPS1 M	ATP2A3 M	ITPR2 M	ORAI1 M	STIM2 M	LY75 M	CD209 M	FPR2 M	CALM2 M
C	S100A8 PHA	S100A9 PHA	ORMDL3 PHA	IPS1 PHA	ATP2A3 PHA	ITPR2 PHA	ORAI1 PHA	STIM2 PHA	LY75 PHA	CD209 PHA	FPR2 PHA	CALM2 PHA
D	S100A8 PHA	S100A9 PHA	ORMDL3 PHA	IPS1 PHA	ATP2A3 PHA	ITPR2 PHA	ORAI1 PHA	STIM2 PHA	LY75 PHA	CD209 PHA	FPR2 PHA	CALM2 PHA
E	S100A8 LpA	S100A9 LpA	ORMDL3 LpA	IPS1 LpA	ATP2A3 LpA	ITPR2 LpA	ORAI1 LpA	STIM2 LpA	LY75 LpA	CD209 LpA	FPR2 LpA	CALM2 LpA
F	S100A8 LpA	S100A9 LpA	ORMDL3 LpA	IPS1 LpA	ATP2A3 LpA	ITPR2 LpA	ORAI1 LpA	STIM2 LpA	LY75 LpA	CD209 LpA	FPR2 LpA	CALM2 LpA
G	18S M	18S M	18S PHA	18S PHA	18S LpA	18S LpA						18S NTC
H	S100A8 NTC	S100A9 NTC	ORMDL3 NTC	IPS1 NTC	ATP2A3 NTC	ITPR2 NTC	ORAI1 NTC	STIM2 NTC	LY75 NTC	CD209 NTC	FPR2 NTC	CALM2 NTC

Table 1 : Pipetting scheme used for this work. Colums 1-12 each represent a gene of interest while rows indicate a stimulating condition (A-F), the housekeeping gene (G) or NTCs (H), respectively.

For pipetting, all reagents were stored and processed on ice. 18S was used as *housekeeping gene* and thus applied next to the 12 genes of interest investigated in this study.

All experiments were run in duplicates. The non-template controls and dissociation curves were used to rule out unspecific amplifications and primer-dimers.
After finishing the pipetting process, the plate was sealed with optical foil (Bio-Rad, Hercules, USA) and then centrifuged at 2500 rpm (rotations per minute) for 15 seconds to eliminate any bubbles.
Afterwards, the plate was placed in the iCycler (Bio-Rad, Hercules, USA) and the protocol was started.

3.3.4.5 Protocol iCycler

Cycle 1: (1x)		95.0°C	2 minutes (= Initial denaturation)
Cycle 2: (40x)	Step 1:	95.0°C	20 seconds (= Denaturation)
	Step 2:	62.5°C	30 seconds (=Annealing + Elongation)
Cycle 3: (1x)		72.0°C	2 Minuten (=Elongation)
Cycle 4: (1x)		95.0°C	30 seconds
Cycle 5: (1x)		55.0°C	30 seconds
Cycle 6: (80x)		55.0°C	5 seconds
Cycle 7: (1x)		20.0 °C	HOLD

3.3.4.6 Gel electrophoresis

Electrophoresis describes the migration of molecules in an electric field depending on their charge and size. Gel electrophoresis is a method used for additional PCR quality control (for further explanation, see below).
In this work, three percent agarose gel plates were used. For this, 6g agarose (Biozym Scientific, Oldendorf, Germany) solved in 200ml of 0.5% buffer (900ml aqua bidest + 100 ml 5xTBE) were heated up to boil until the solution was clear. After cooling down to 80°C, 70 µl ethidium bromide (Biorad, Hercules, USA), which intercalates with DNA and visualizes it in UV light, were added and the gel (14x23cm) was cast. The gel chamber (30x27x11cm, buffer volume 800ml, Thermo Scientific, Waltham, USA) was prepared with several combs, forming pockets in the gel after their removal when the gel was completely cooled down.
For performing the gel electrophoresis, 2µl loading dye was added to all PCR products as well as the NTCs.

PCR products, NTCs and the “ladder”, a solution containing DNA fragments of known sizes, used to quantify the size of the PCR products, were pipetted into the gel’s pockets.
The gel chamber was then connected to 120V voltage and 400mA current for 40 minutes. Afterwards, the gel was evaluated and then photographed under UV light.

3.3.4.7 qRT-PCR analysis

After completion of the iCycler protocol, data were analysed with the associated program (Bio-Rad, Hercules, USA).

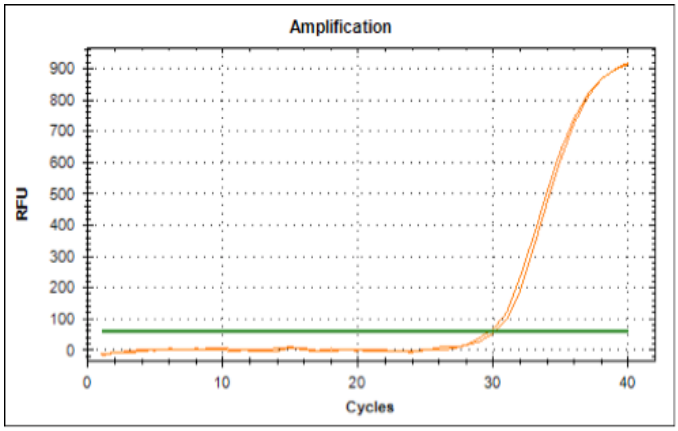

Figure 5: Exemplary amplification of cDNA. The both lines each represent one of the duplicates.

The x-axis shows the number of PCR cycles, while the y-axis shows the intensity of the fluorescence signal. The green line (y-axis value 50) is the visualization of the C_t, in this case set by the iCycler and not manually. It should lie at the beginning of the exponential phase of amplification. The C_t value is what later is used for statistical analysis.

3.3.4.8 Quality control

3.3.4.8.1 Melting curve analysis

Melting curve analysis is a method to check for specificity in PCR. The melting curve is created by the iCycler by constantly measuring the fluorescent signal while raising the temperature in steps of 0.5°C. The PCR product denaturates at a specific temperature, represented ideally in a narrow, high peak in the melting curve. Unspecific amplification, such as primer dimers, can be recognized by low, broad melting curves.

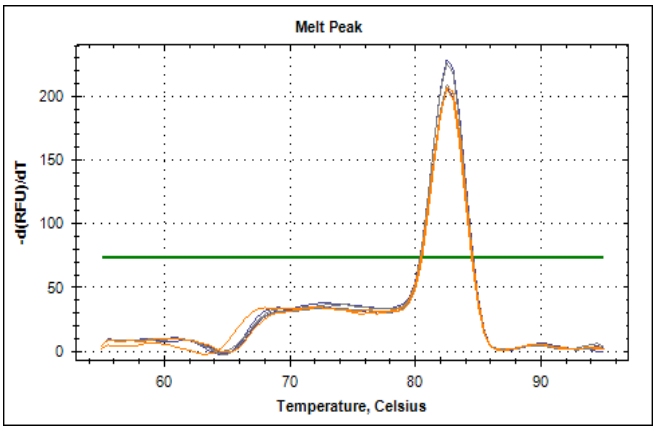

Figure 6: Exemplary melting curve with typical characteristics of specific amplification.

The specifity of all data generated by the iCycler was primarily assessed through melting curve analysis. In case of unspecific amplification (see Fig.7), the duplicate in question was repeated in order to obtain specific results.

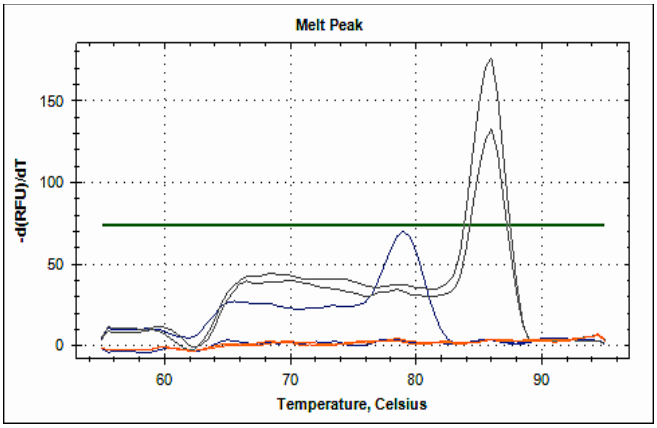

Figure 7: Exemplary melting curves. In blue: unspecific amplification. In dark grey: height difference in two duplicates. In orange: two duplicates, none of which were detectable.

If the height of the melting curve of two duplicates showed differences equivalent to a difference in C_t values greater than 0.7, they were also repeated (see fig.7). This was done in order to minimize the influence of technical mistakes and other disturbances. If a technical error was already noted during the pipetting process, this duplicate was excluded and only the other duplicate was used.

If both duplicates failed to reach a detectable C_t after 40 cycles of amplification but a technical error could be excluded they were both marked as *"non-detects"* and set to an artificial C_t value of 40, which represents the last possibly measured cycle, so they were not lost to analysis.

3.3.4.8.2 Gel electrophoresis

PCR products were separated via gel electrophoresis for additional quality control. Specificity was evaluated by comparing the expected size of the amplification product (around 200bp) with the scale provided by the ladder.

Also, the electrophoresis was another mean to control the duplicates' consistency. If the PCR product consisted only of the specific sequence demarcated by the primer used, a single, sharply outlined band showed in the electrophoresis.

3.3.5 Statistical analyses[5]

Data analysis was performed with SPSS Statistics and *R*.

Epidemiological parameters were assessed for differences in phenotypes for the study population and screened for possible confounders. Continuous parameters were analysed with the Wilcoxon test, categorical variables with the Chi-square test.

The effect of stimulation with either PHA or LpA in comparison with unstimulated cells was assessed using the t-test after calculating the fold change after stimulation.

$$\textbf{Fold change} = (\log_2 - \Delta\Delta C_t)$$

For the analysis of the PCR data, the ΔC_t was calculated by using 18S as housekeeping gene, as it is ubiquitously and stably expressed.

$$\Delta C_{t\,(\text{gene of interest})} = C_{t\,(\text{gene of interest})} - C_{t\,(18S)}$$

As all experiments were run in duplicates, mean ΔC_t values were calculated for all duplicates in the next step.

$$\Delta C_{t\,(\text{mean})} = \tfrac{1}{2}\,(\Delta C_{t\,(\text{duplicate A})} + \Delta C_{t\,(\text{duplicate B})})$$

Differences in the expression rates between two subgroups were assessed with the Wilcoxon two sample rank sum test.

Statistical significance was defined as $p \leq 0{,}05$. P values with $p \leq 0{,}1$ were defined as trends.

As no confounders were found in our analysis, a stratified analysis of data was not necessary.

5 For the statistical analysis, the author was supported by the laboratory's statistician.

4 Results

4.1 Study population

4.1.1 Characteristics

Epidemiological and clinical data about the children included in PAULINA or PAULCHEN, respectively, were collected from detailed questionnaires filled in by the parents at birth as well as at age three and six[6]. For more detailed information about the design of each study, see chapter 3.2. As the inclusion and exclusion criteria as well as the parts of the questionnaires relevant for this work were equivalent in PAULCHEN and PAULINA, they will no longer be distinguished in the following.

4.1.2 Assessment of outcome at age six

Based on the information from the questionnaires, patients were assigned to one of four previously defined subgroups – multitrigger wheeze, persistent viral wheeze, transient early viral wheeze and healthy controls. Those subgroups, each comprising a specific clinical outcome, were later compared to each other in order to find differences in gene expression in CBMCs at birth. In order to avoid the creation of too many subgroups, categorization focused on a small number of questions.
The definitions applied to the three-year follow-up were equivalent to those applied to the six-year follow-up.

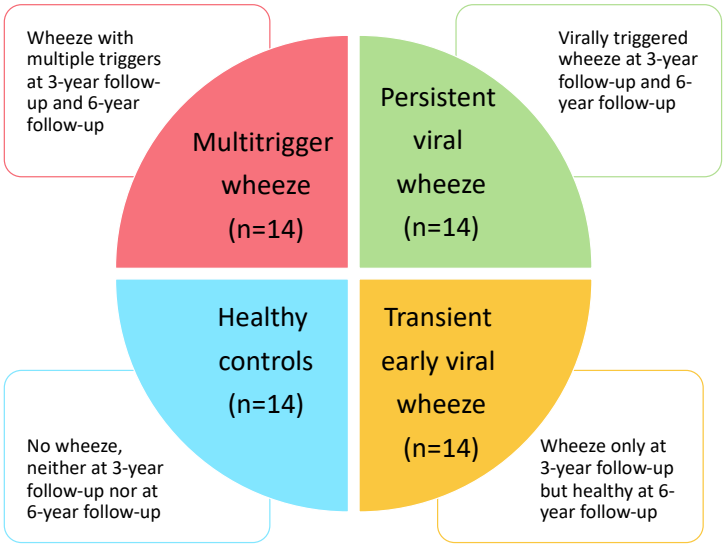

Figure 8 : Schematic representation of the four subgroups used in this work.

6 All questionnaires can be found in the appendix (chapter 9.6).

Healthy controls (HC) were defined as children who did not have wheeze in both the three-year and the six-year follow up. Children whose parents answered “yes” to the question “has your child ever had wheeze” were classified as *“any wheeze”.* Additionally, a (repeated) doctor’s diagnosis of obstructive bronchitis was also classified as the child having shown wheeze. This large group was then further divided.

“Transient early viral wheeze” (TEV) was defined to include children who classified as *“viral wheeze”* in the three-year follow up, with wheezing only during viral infection (see below), but whose parents in the six-year follow-up, answered “no” to the question “did your child ever show wheeze in the last three years”.

Children whose parents answered “yes” to the question “has your child shown wheeze in the last three years” in both three- and six-year follow-up could either classify as *“persistent viral wheeze”* or *“multiple trigger wheeze”*.

Of those children, *“persistent viral wheeze”* (PV) contains all whose parents indicated that they only wheezed when they had an infection of the airways (i.e. wheezy bronchitis) but not outside of these episodes. This had to be stated in both the three-year and the six-year follow-up.

For *“multiple trigger wheeze”* (short: multitrigger or MT), parents had to either report that their child’s wheeze was also present without infection and triggered either by effort, contact to animals, house dust, grass, or others, or a doctor’s diagnosis of obstructive bronchitis. In case of an additional doctor’s diagnosis of an allergic comorbidity, like rhinoconjuctivitis, or an allergic sensitization (either parent-reported, diagnosed by a doctor, or a positive specific IgE if it was available) was indicated in the questionnaire, the child in question was also classified as MT.

In contrast to PV, this could be stated in either the three- or the six-year follow-up, or both. Children who were pulmonally healthy in the three-year follow up but filed as MT in the six-year follow up were summarized as *“late-onset-multitrigger wheeze”* (LOM). Viral wheeze in the three-year follow-up, which changed to multitrigger wheeze in the six-year follow-up, was classified as *“viral to multitrigger wheeze”* (VM). In contrast, the group who showed multitrigger wheeze in both follow-ups was called *“persistent multitrigger wheeze”* (PM). For reasons of case numbers, these three individual subgroups were summarized as MT for sample selection and for a part of the analysis.

For more detailed information on the questions asked, see the complete questionnaires in the appendix as well as the summary table below.

Phenotype	*Question 1: "Has your child ever had wheezing?"*	*Question 2: "Has your child wheezed in the past 3 years?"*	*Additional questions (for more exact definition of phenotype)*
Pulmonary healthy (H)	No	No	'Has your child been prescribed medication for wheezing or shortness of breath in the last 3 years?': no 'Has your child been diagnosed with obstructive or convulsive bronchitis or asthmatic bronchitis?': no
Multitrigger wheeze (MT)	Yes	Yes/no	'What triggers the wheezing?': at least two different triggers 'How often does your child wheeze when they are not having an acute infection?': at least once a month Doctor's diagnosis of obstructive bronchitis
	Yes	Yes	Positive allergy test Positive for rhinoconjunctivitis Positive for atopic dermatitis
Viral wheeze (V)	Yes	Yes/no	'Is your child completely symptom-free between the wheezing episodes?': yes 'How often does your child wheeze when they're not having an acute infection?': never

Table 2 : Summary table of questions especially relevant for phenotype definition.

4.1.3 Sample selection

Sample selection was based on a case-control design matched for potential confounders. As the limiting group was, consistent with known prevalence, multitrigger wheeze with n=14, an equal number was selected from the other, larger groups. Those were selected aiming for high homogeneity regarding the variables shown in Table 3. Ultimately, a number of n=56 were selected for the analyses of this work. As possible confounders were eliminated through the described matching

process, there was no need for stratified analyses. The allocation of the samples chosen is shown in Figure 9.

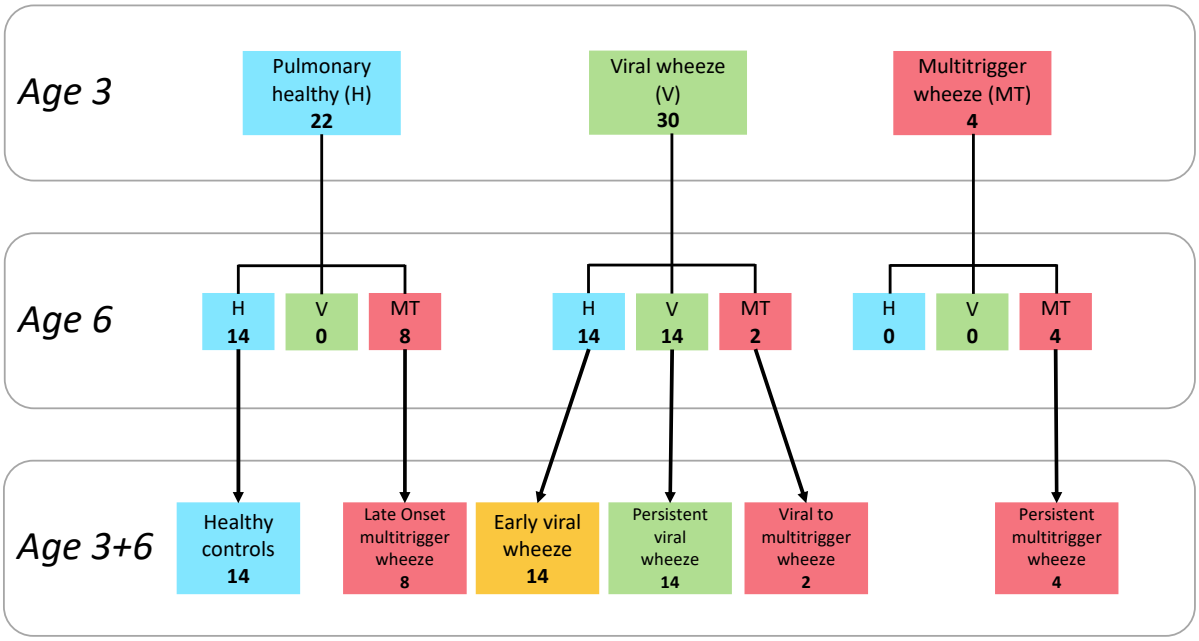

Figure 9: Schematic representations for the subgrouping of samples used in this work. N=14 for the MT group result from summarizing late onset multitrigger wheeze, viral to multitrigger wheeze and persistent multitrigger wheeze.

The epidemiological and socioeconomic characteristics of this smaller cohort are shown in the following table.

	MT (n=14)	**HC** (n=14)	**PV** (n=14)	**TEV** (n=14)	
Female sex	43% (6)	43% (6)	43% (6)	29% (4)	p=0.83 *
Gestational age (weeks)	40.1	39.7	40.1	39.9	p≥0.1 #
Birth weight (g)	3693	3411	3660	3628	p≥0.1 #
Birth length (cm)	52.2	51.5	52.6	52.3	p≥0.15 #
Family history					
Mother asthmatic	14% (2)	7% (1)	43% (6)	21% (3)	p=0.12 *
Mother atopic	57% (8)	64% (9)	71% (10)	64% (9)	p=0.89 *
Father asthmatic	7% (1)	21% (3)	0% (0)	7% (1)	p=0.24 *
Father atopic	57% (8)	50% (7)	21% (3)	57% (8)	p=0.18 *
Maternal smoking					p=0.67 *
No	86% (12)	86% (12)	64% (9)	86% (12)	
Yes	0% (0)	0% (0)	7% (1)	7% (1)	
Until pregnancy	7% (1)	14% (2)	14% (2)	0% (0)	
Quit before pregnancy	7% (1)	0% (0)	14% (2)	7% (1)	
Maternal education					p=0.05 *
Basic school certificate	14% (2)	0% (0)	14% (2)	0% (0)	
Secondary school certificate	29% (4)	7% (1)	14% (2)	0% (0)	
High school degree	14% (2)	43% (6)	7% (1)	14% (2)	
University	43% (6)	50% (7)	64% (9)	86% (12)	

Table 3: Description of the analysed subgroup of the birth cohort. Mother/father atopic: doctor's diagnosis of atopy or atopic disease (hay fever, atopic dermatitis). Mother/father asthmatic: doctor's diagnosis of asthma. Maternal education: highest degree or certificate reached by the mother. MT multitrigger wheeze, HC healthy control, PV persistent viral wheeze, TEV transient viral wheeze. Percentages and absolute number of subjects (indicated in brackets) shown. Test statistic based on the Chi-Square-Test for categorical variables (*) and ANOVA for continuous variables (#).

4.2 Gene expression[7]

4.2.1 Description

In general, most genes from most children were detectable, i.e. their C_t was below the detection limit of 40 cycles[8]. However, several non-detectables, with a C_t above the detection limit also occurred. This is to be expected in CBMCs, as gene expression is generally lower than in other cell populations like e.g. PBMCs (peripheral blood mononuclear cells), and especially in unstimulated CBMCs. In case both duplicates showed a C_t >40 and technical issues as possible cause were ruled

7 For a better overview, see the gene map in the appendix.

8 In qRT-PCR, a low C_t value indicated high gene expression, and vice versa.

out, an artificial C_t of 40 was applied in order to include those values in the analysis. The following tables give an overview of the characteristics of gene expression. As visible in Table 5, only CD209 featured less than 80% detectable values. This censoring was considered for the statistical analysis.

Characteristics of overall gene expression	
Share of detectable values	93.65%
Share of non-detectable values	6.35%
Censored values	8.5%

Table 4: Descriptive display of overall gene expression. Non-detectable: C_t values >40. Censored values: exclusion because of known technical mistake. For one ID there was no sufficient material for measurement of gene expression in LpA stimulation (visible in the analysis as n=13 instead of n=14).

Characteristics of gene expression per gene		
Candidate gene	***Share of non-detectable values***	***Share of detectable values***
ATP2A3	1.53%	98.47%
CALM2	0.59%	99.41%
CD209	20.24%	79.76%
FPR2	4.24%	95.76%
IPS1	1.75%	98.25%
ITPR2	0%	100%
LY75	1.17%	98.83%
ORAI1	2.99%	97.01%
ORMDL3	0%	100%
S100A8	0%	100%
S100A9	0%	100%
STIM2	2%	97.62%
18S	0%	100%

Table 5: Descriptive display of gene expression, sorted by candidate genes. Non-detectable: C_t values >40.

4.2.2 Effect of stimulation

The following figure illustrates the expression of the candidate genes after stimulation with either PHA or LpA compared to expression levels in unstimulated cord blood mononuclear cells (CBMCs) for the cohort that was analysed for this work.

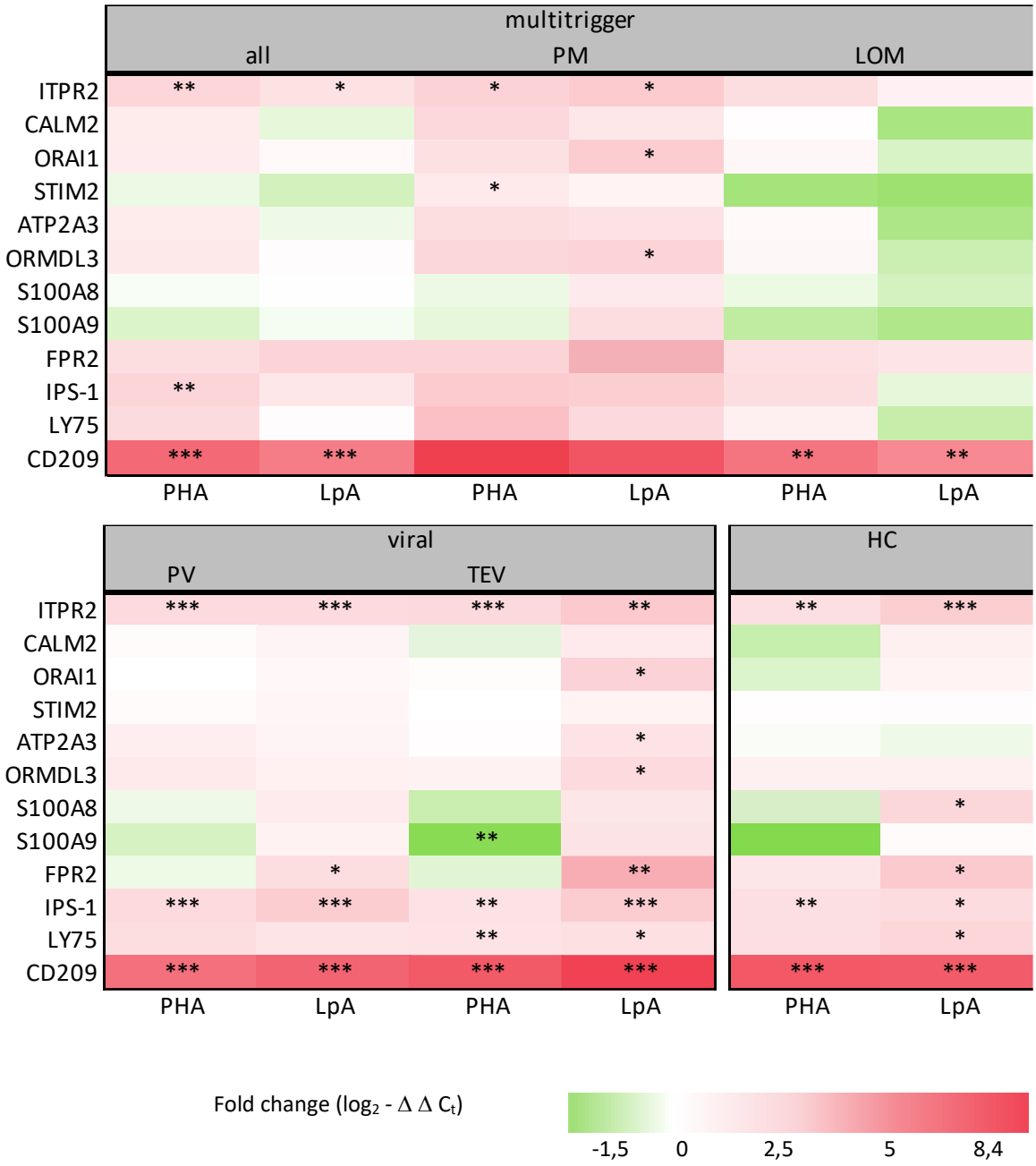

Figure 10: Graphic representation of fold change in comparison to unstimulated cells after stimulation for 72h with PHA and LpA, respectively. Negative values (represented in green) show downregulation, positive values (represented in red) show upregulation. *** p<0,0001, ** p<0,001, *p<0,05 (t-test). PM persistent multitrigger, LOM late-onset multitrigger, PV persistent viral, TEV transient early viral, HC healthy controls.

4.2.4 Gene expression analysis in the wheeze subgroups

For this paragraph the four subgroups multitrigger wheeze, persistent viral wheeze, transient early viral wheeze and healthy controls (see fig.8) were analysed. The results are organized in two parts, *calcium signalling* and *innate signalling*, according to the biological functions of each gene of interest.

4.2.4.1 Expression of genes associated to calcium signalling

4.2.4.1.1 Results for each gene

In the following sections, the results for all calcium-related genes investigated in this work will be displayed, preceded by a short summary of the gene's biological functions.

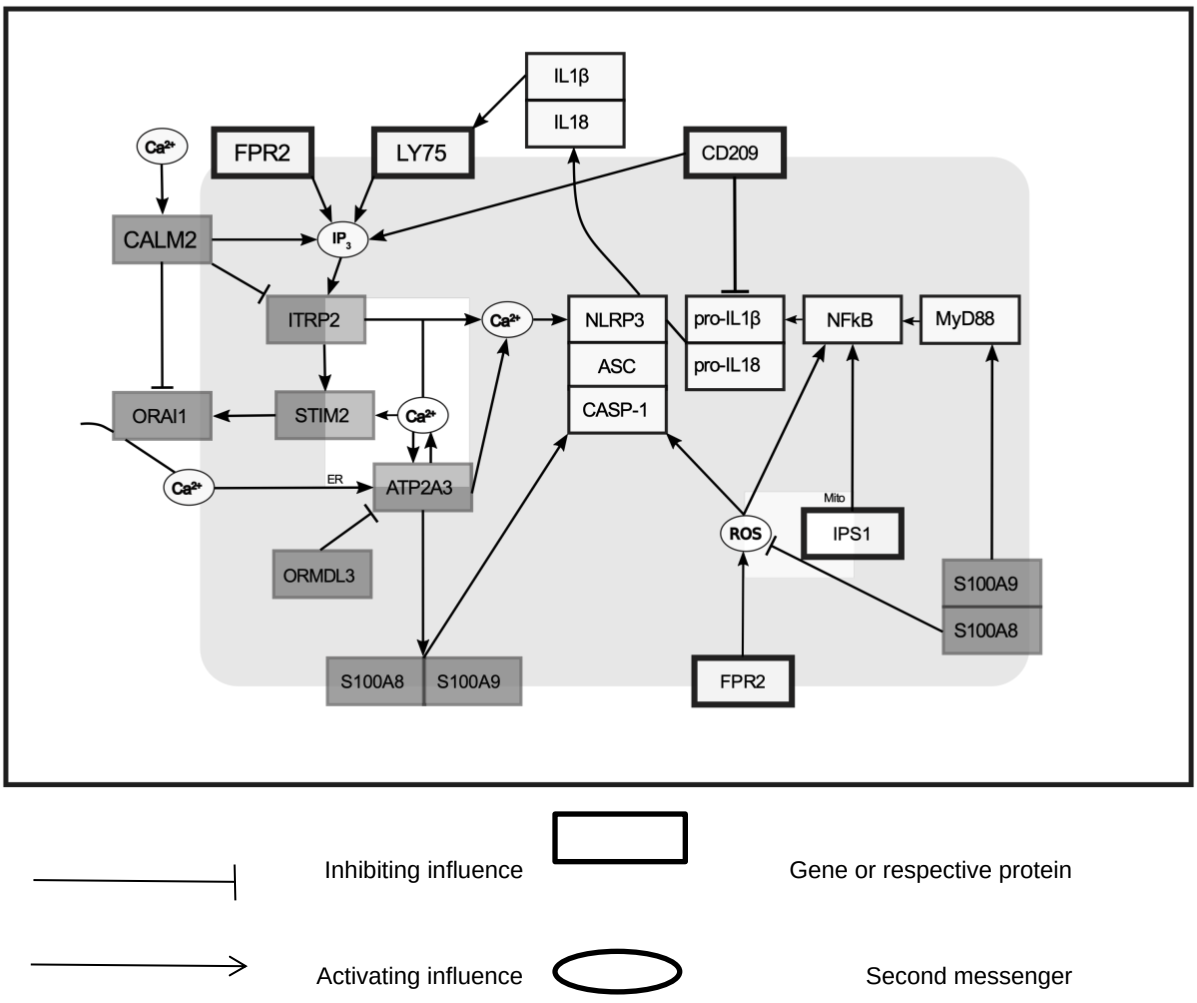

Figure 11: Overview of the candidate genes in a schematic cell (light grey background). Genes related to calcium signalling are shown in grey boxes. Genes related to innate immunity are marked in bold.

4.2.4.1.1.1 ITPR2

ITPR2 is a signal integrator that recognizes IP_3, but also ROS and is located in the endoplasmatic reticulum. Upon activation, it initiates calcium release from the ER stores into the cytoplasm.

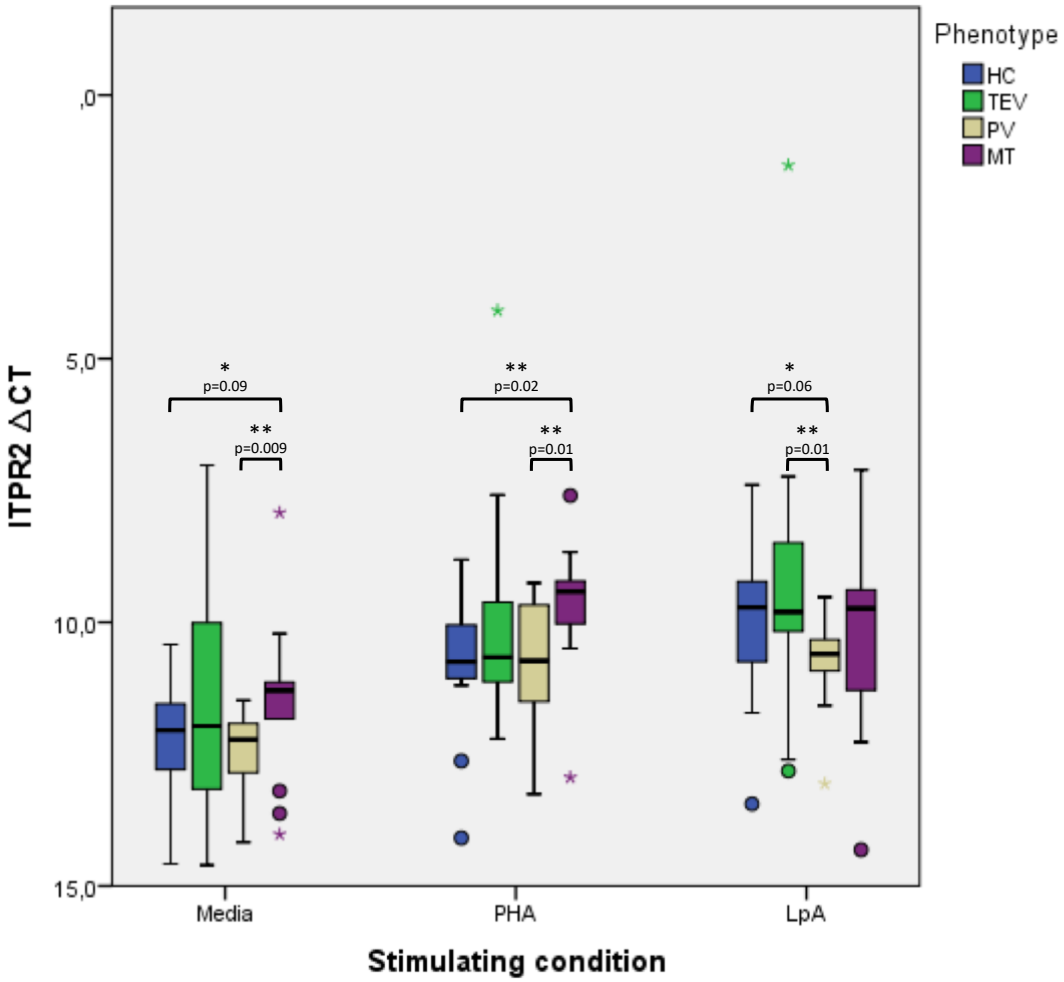

Figure 12: Graphical representation of the results for ITPR2 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

Differences between the four groups were seen in unstimulated cells, as the MT group showed a significantly higher expression of ITPR2 than the PV group (p= 0.009). The same was visible in comparison of MT to healthy controls, but only as a trend (p= 0.09). After stimulation with PHA, this effect was strengthened in MT vs. HC (p=0.02) and remained significant for the comparison of MT vs. PV (p=0.01). Cells stimulated with LpA showed a significantly lower expression of ITPR2 in PV when compared to TEV (p=0.01) and a trend in the same direction when compared to healthy controls (p=0.06).

4.2.4.1.1.2 CALM2

CALM2 is a receptor for elevated extracellular calcium levels located in the outer membrane of the cell. Its downstream signalling activates the NLRP3 inflammasome as well as the NFκB pathway through CALMKII.

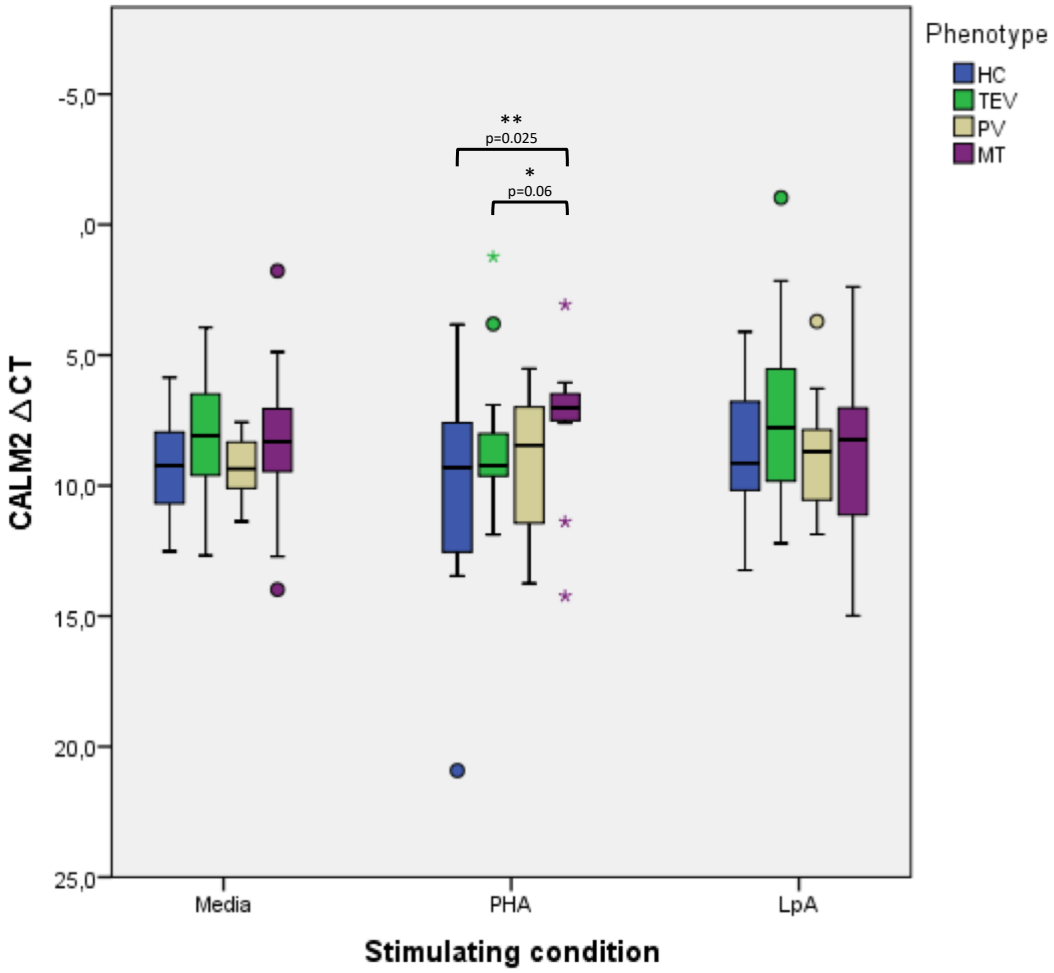

Figure 13: Graphical representation of the results for CALM2 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

For CALM2, effects were only visible after PHA stimulation. The MT group showed a trend for higher gene expression when compared to TEV (p=0.06) and a significantly higher expression than the healthy controls (p=0.025).

4.2.4.1.1.3 ORAI1

ORAI1 is part of a calcium channel in the outer cell membrane needed for store-operated calcium entry after the depletion of the ER's stores.

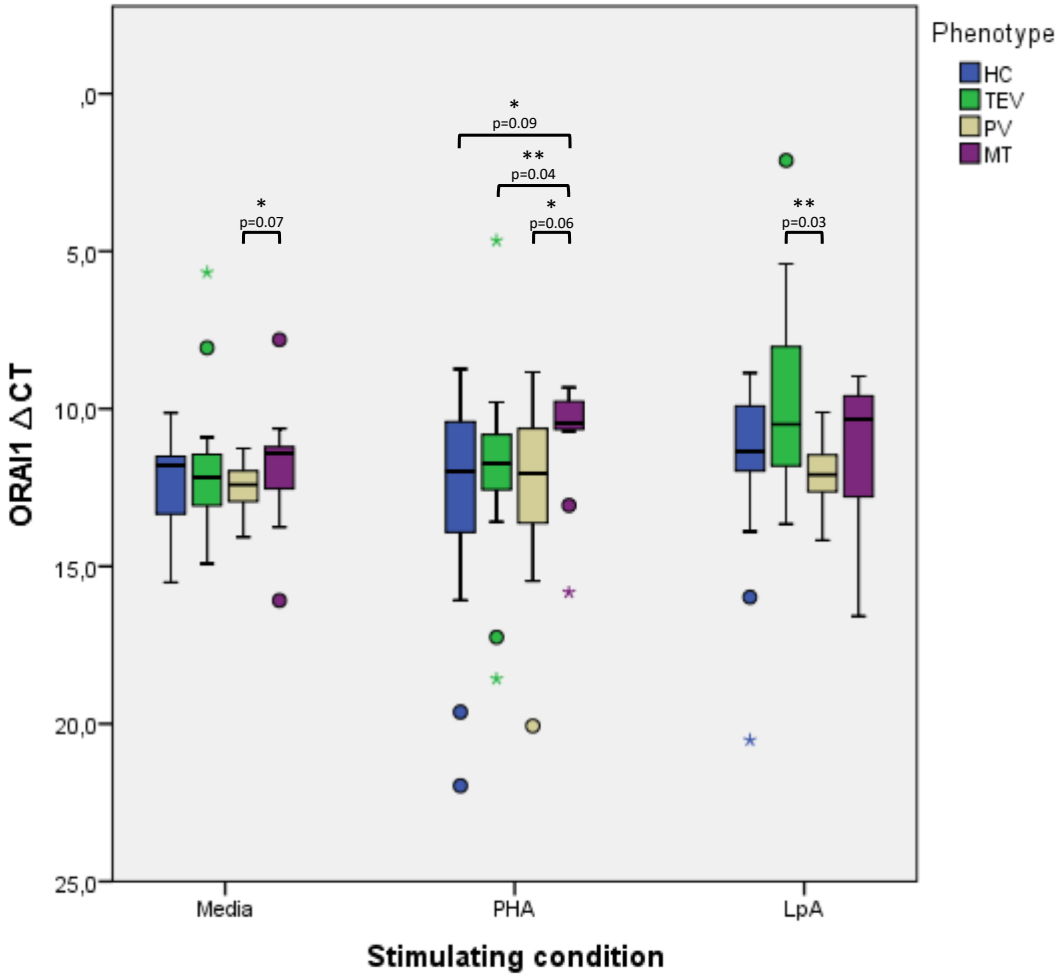

Figure 14: Graphical representation of the results for ORAI1 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

An elevated expression of ORAI1 for the MT group was already detected as a trend in unstimulated cells when compared to the *persistent wheeze* group (p=0.07). The same trend was visible in cells stimulated with PHA (p=0.06). For PHA stimulation, the MT group showed significantly higher expression in comparison with the TEV group (p=0.04) and trend wise in comparison with the healthy controls (p=0.09). In LpA, the *persistent wheeze* group showed significantly lowered expression opposed to the TEV group (p=0.03).

4.2.4.1.1.4 STIM2

STIM2 is a protein located in the ER, sensing depletion of the ER's calcium stores. If such depletion occurs, STIM2 initiates store-operated calcium entry.

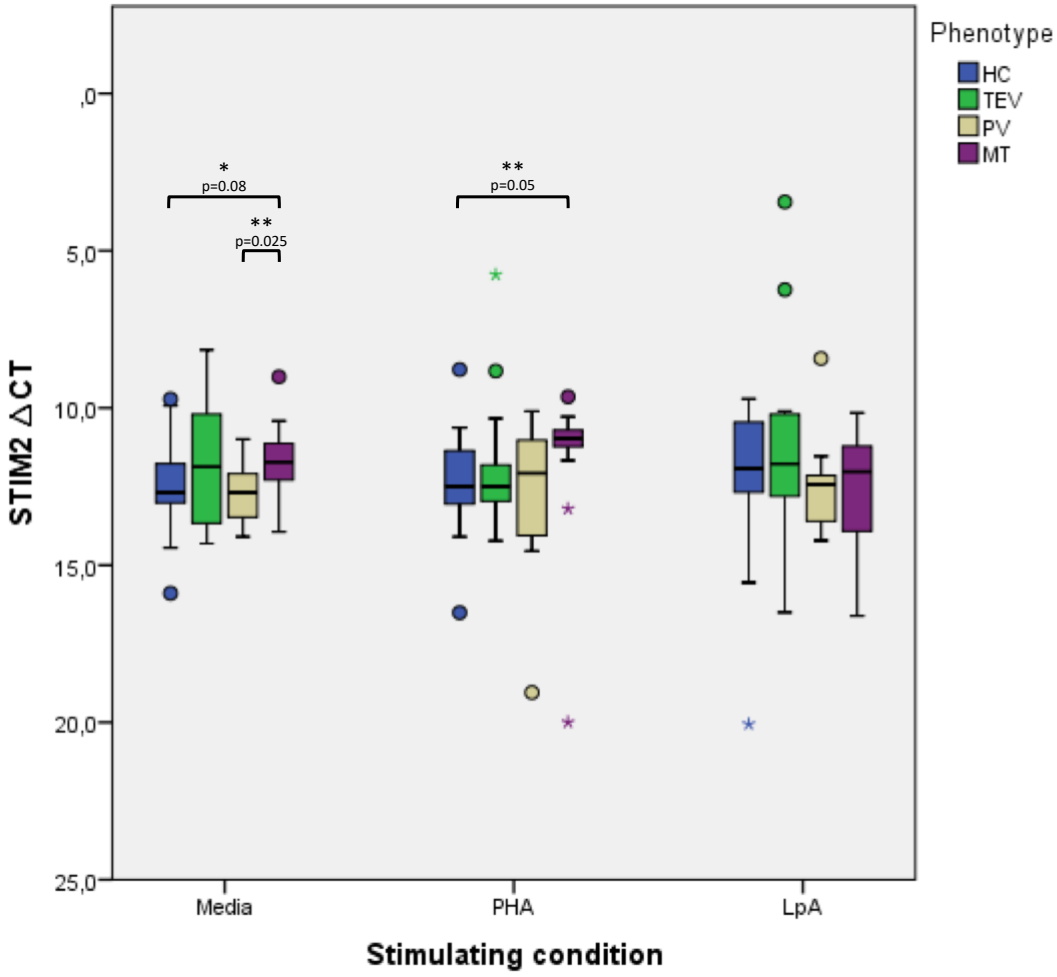

Figure 15: Graphical representation of the results for STIM2 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

STIM2 was significantly higher expressed in the MT group when compared to the PV group (p=0.025), with the same effect visible as a trend in comparison to healthy controls (p=0.08), both in cells without stimulation.

In PHA, the comparison MT vs. HC showed a significantly elevated expression of STIM2 in the *multitrigger wheeze* group (p=0.05).

4.2.4.1.1.5 ATP2A3

ATP2A3 is a SERCA (sarcoplasmatic/endoplasmatic calcium ATPase) located in the ER and responsible for restoring physiological intracellular calcium levels after store-operated calcium-entry.

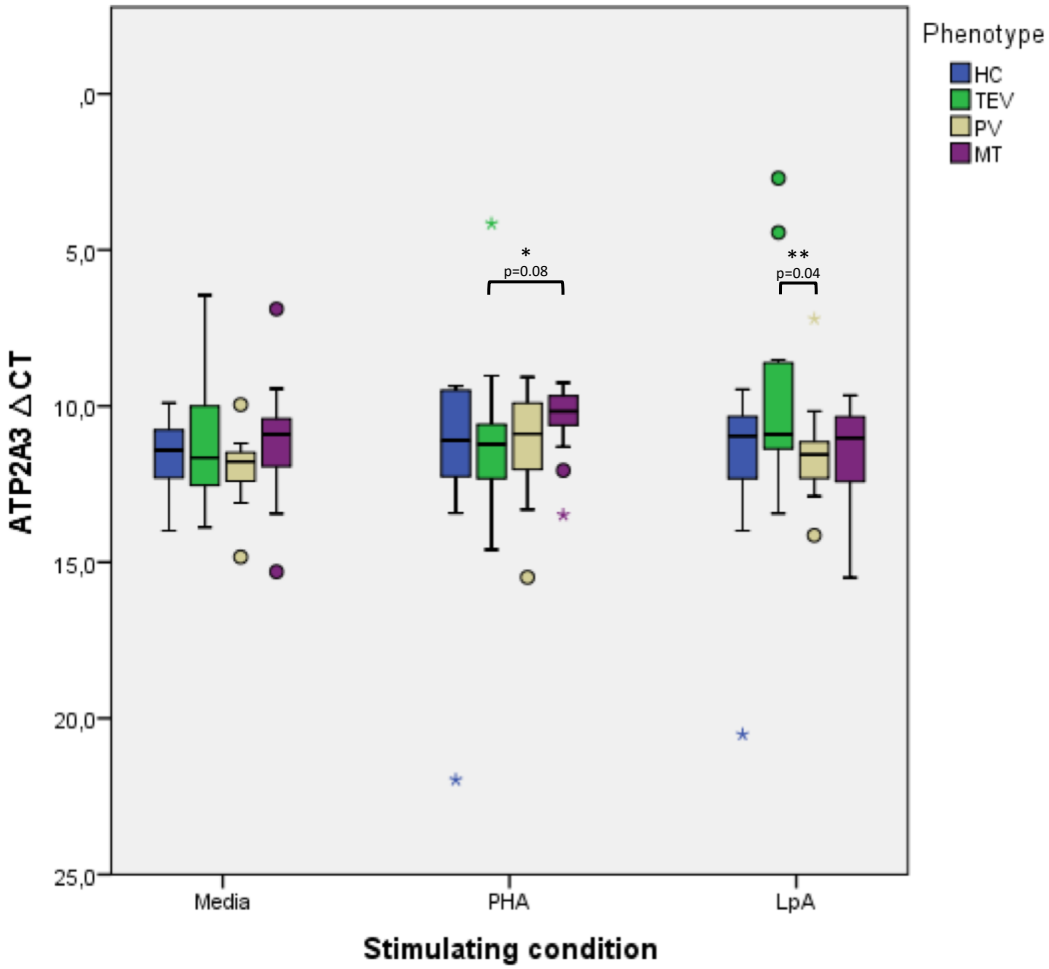

Figure 16: Graphical representation of the results for ATP2A3 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

A trend for higher expression in the MT group compared to the TEV group was detected in cells stimulated with PHA (p=0.08). For PV vs. *transient early viral* wheeze in LpA there was significantly lower expression of ATP2A3 (p=0.04).

4.2.4.1.1.6 ORMDL3

ORMDL3 is a protein responsible for inhibiting and thus controlling the SERCA ATP2A3.

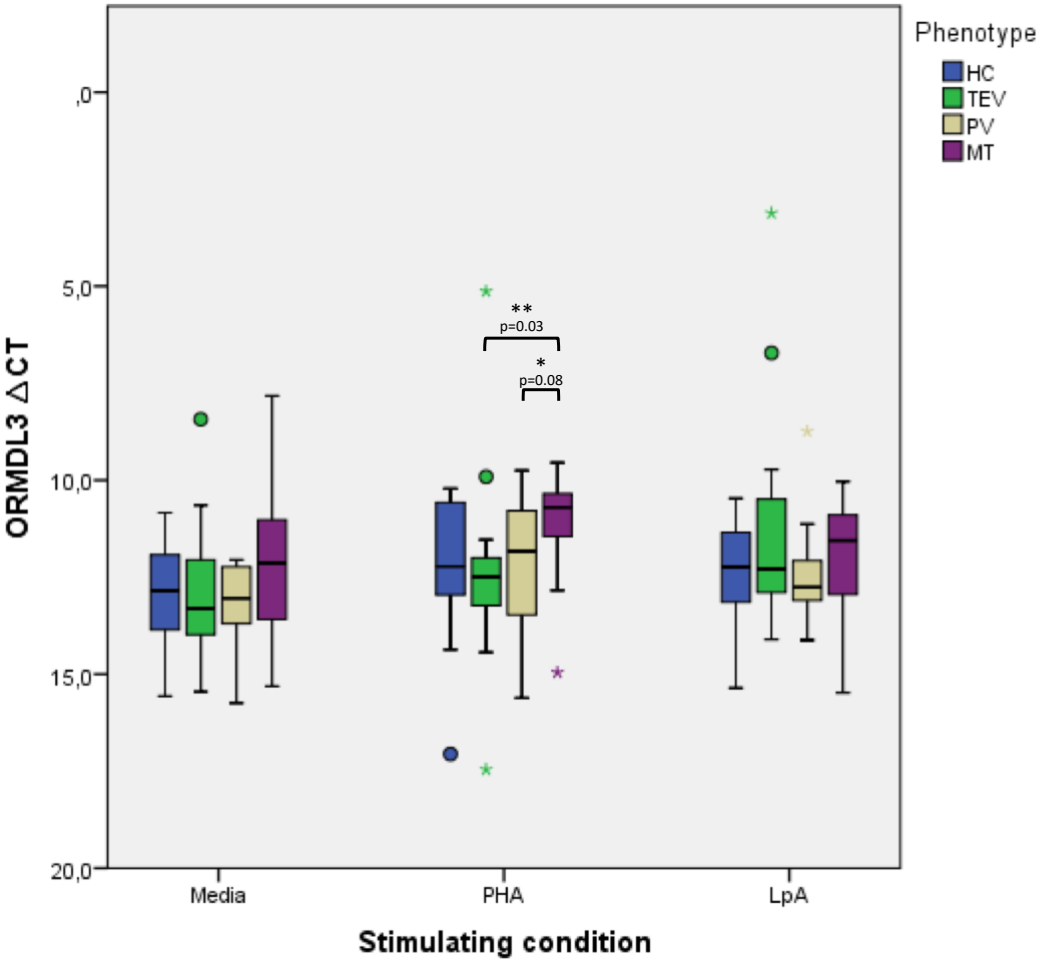

Figure 17: Graphical representation of the results for ORMDL3 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate* trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

After PHA stimulation, expression of ORMDL3 was significantly higher in the MT group when compared to *transient early viral* wheeze (p=0.03). The same effect was visible as a trend in the MT vs. PV comparison (p=0.08).

4.2.4.1.1.7 S100A8

S100A8 is a protein that is abundantly found in the cytoplasm of neutrophils. Upon activation of the neutrophil, it changes its location to the outer cell membrane. S100A8 has chemotactic functions, acts as DAMP and is involved in the cell redox state. However, through the liberation of arachidonic acid it also seems to have anti-inflammatory properties.

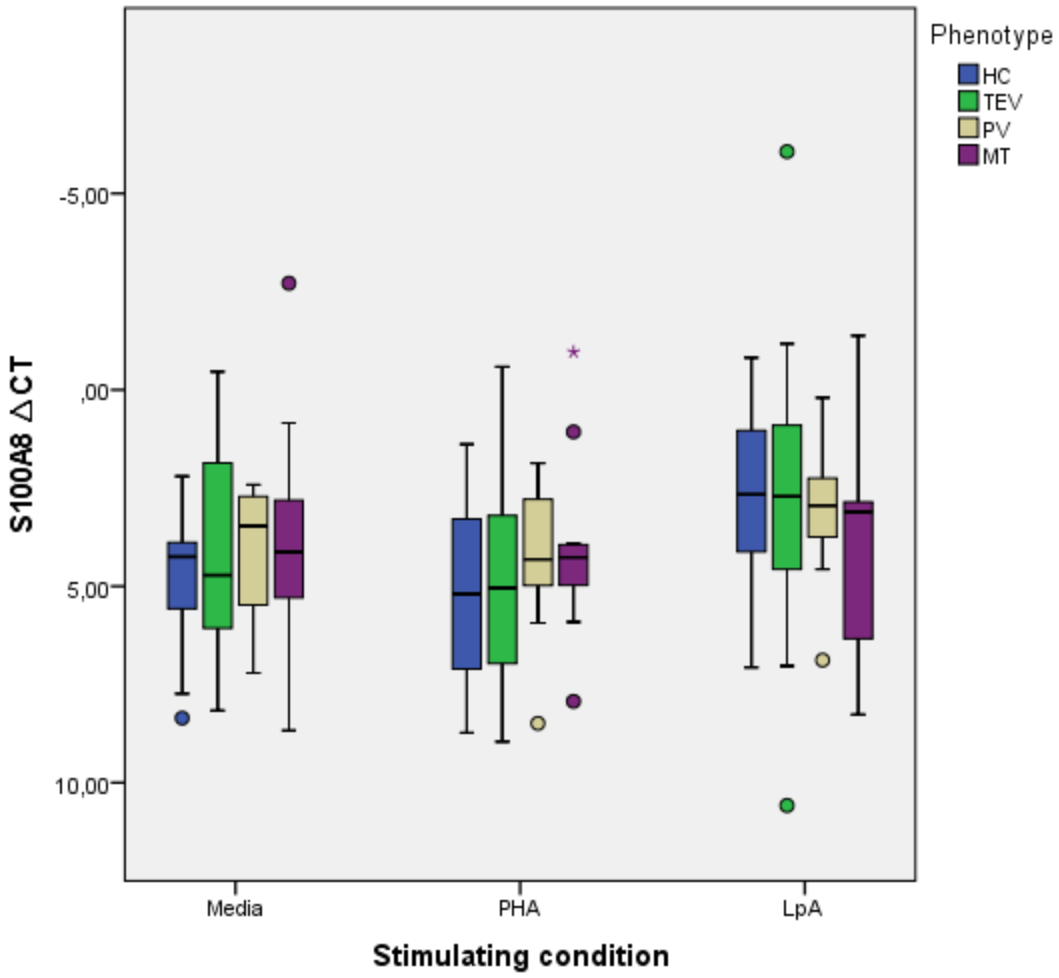

Figure 18: Graphical representation of the results for S100A8 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate* trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

For S100A8, neither significant effects nor trends were found in this analysis.

4.2.4.1.1.8 S100A9

S100A9 is mainly found in complex with S100A8, and is then called calprotectin. It has similar functions as S100A8, but a less pronounced anti-inflammatory function than S100A8.

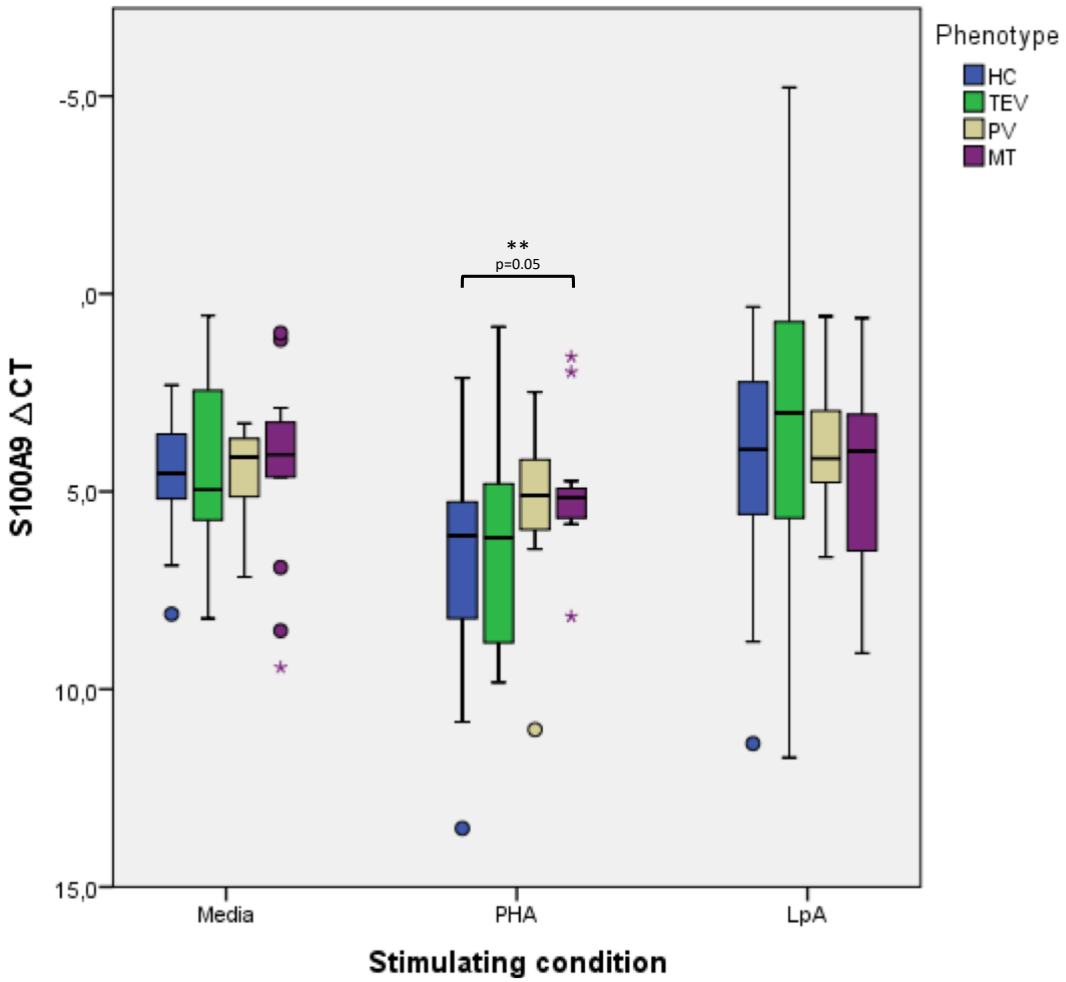

Figure 19: Graphical representation of the results for S100A9 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate* trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

Significantly higher expression of S100A9 in cells stimulated with PHA was observed when the MT group was compared to healthy controls (p=0.05).

4.2.4.2 Expression of genes associated to innate signalling

4.2.4.2.1 Results for each gene

In the following sections, the results for all innate-immunity-related genes investigated in this work will be displayed, preceded by a short summary of the gene's biological functions.

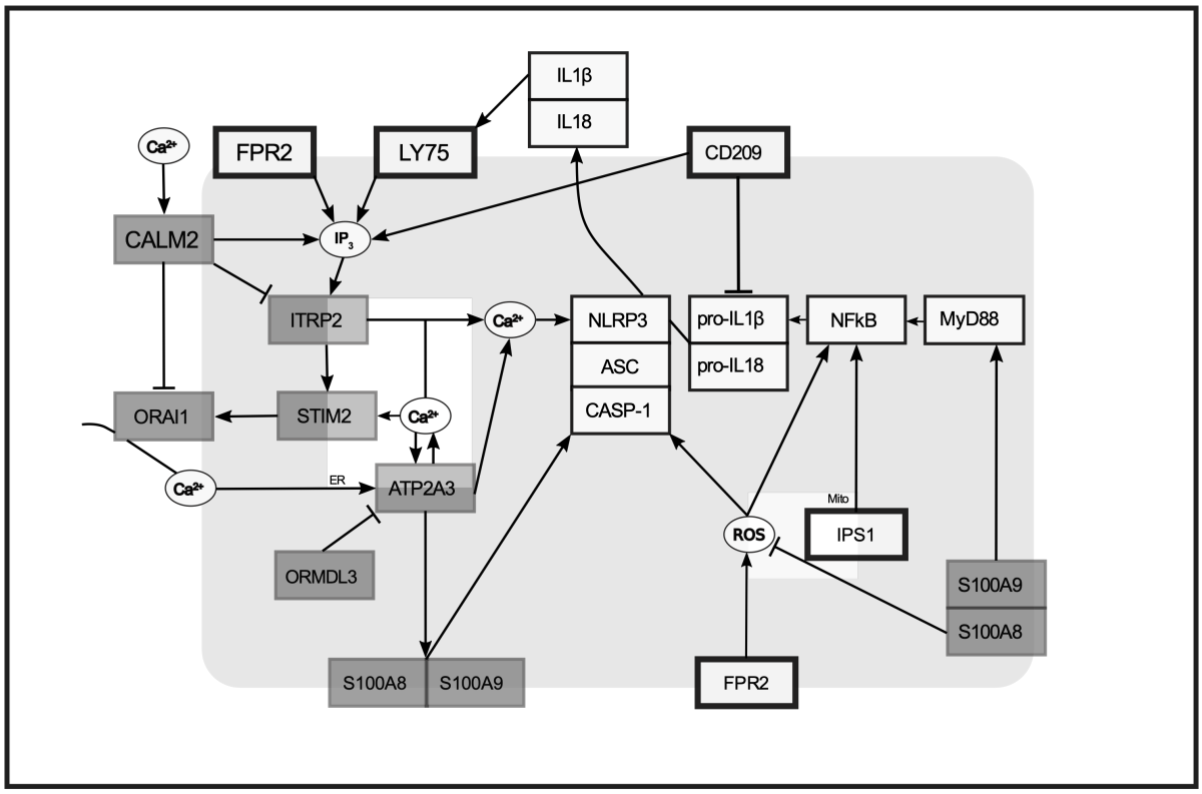

Figure 20: Overview of the candidate genes in a schematic cell (light grey background). Genes shown in this paragraph, related to innate immunity, are marked in bold (FPR2, LY75, CD209 and IPS1). For a detailed legend see figure 11.

4.2.4.2.1.1 FPR2

FPR2 is a protein in the outer cell membrane, acting as a PRR for bacterial signalling peptides and formylpeptide. Additionally, it recognizes lipoxins and is involved in IP_3 generation, thus contributing to the calcium-signalling pathway.

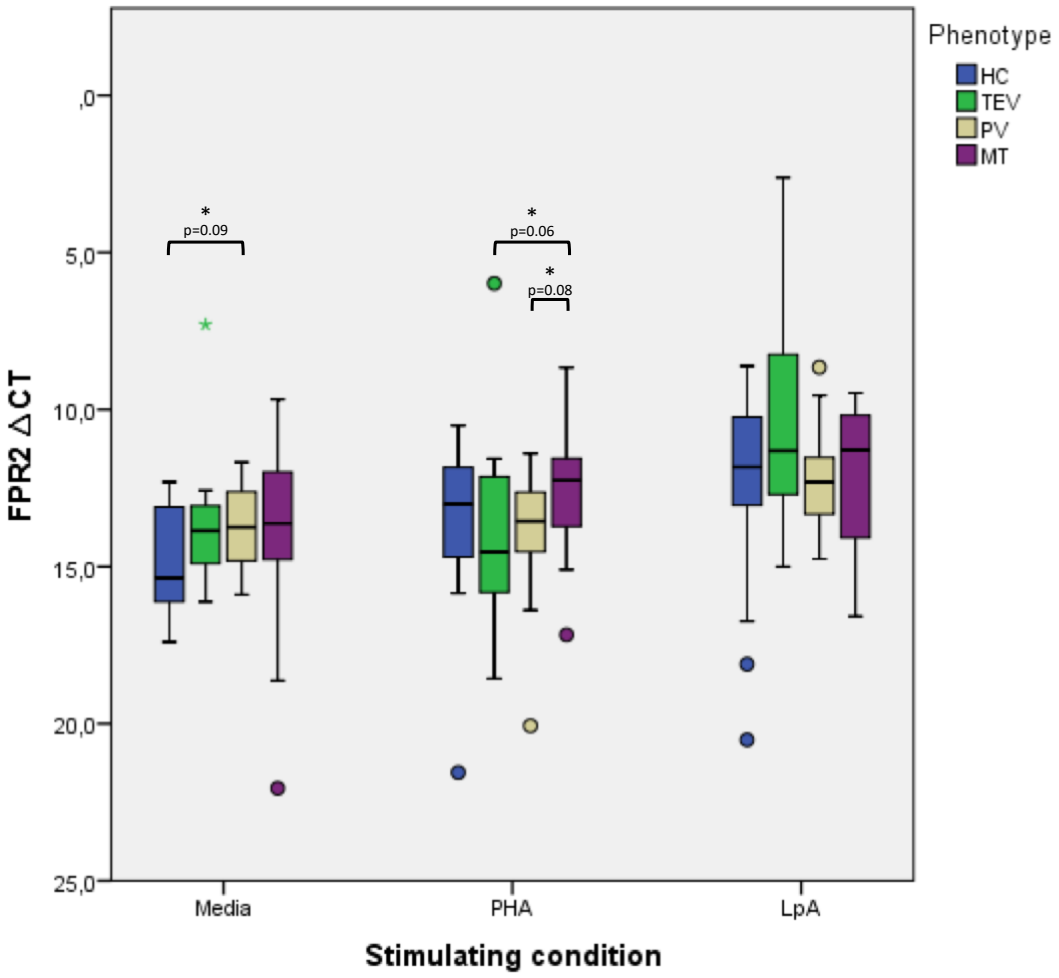

Figure 21: Graphical representation of the results for FPR2 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate* trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

Trend wise, FPR2 expression was elevated in the MT group after stimulation with PHA, in comparison to both the PV and the TEV group (p=0.08 and p=0.06, respectively). In unstimulated cells, a trend for higher expression of FPR2 in the *persistent viral* wheeze group when compared to healthy controls was noticed (p=0.09).

4.2.4.2.1.2 IPS-1

IPS-1 is a downstream adaptor signalling protein for other RIG-I-like-receptors, located in the mitochondrium. Upon activation, it influences the NFκB-pathway and leads to ROS generation.

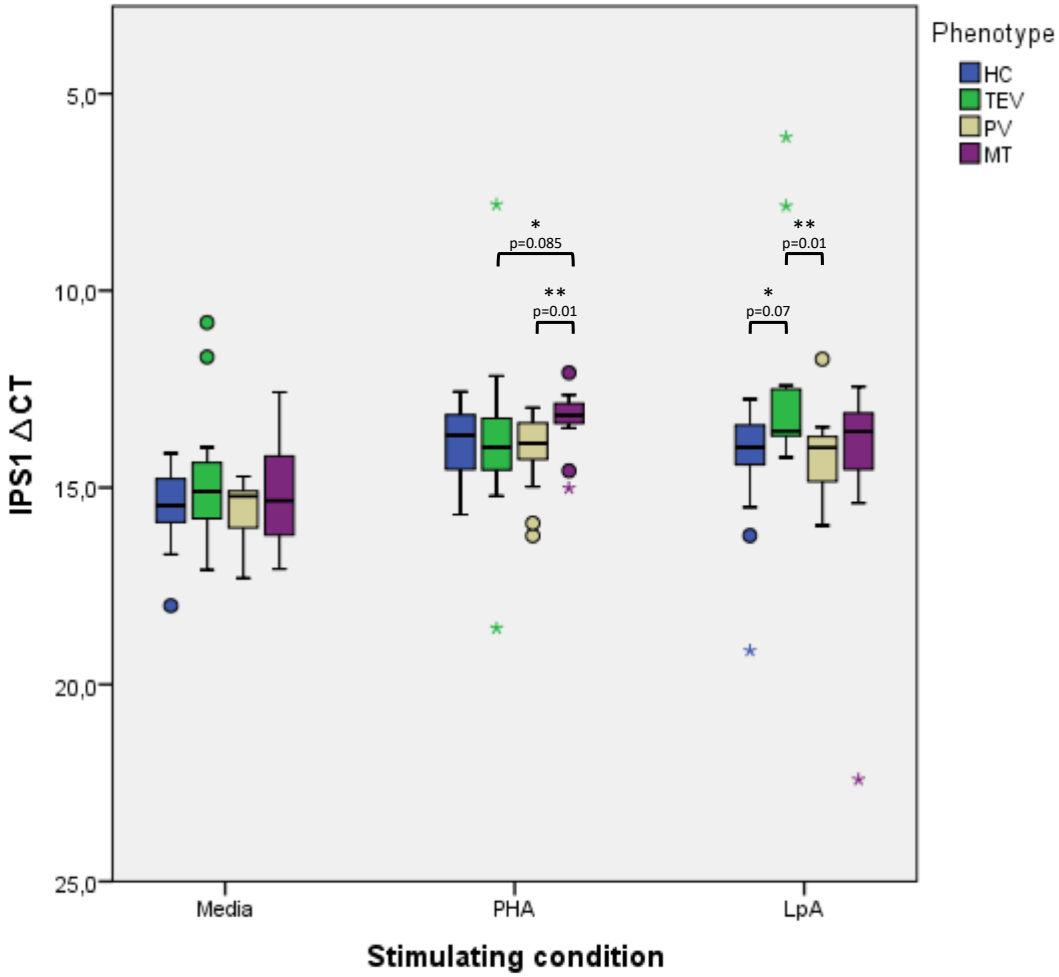

Figure 22: Graphical representation of the results for IPS1 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate* trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

IPS-1 expression was shown to be elevated in the MT group after PHA stimulation, significantly in comparison to the PV group (p=0.01) and as a trend in comparison to the TEV group (p=0.085). In addition, the TEV group showed a trend fhigher expression after LpA stimulation when compared to healthy controls (p=0.07). Also, in LpA-stimulated cells the expression of IPS-1 in the PV group was significantly lower than in the TEV group (p=0.01).

4.2.4.2.1.3 LY75

LY75 is a PRR for cell death material for virally infected or apoptotic cells, located on the outer cell membrane. In a steady state, it has tolerance-inducing properties.

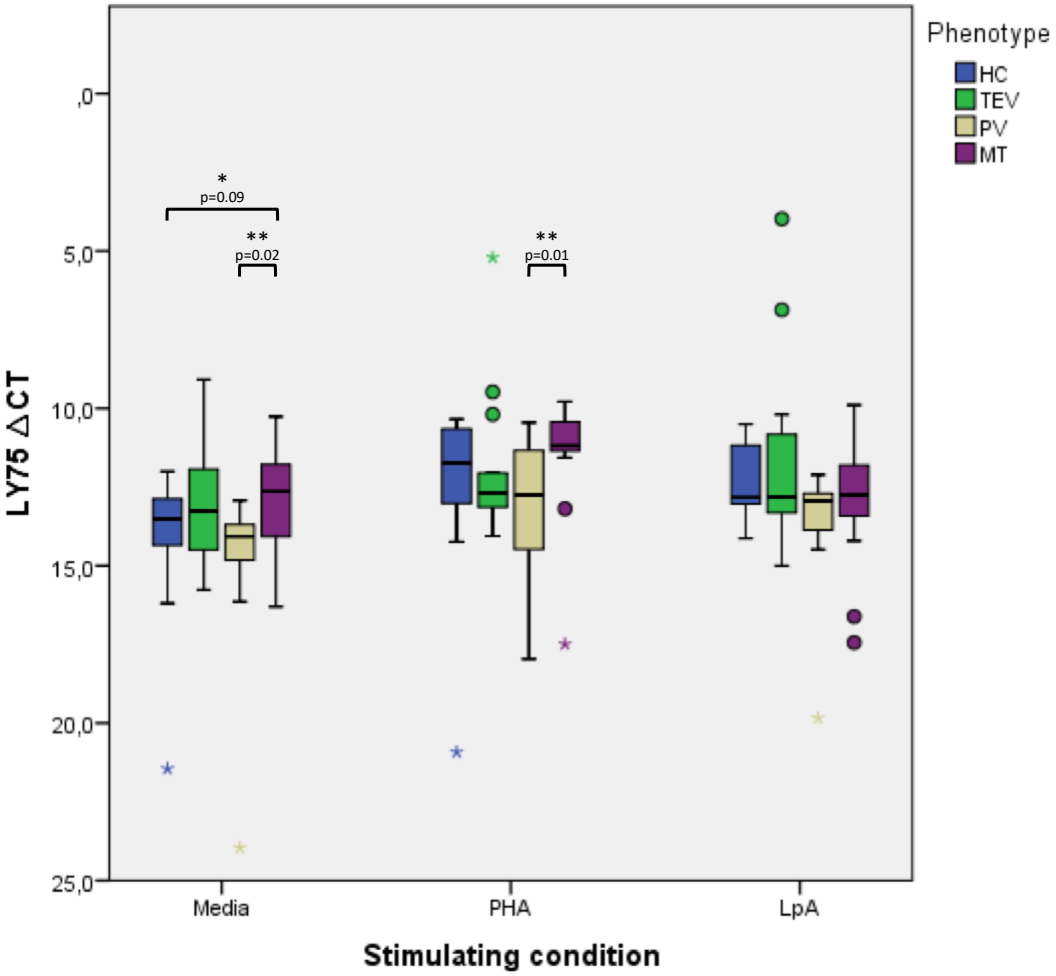

Figure 23: Graphical representation of the results for LY75 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate* trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

Effects for higher expression in *multitrigger* wheeze were visible at a significant level in both unstimulated and PHA-stimulated cells (p=0.02 and p=0.01, respectively) when compared to the PV group. The same effect was visible as a trend in unstimulated cells (p=0.09) in comparison to the healthy controls.

4.2.4.2.1.4 CD209

CD209 also is a PRR, detecting carbohydrates and Der-p, the main allergen of house dust mite. It can activate the arachidonic acid cascade and regulate the NFκB-pathway via acetylation.

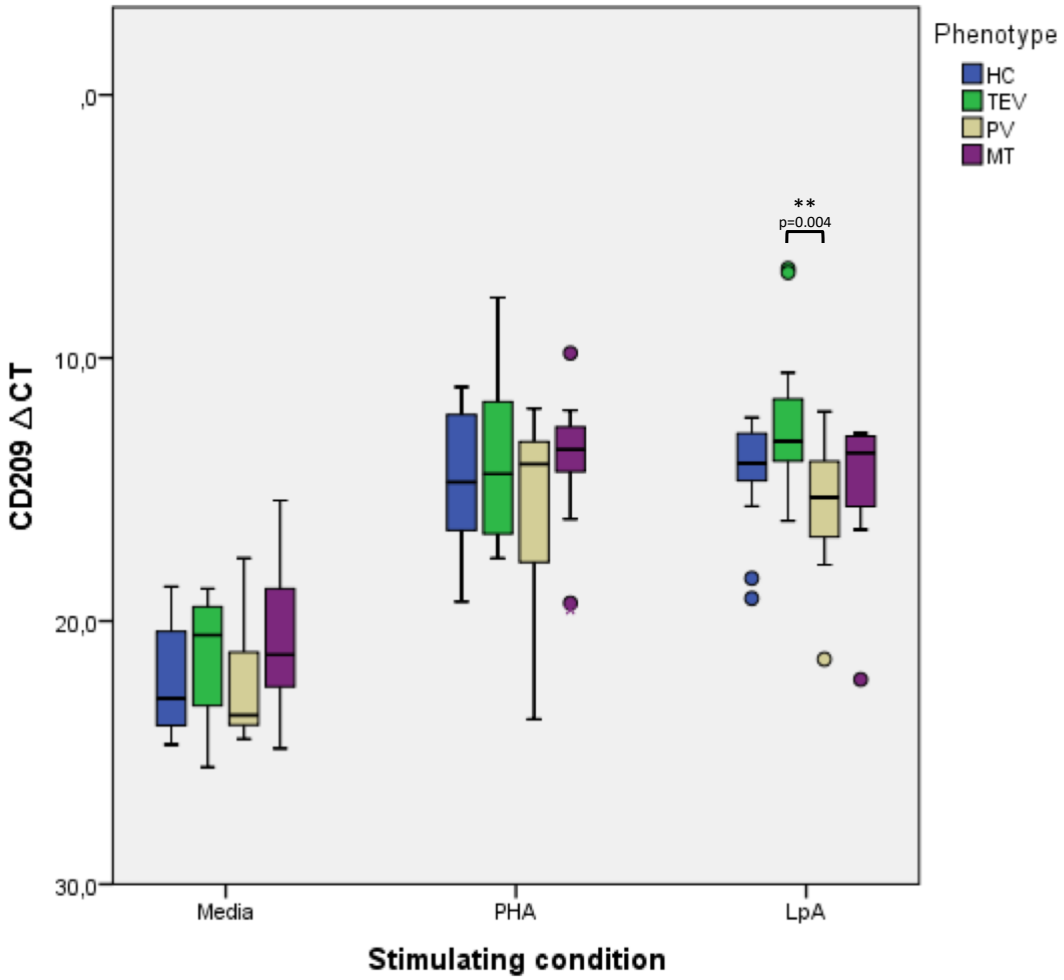

Figure 24: Graphical representation of the results for CD209 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate* trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

For CD209, a significantly lowered expression after LpA stimulation in the persistent viral wheeze group when compared to the TEV group was observed (p=0.004).

4.2.5 In-depth analysis of the *multitrigger* group

After comparing the four original, larger subgroups, a second analysis was conducted. The *multitrigger* group was divided in the three subtypes *late-onset multitrigger* (LOM), *viral to multitrigger* (VM) and *persistent multitrigger* wheeze (see fig.9). This was done because the MT group showed the most significant findings and by subdividing it, those effects could be traced back to the distinct subgroups causing them. However, due to the limited size of the multitrigger group (n=14), case numbers for this in-depth analysis were also small (n=2 for VM, n=4 for PM and n=8 for LOM), meaning all results need to be interpreted with this limitation in mind. Again, the results are organized in two parts, *calcium signalling* and *innate signalling*, according to the biological functions of each gene of interest. Additionally, the changes in contrast to the analysis of the four original subgroups are commented on.

4.2.5.1 Expression of genes associated to calcium signalling

4.2.5.1.1 Results for each gene

In the following sections, the results from the in-depth analysis of the *multitrigger* wheeze subgroup for the calcium-related genes investigated in this work will be displayed, followed by a comment on the changes in regard to the analysis of the original four wheeze subgroups. In the interest of clarity, effects only concerning the PV, TEV and HC groups among one another are not shown in the figures.

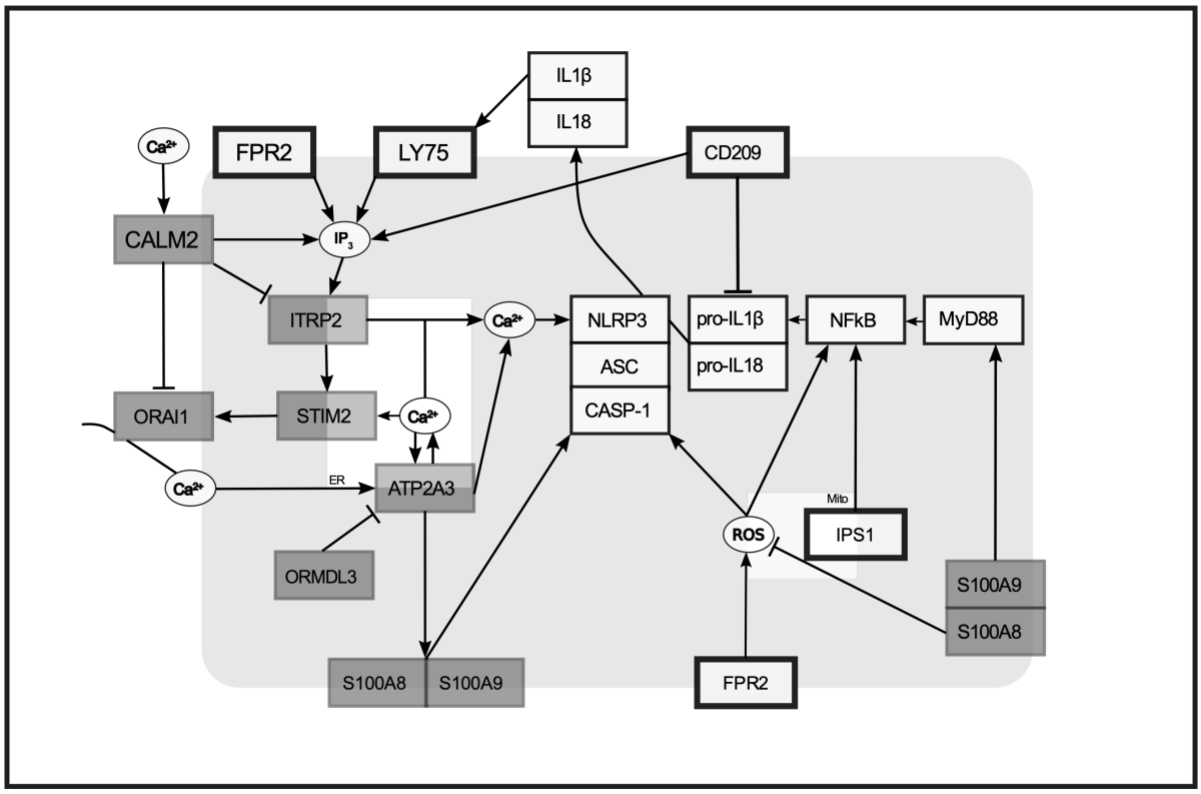

Figure 25: Overview of the candidate genes in a schematic cell (light grey background). Genes shown in this paragraph, related to calcium signalling, are marked in grey boxes (CALM2, ORAI1, ITPR2, STIM2, ATP2A3, ORMDL3, S100A8, S100A9). For a detailed legend see figure 11.

4.2.5.1.1.1 ITPR2

For ITPR2, the previous effect in MT vs. PV for PHA stimulation changes to an effect in PM vs. PV. Also, new significant effects in LpA appear, both concerning the PM group. In regard to the comparison of MT to healthy controls, it can be seen that both the LOM and PM group were involved. However, the trend for higher expression in MT vs. HC in unstimulated cells disappears.

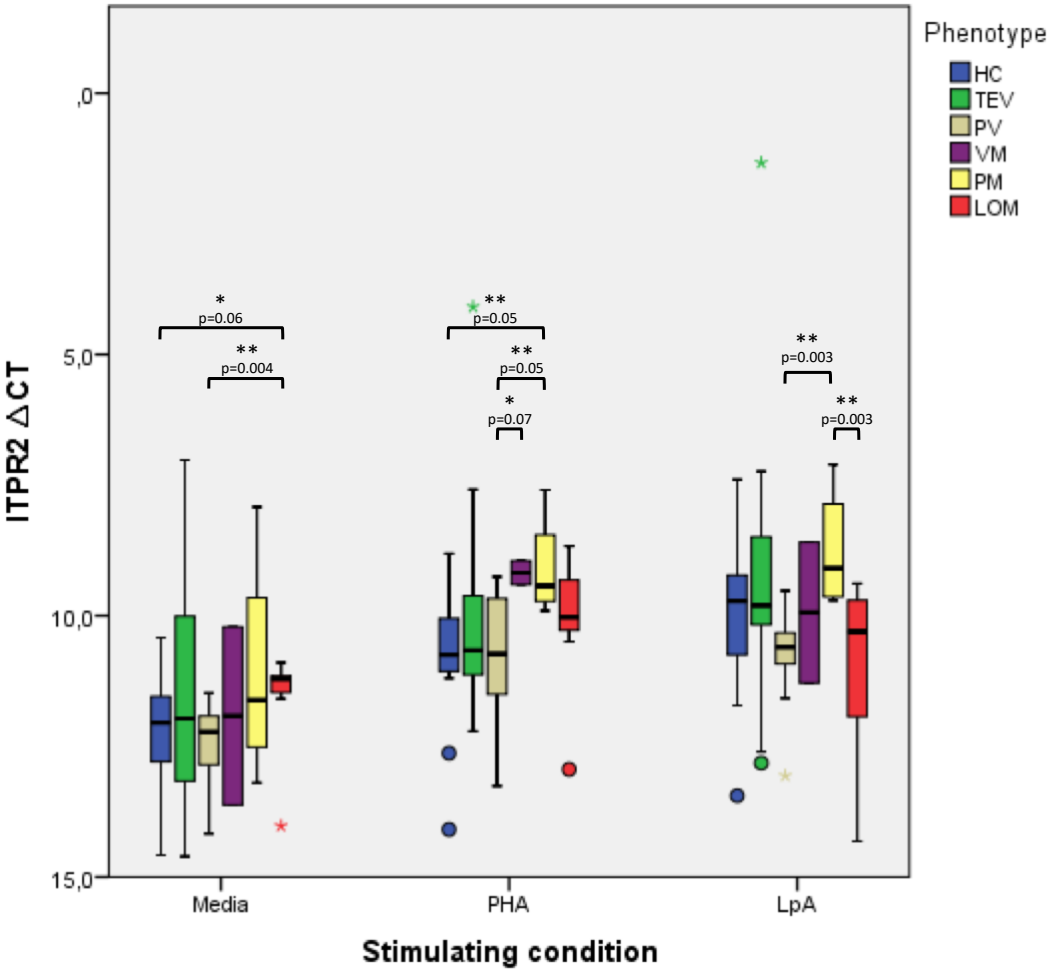

Figure 26: Graphical representation of the results for ITPR2 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

In unstimulated cells, effects were visible in the LOM group, significantly in comparison to PV (p=0.004) and as a trend in comparison to the healthy controls (p=0.06). A trend for higher expression in PHA when comparing the VM group to the PV group was noticed (p=0.07). Elevated expression in PHA for the comparison PM vs. PV (p=0.05) and PM vs. HC (p=0.05) were observed.

In the PM group, expression was significantly higher in comparison to LOM (p=0.03) and PV (p=0.003) wheeze in LpA-stimulated cells.

4.2.5.1.1.2 CALM2

In this detailed analysis of the MT group, several trends for the whole *MT* group are strengthened to significant effects mediated by the PM group. Additionally, the different MT subgroups also differ amongst each other, namely the PM and the LOM subtype. Also, a new trend for LOM vs. TEV emerges in LpA.

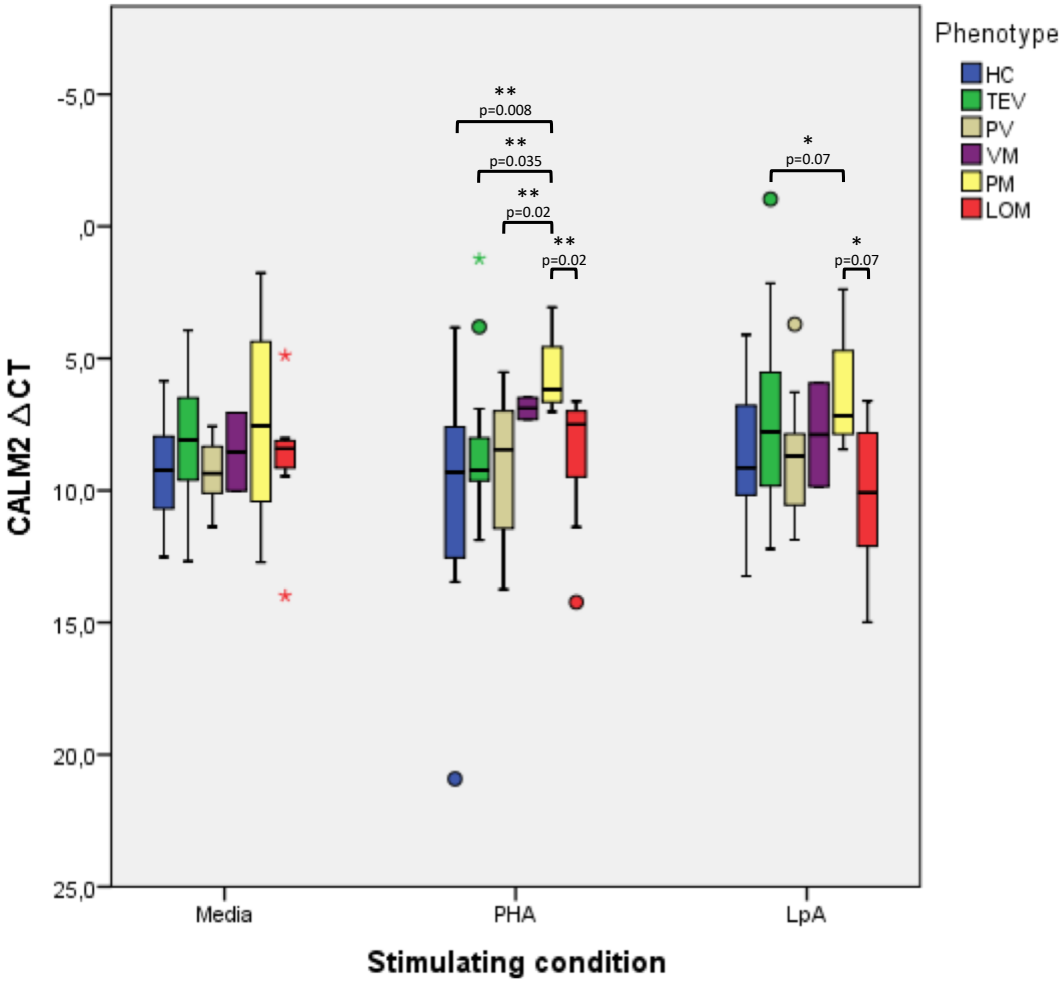

Figure 27: Graphical representation of the results for CALM2 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

Most effects for CALM2 could be found in comparisons involving the PM group.

In PHA, the *persistent multitrigger* group showed significantly higher expression than all the other groups (except VM): in comparison to LOM (p=0.02), to PV (p=0.02), to TEV (p=0.035) and to HC (p=0.008). In LpA, this effect was also visible as a trend when the PM group was compared to LOM (p=0.07).

Additionally, the LOM group showed a tendency to lower expression than the TEV group (p=0.07) after LpA stimulation.

4.2.5.1.1.3 ORAI1

In comparison to the four-group analysis, it emerges that effects previously attributed to the MT group can be traced to the PM group. In addition, one of the new significant effects is distinguishing the two MT subgroups PM and LOM through higher expression of ORAI1 in the PM group. The trend for higher expression in MT in comparison to HC after PHA stimulation disappears.

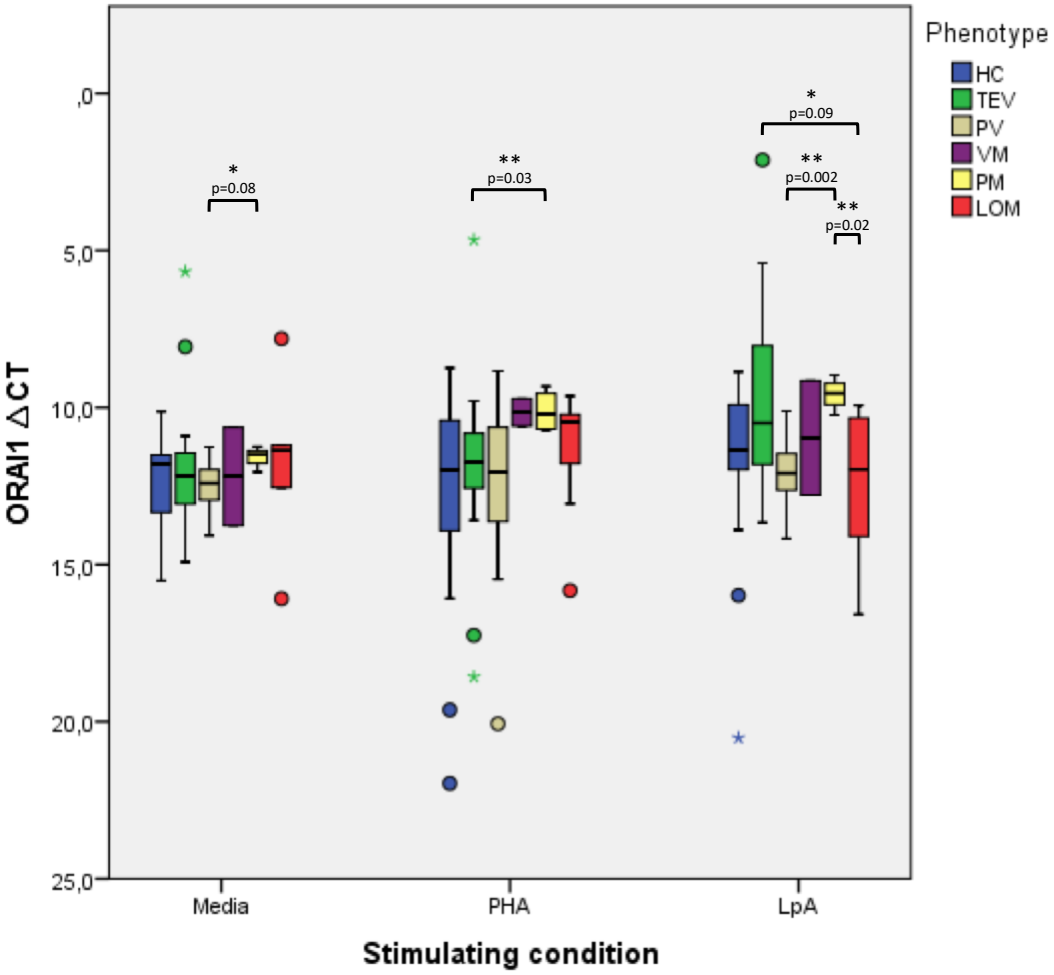

Figure 28: Graphical representation of the results for ORAI1 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

For ORAI1, the PM group showed high expression in all three stimulating conditions. In unstimulated cells, expression was trendwise higher than in the PV group (p=0.08). This effect was stronger in cells stimulated with LpA (p=0.002).
In LpA, PM also showed significantly higher expression than the LOM group (p=0.02). After stimulation with PHA, higher expression than in the TEV group was observed (p=0.03). Regarding trends, the ORAI1 expression in LOM was lowered in LpA when compared to TEV (p=0.09).

4.2.5.1.1.4 STIM2

Here, a previously significant effect for MT vs. PV in unstimulated cells is divided into two trends concerning PM and LOM in comparison to PV. However, there are also new trends in comparisons involving the PM group, in both PHA and LpA. The significant effect of MT vs. healthy controls becomes a significantly higher expression in PM vs. HC.

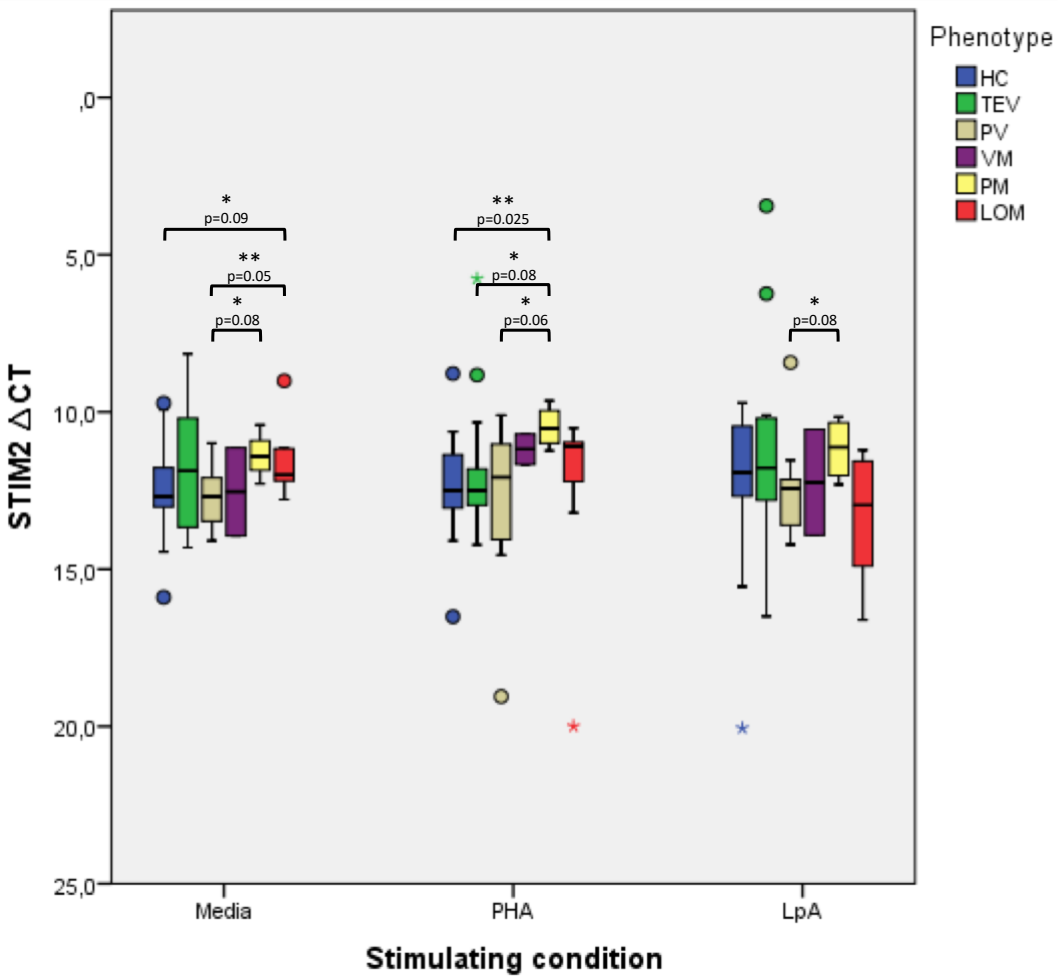

Figure 29: Graphical representation of the results for STIM2 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

A trend for higher expression of STIM2 was observed in the comparison of PM vs. PV in all three stimulating conditions (unstimulated p=0.08, PHA p=0.06, LpA p=0.08). For PHA stimulation, the trend was also visible in PM vs. TEV (p=0.08). Also, PM showed significantly higher expression after PHA stimulation when compared to healthy controls (p=0.025). LOM showed higher expression of STIM2 in unstimulated cells than the PV group did (p=0.05) and a trend-wise higher expression than healthy controls (p=0.09).

4.2.5.1.1.5 ATP2A3

Compared to the four-group analysis, the previous trend in MT vs. PV is strengthened to significance in PM vs. PV. Two new significant effects emerge for the LOM group, one of them distinguishing it from the PM group.

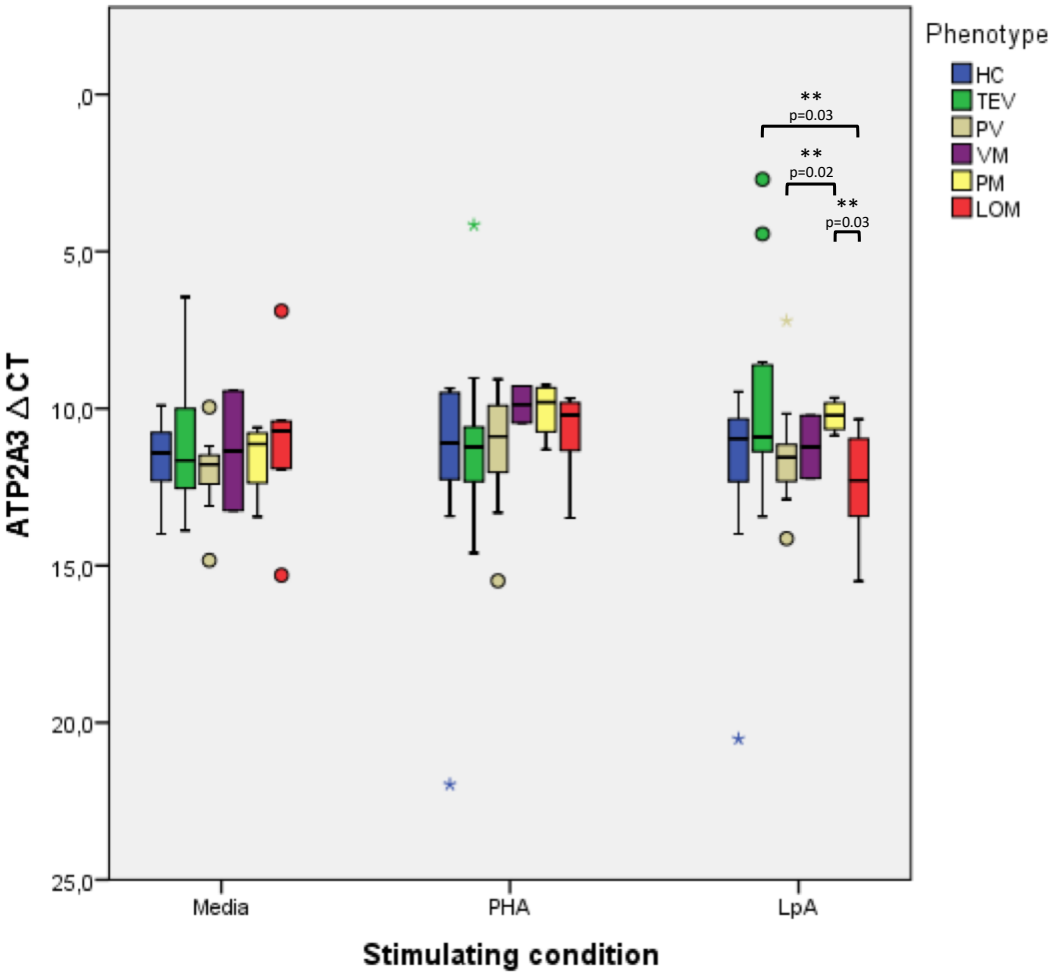

Figure 30: Graphical representation of the results for ATP2A3 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

In LpA, the *persistent multitrigger* group showed significantly higher expression than the LOM group (p=0.03) as well as the *persistent viral group* (p=0.02).

The LOM group showed lower expression of ATP2A3 when compared to the TEV group (p=0.03).

4.2.5.1.1.6 ORMDL3

In the in-depth analysis, there are many changes to the earlier analysis of the four wheeze subgroups. A new trend in unstimulated cells emerges for LOM vs. PV. Previously visible effects in PHA disappear (MT vs. PV and MT vs. TEV). Instead, new effects in LpA appear, both of them involving the PM group.

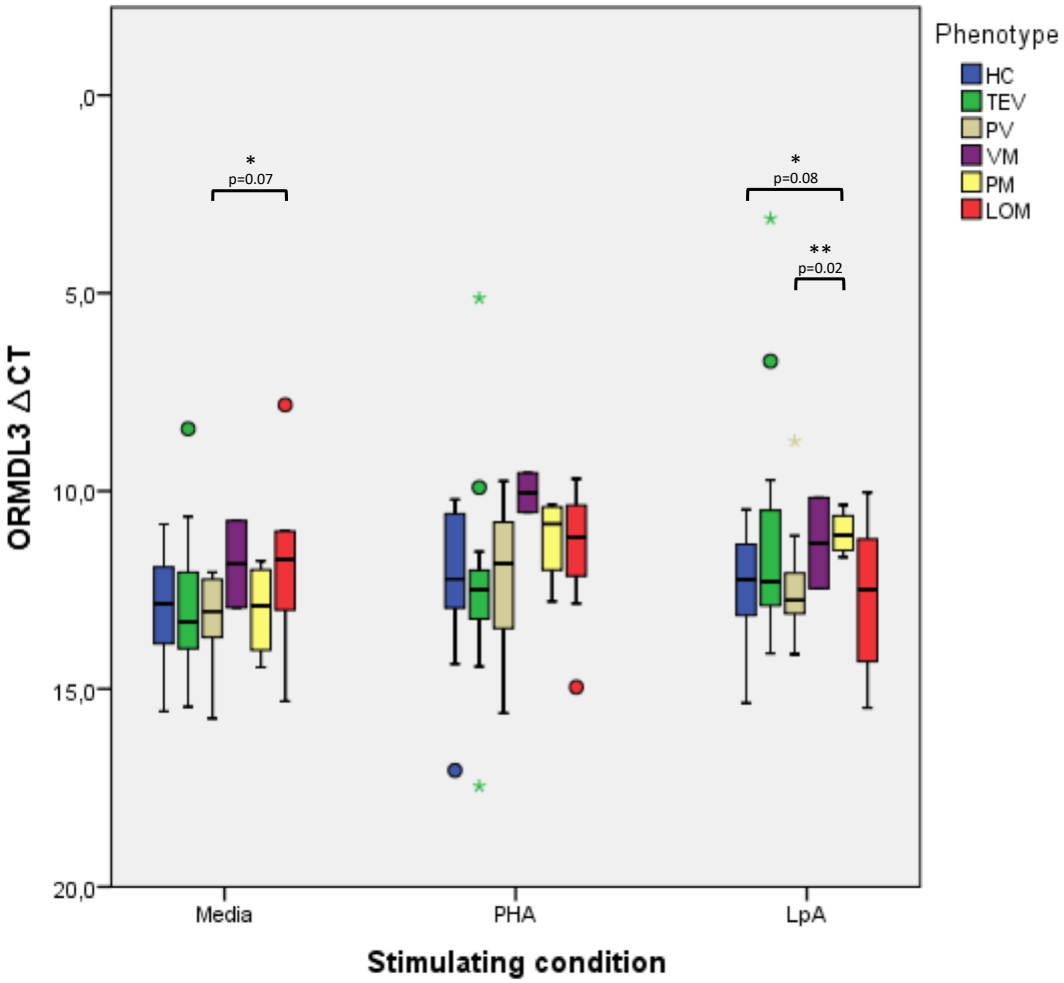

Figure 31: Graphical representation of the results for ORMDL3 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

For ORMDL3, expression in cells stimulated with LpA was significantly higher in the PM group as opposed to the PV group (p=0.02), a trend also visible in comparison to healthy controls (p=0.08).

In unstimulated cells, the *late-onset multitrigger* group showed a trend higher expression than the PV group (p=0.07).

4.2.5.1.1.7 S100A8

While in the four-group comparison there were neither trends nor significant effects for S100A8, in this more detailed analysis of the MT group three new effects concerning *late-onset multitrigger* group emerge for cells stimulated with LpA.

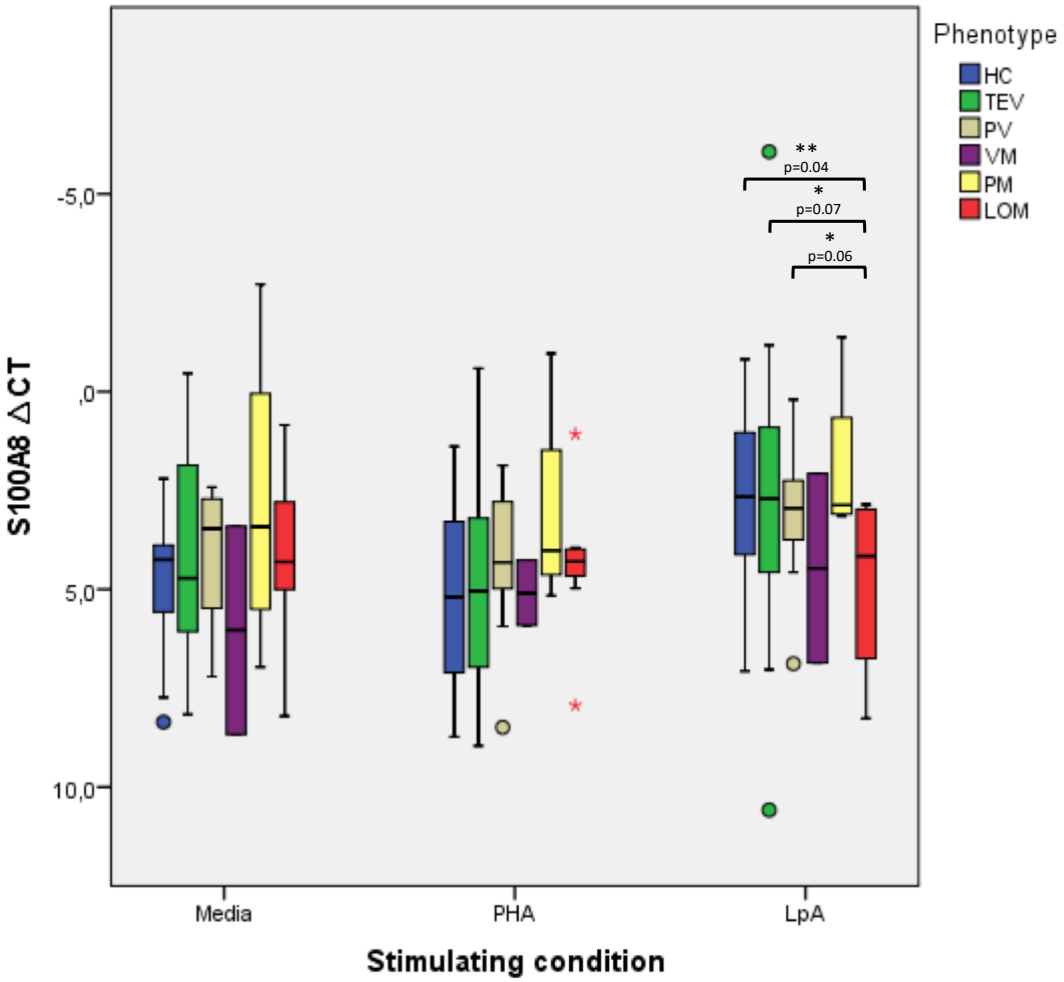

Figure 32: Graphical representation of the results for S100A8 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

The LOM group showed a trend for lower S100A8 expression than the TEV group (p=0.07) as well as the PV group (p=0.06). In comparison to healthy controls, S100A8 expression is significantly lower in the LOM group (p=0.04).

4.2.5.1.1.8 S100A9

Parallel to S100A8, a new effect concerning expression in LOM group after LpA stimulation appears in this analysis. However, a previously significant effect for MT vs. HC diminishes to a trend. Additionally, a new significant effect distinguishing the LOM group from the *persistent multitrigger* group emerges.

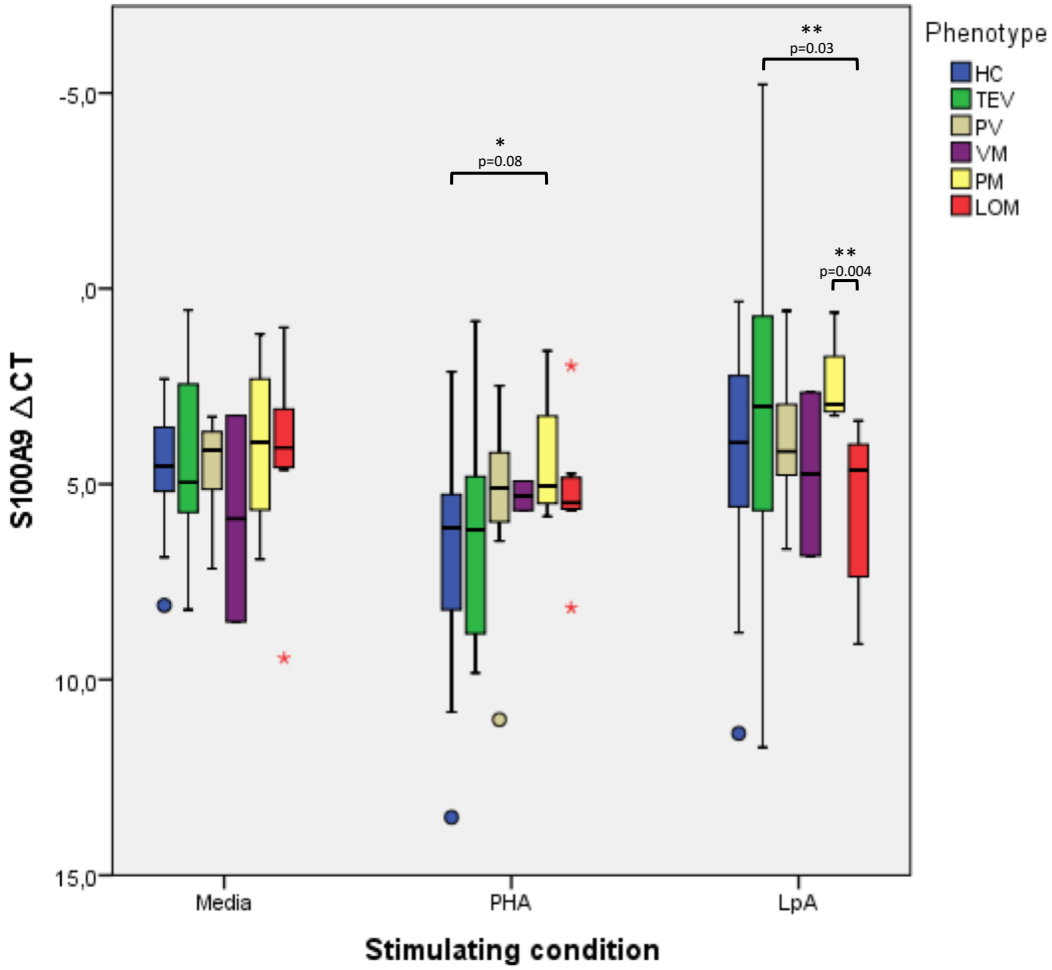

Figure 33: Graphical representation of the results for S100A9 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

In PHA, there is a trend for higher expression of S100A9 in PM when compared to healthy controls (p=0.08).

In LpA, the TEV group shows higher expression than the LOM group (p=0.03). Also, in the comparison PM vs. LOM, the *persistent multitrigger* group has a higher expression (p=0.004).

4.2.5.2 Expression of genes associated to innate signalling

4.2.5.2.1 Results for each gene

In the following sections, the results from the in-depth analysis of the *multitrigger* wheeze subgroup for the innate-immunity-related genes investigated in this work will be displayed, followed by a comment on the changes in regard to the analysis of the original four wheeze subgroups. In the interest of clarity, effects only concerning the PV, TEV and HC groups among one another are not shown in the figures.

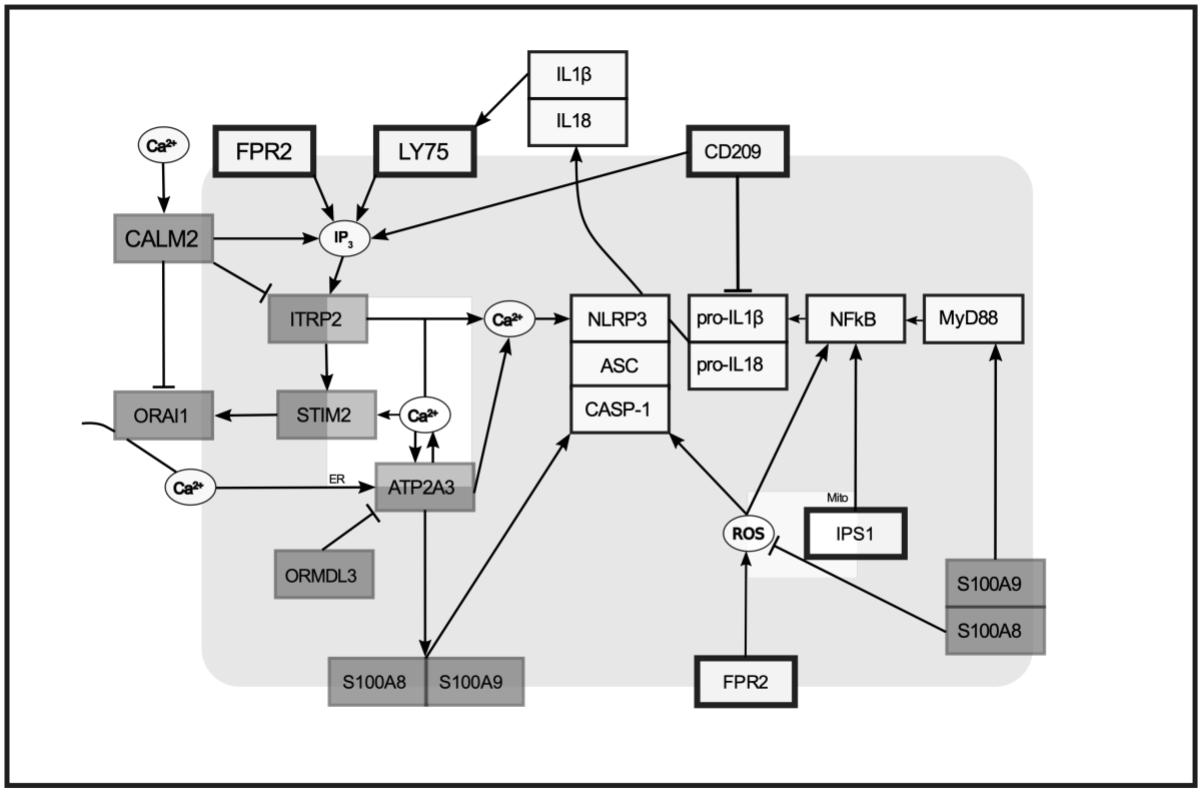

Figure 34: Overview of the candidate genes in a schematic cell (light grey background). Genes shown in this paragraph, related to innate immunity, are marked in bold (FPR2, LY75, CD209 and IPS1). For a detailed legend see figure 11.

4.2.5.2.1.1 FPR2

For FPR2, trends already visible in PHA for the whole MT group changed to significant differences in the PM group. In addition, new effects appear for cells stimulated with LpA, one of them differentiating the LOM from the PM group. Also, a new trend concerning the comparison LOM vs. TEV in LpA-stimulated cells emerges.

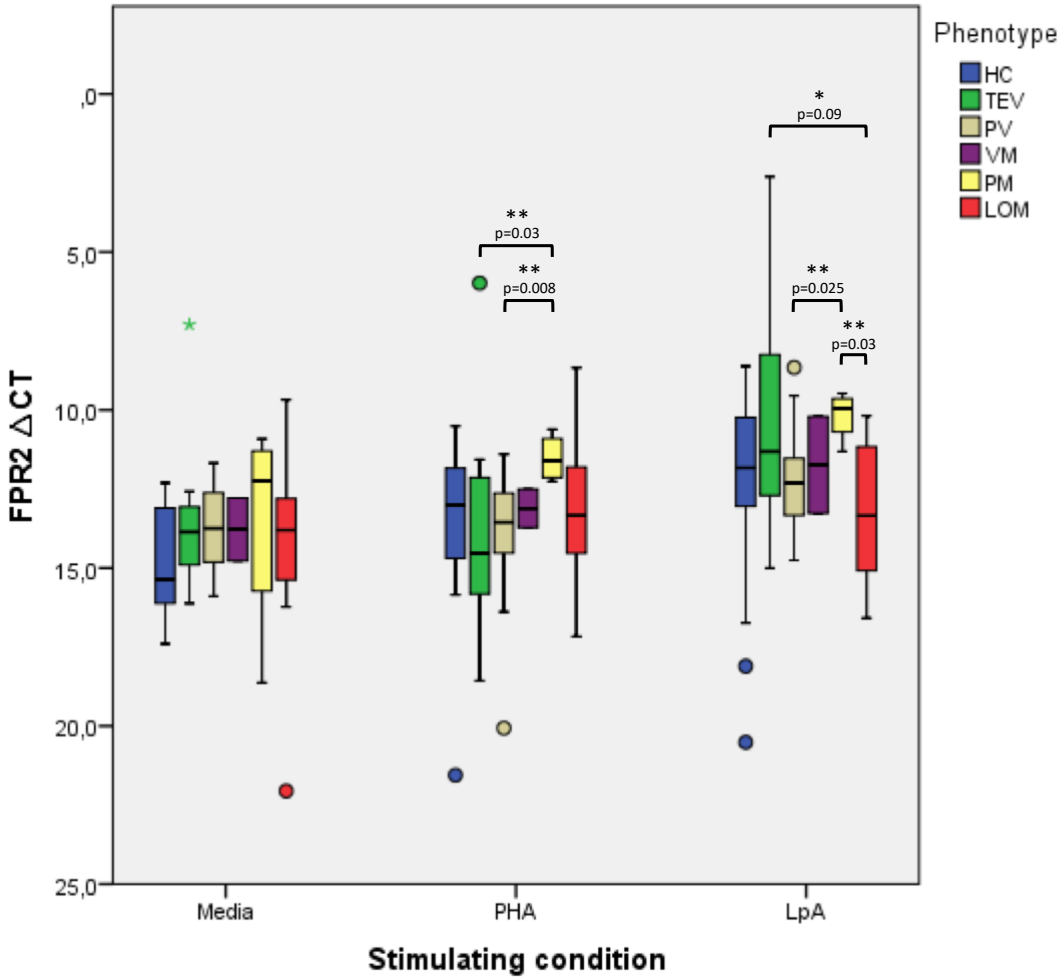

Figure 35: Graphical representation of the results for FPR2 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

In PHA, there is a significantly higher expression in the PM group when compared to *persistent viral* wheeze (p=0.008) as well as compared to *transient early viral* wheeze (p=0.03). Similarly, in LpA the PM group showed elevated expression in comparison to the LOM group (p=0.03) and to the PV group (p=0.025), as well.

In LpA there was also a trend for lower FPR2 expression in the LOM group when compared to the TEV group (p=0.09).

4.2.5.2.1.2 IPS-1

For IPS-1, several new effects involving the PM group emerge for LpA- as well as PHA- stimulated cells. A new effect differentiating the LOM group from the TEV group was discovered.

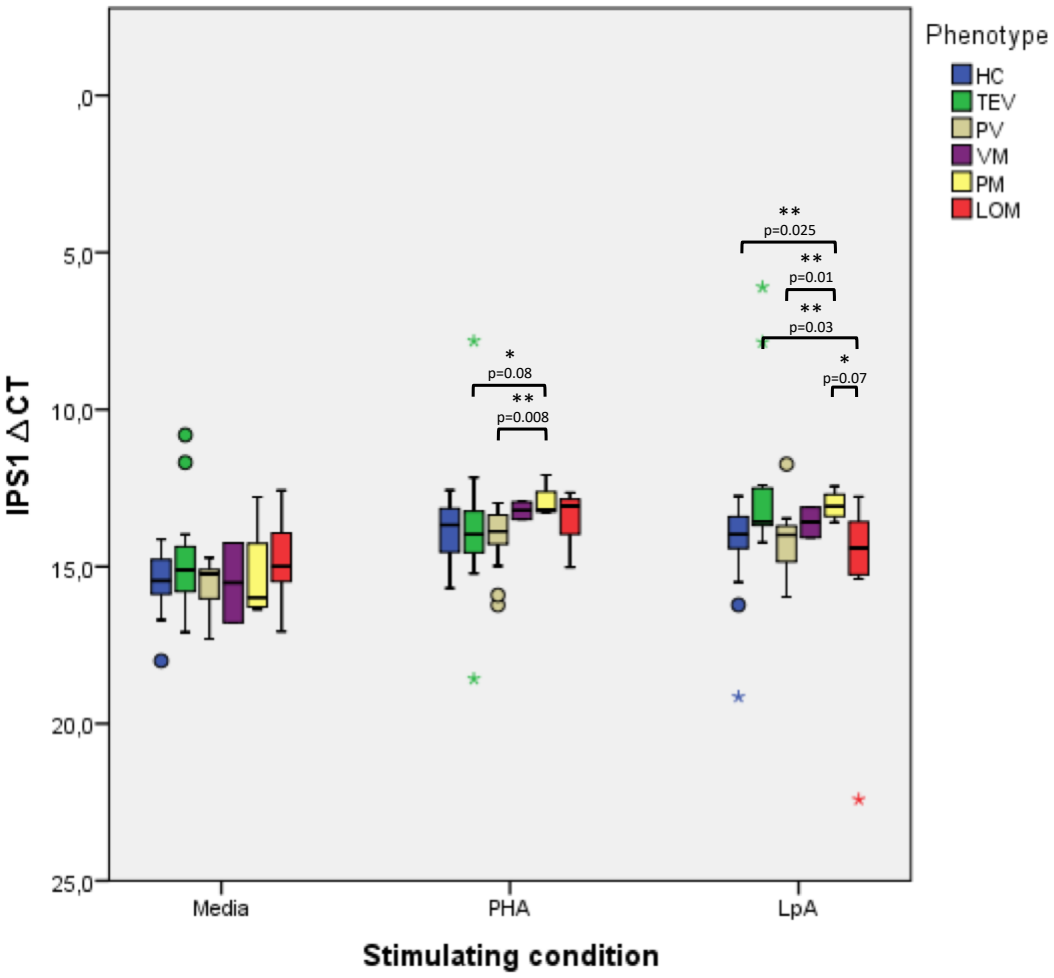

Figure 36: Graphical representation of the results for IPS1 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

In general, the *persistent multitrigger* group showed the highest expression in this comparison. In LpA, expression of IPS-1 in PM was significantly higher than in the PV group (p=0.01) as well as the healthy controls (p=0.025). Trend-wise, this effect was also noticeable in the comparison PM vs. LOM (p=0.07). In cells stimulated with PHA, parallel effects were observed: IPS-1 expression was higher in PM than in the PV group (p=0.008), and higher than in the TEV group, although only as a trend (p=0.08). Also, the LOM group showed a lower expression than the TEV group after LpA-stimulation (p=0.03).

4.2.5.2.1.3 LY75

In comparison to the previous analysis, multiple new effects showing a higher expression emerge for the PM group in cells stimulated with PHA as well as in cells after LpA-stimulation. Additionally, there are new effects visible in unstimulated cells for the comparison LOM vs. PV and healthy controls.

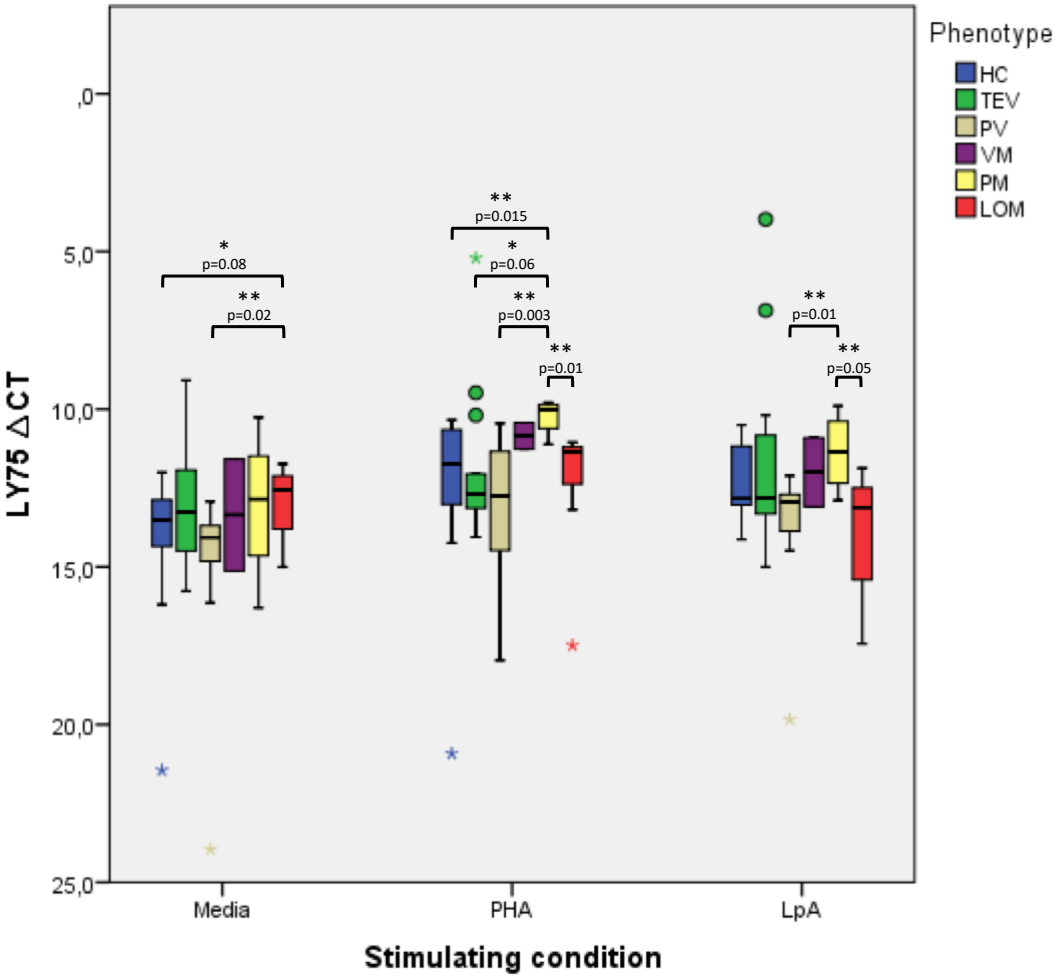

Figure 37: Graphical representation of the results for LY75 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

In LpA, the PM group showed significantly higher expression than both LOM (p=0.01) and PV (p=0.05). In PHA, exactly the same effect was visible for both the comparisons PM vs. LOM (p=0.01) and PM vs. PV (p=0.003). Additionally, LY75 expression in the PM group was significantly elevated when compared to healthy controls (p=0.015). As a trend, higher expression in PM in comparison to the TEV group was observed (p=0.06). In unstimulated cells, expression of LY75 in the PM group compared to the *persistent viral* wheeze group was significantly elevated (p=0.02). There was a parallel trend in LOM vs. healthy controls (p=0.08).

4.2.5.2.1.4 CD209

In contrast to the analysis of the original four wheeze subgroups new, partly significant effects appeared for cells after stimulation with LpA appeared, involving the PM as well as the LOM group.

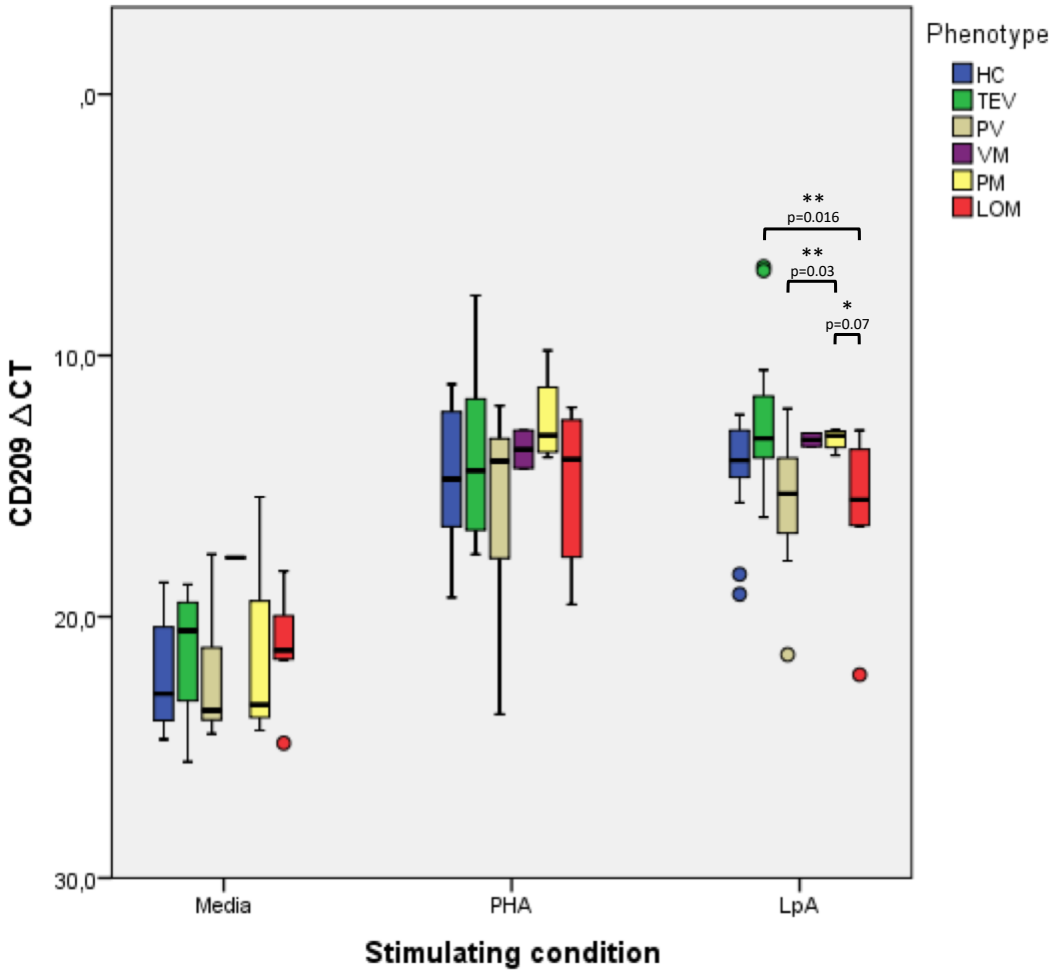

Figure 38: Graphical representation of the results for CD209 in boxplots, sorted by stimulating condition on the x-axis and by phenotype as indicated in the legend. Y-axis inverted to illustrate a lower ΔCT value as higher expression rate. * trend found in the comparison of the two indicated subgroups (p ≤ 0.1), ** significant findings in the comparison of the two indicated subgroups (p ≤ 0.05) for Wilcoxon two sample rank sum test.

In the PM group, CD209 expression was significantly higher than in PV (p=0.03) and trend-wise higher than in the LOM group (p=0.07) in cells stimulated with LpA. Also, in LpA, expression of CD209 was lower in the LOM group when compared to *transient early viral* wheeze (p=0.016).

5 Discussion

Despite great efforts in research, asthma remains one of the most common chronic diseases in childhood, developing already early in life. To improve the current situation of asthma patients, which is severely lacking in many dimensions, new, targeted, more effective therapeutic strategies and predictive tools are urgently needed [169]. However, to identify possible targets for therapy as well as to correctly deliver individualized therapy to the patients, it is necessary to define precise and consistent asthma endotypes.

This thesis investigated the gene expression of immunological pathways in cord blood mononuclear cells. In combination with the detailed follow-ups of the study population, this study seeks to identify possibly pathogenetic gene expression patterns within the immune system. A long-term goal could be the development of selected candidate genes as new biomarkers that could possibly be used to predict the outcome of childhood asthma.

In the following paragraphs, the results will be discussed against the background of the current state of relevant studies.

5.1 Overview of the main findings

In CBMC samples selected from the PAULINA/PAULCHEN birth cohort, expression of all candidate genes could be detected via qRT-PCR. While gene expression in CBMCs is often only detectable after stimulation, in this study all candidate genes could be detected even in unstimulated cells. Expression varied, trend-wise and significantly, among the subsets of children with *transient early viral, persistent viral* or *multitrigger* wheeze, and also in comparison to healthy controls. Despite the small sample size (n=14 per group, lower for the in-depth analysis of the multitrigger group), highly significant differences in gene expression levels could be detected. Even so, this limitation needs to be kept in mind when interpreting the results from this work.

What sets apart these results is the fact that instead of single findings for stand-alone genes with often only mild impact on phenotypes, consistent patterns of elevated or lowered gene expressions could be detected for a whole signalling pathway [35].

5.1.1 Main findings for *transient early viral* wheeze

Gene expression patterns for *transient early viral* wheeze showed a level of gene expression below that of persistent multitrigger wheeze, visible in significant effects for FPR2, CALM2 and ORAI1 as well as in trend-wise effects for LY75, STIM2 and IPS1, all after stimulation with PHA. In comparison to persistent viral wheeze, TEV wheeze showed significantly higher expression of ITPR2, CD209, ORAI1, ATP2A3 and IPS1, all visible after stimulation with LpA. This gene expression pattern is mostly parallel to that of healthy controls. This theory was tested in a three-group comparison[9] merging HC and *transient early viral* wheeze (TEV).

These findings fit the description of *transient early* wheeze in other studies as very common and associated with viral infection but unrelated to atopy, generally disappearing without any intervention [170, 171].

Accordingly, cohort studies have shown a good prognosis in terms of wheeze frequency in later childhood. Martinez et al. reported that 60% of children who experienced viral wheeze until their third birthday outgrow their symptoms by age 6 [12]. Additionally, a follow-up study to the Tucson birth cohort proposed children who wheezed before age three but not at age six were shown to be as unlikely to experience wheeze again after age six as children who had never wheezed [172].

Children with TEV wheeze have been consistently reported to have impaired lung function in infancy. However, lung function in later childhood has been controversially discussed as it had recovered when the children reached school age in some cohorts, whereas it stayed at a comparatively low level in others [12, 173].

Still, the major part of children who wheeze transiently early in life have a good prognosis in terms of respiratory function and show no clinical symptoms at school age [174-177].

Results from this study indicate that TEV wheeze does not show altered gene expression levels at birth, neither in genes related to calcium signalling nor antiviral innate immunity. However, these children still experience wheeze. A possible explanation might be the involvement of other pathogenetic factors besides immune regulation at birth. Consistently being reported as risk factor is smoking during pregnancy. Some studies propose a direct influence of in-utero smoke exposure through impaired lung growth and maturation resulting in congenitally smaller

9 As there were no vast differences to the conducted four-group analysis, these data are not shown.

airways, a predisposition for wheeze while others suggests it is mediated through lower birth weight [38, 178-180]. However, in this study smoking during pregnancy could be excluded as possible confounder, pointing out that this theory might only be true in some cases. Other influencing factors reported for TEV wheeze include the mother's vitamin D status as potentially protective through the development of the foetal lung and thus wheeze as well as a potential influence of young maternal age and high maternal BMI [181, 182]. While no evidence for this as an influence through socioeconomic status could be found in this study, it has also been proposed that the in-utero exposure to systemic inflammation in obesity can hinder the development of the child's immune system [180, 183]. The importance of immune maturation is underlined by studies that showed exposure to animal sheds on farms lowers the risk for TEV wheeze, suggesting that early life exposure to diverse bacteria could hold an immunomodulatory protective effect [184].

In summary, findings from this work indicate that transient early viral wheeze has a gene expression level similar to that of healthy controls. This indicates a positive outlook for these children as there seems to be no underlying major modification in immune regulation at play. A hypothesis as to why TEV patients experience wheeze anyway that would be consistent with findings from this study is that multiple potentially modifiable influences lead to a limited period of susceptibility to lower respiratory tract infection. This in turn causes oedema, effectively diminishing the already small airway diameter of these children, resulting in wheeze [12, 17, 185-187].

As transient early wheeze currently can only be defined to have been transient in retrospect, most of the patients in this group are treated with ICS, accounting for the major part of ICS prescriptions in preschool children. However, the benefit of ICS for these patients is minimal, as they have been shown to be likely to remit without intervention eventually. As ICS have grave side effects like growth retardation, this is not to be taken lightly [173, 188]. A way to, indirectly, improve therapeutic management of these children through the findings of this work could lie in early identification of children at risk for persistent wheeze, which will be discussed in the following paragraphs.

5.1.2 Main findings for *persistent viral* wheeze

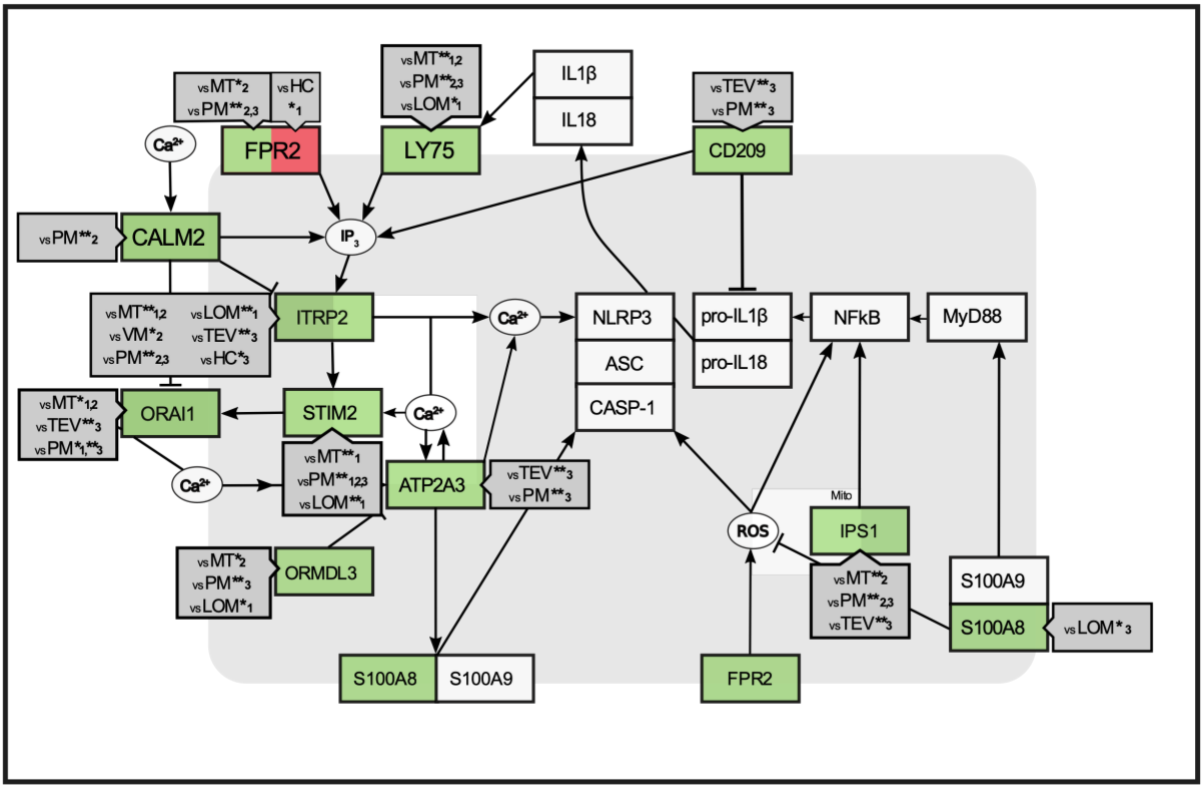

Figure 39: Overview of the significant findings for persistent viral wheeze, based on differences in ΔC_t values. HC healthy controls, TEV transient early viral wheeze, PV persistent viral wheeze, PM persistent multitrigger, LOM late-onset multitrigger, MT multitrigger. Red background marks higher expression, green background marks lower expression. All subgroups indicated in the grey boxes have been compared to persistent viral wheeze. * trend like results (p ≤ 0,1); ** significant results (p ≤ 0,05). Stimulating conditions can be seen next to the level of significance: 1 unstimulated, 2 phytohemagglutinin, 3 lipid A. For a detailed legend see chapter 9.5 in the appendix.

Children with *persistent viral* wheeze showed an overall trend for a low level of expression of the candidate genes with varying levels of significance depending on the respective subgroup comparison. The only exception to this overall pattern is a trend for higher expression of FPR2 in healthy controls.

A large number of candidate genes – including CALM2, ITPR2, ORAI1, STIM2, ATP2A3, ORMDL3, IPS1, LY 75 and CD209 - show significantly lower expression in children with persistent viral wheeze in comparison to persistent multitrigger wheeze, after both stimulation with LpA and PHA, respectively. In comparison to late-onset multitrigger wheeze, PV showed significantly lower expression of ITPR2 and STIM2 as well as a trend-wise lower expression of ORMDL3 and LY75 in unstimulated cells. The effects distinguishing PV from TEV wheeze have already been in summarized in the chapter above, but fit with the overall pattern of lower expression of the candidate genes in persistent viral wheeze.

As discussed above, it is important to single out children at risk for persistent wheeze from the larger pool of children that experience wheeze before the age of three. As most transient early wheeze episodes are connected to viral infection, it is of special interest to identify mechanisms that distinguish between transient early viral and persistent viral wheeze. A possible predictive pattern that has been identified is that children with “troublesome early viral” wheeze are likely to also show viral wheeze at age six, which fits description of *persistent viral* wheeze used in this work [189]. Troublesome, in this case, means severe forms of early viral wheeze. Consistently, in other studies children with wheeze caused by severe bronchiolitis that required hospitalization before the age of three have been shown to be at high risk to develop recurrent and persistent wheeze and subsequent asthma [190, 191]. The comparison TEV vs. PV wheeze from this work show lower expression of genes related to innate antiviral immunity in PV wheeze, such as IPS1 or CD209, after stimulation with LpA. Additionally, several genes in the calcium signaling pathway, such as ITPR2, ATP2A3 and ORAI1 show the same pattern. ITPR2, for example, is a central signal integrator at the starting point of the calcium signaling pathway, so a diminished expression, and eventually an impaired function could potentially cause disturbances in the whole signaling pathway [105]. Hypothetically, this impaired response could facilitate more severe and recurrent infections, in accordance with the reports of severe early viral wheeze being a possible precursor of persistent viral wheeze [175].

It has been repeatedly proposed that an important part of PV wheeze pathogenesis lies in aberrant stabilization in response to recurrent viral infection and delayed maturation of the immune system, especially of Th_1-type immunity. This results in a susceptibility for lower respiratory tract infections that create airway inflammation, disturbing the postnatal maturation or development of the respiratory system [2, 187, 192]. In keeping, other reports show that physiologically, innate immune cells are kept in an “alerted state” through exposition to bacteria in order to be ready to ward off viral infections through the release of IL-1β when sensing an inflammasome trigger. The authors suggest that impairment of these mechanisms, either through not enough exposition to bacteria or through altered function of the inflammasome and its triggers could result in a reduced capacity to clear viral infection, contributing to the susceptibility to lower respiratory tract infection in *persistent viral* wheeze [193].

Results from this work show altered gene expression for several genes connected to inflammasome function, albeit in a small sample and different subgroup comparisons, as visualized in fig. 39. The most interesting results for a candidate gene from this work come from ITPR2, for which PV wheeze showed significant or trend-wise lowered expression of ITPR2 in comparison to all other subgroups. As described above, ITPR2 holds a central role in the calcium signalling pathway that regulates the intracellular level of calcium ($[Ca^{2+}]_i$). A high $[Ca^{2+}]_I$ has been reported to activate the inflammasome, so hypothetically disturbances in the pathway controlling $[Ca^{2+}]_i$ could lead to impared function of the inflammasome [138, 140]. The same gene expression pattern is also visible for other genes of the calcium signalling pathway such as STIM2, ORAI1, ATP2A3 and ORMDL3, albeit less pronounced.
The underlying causes for this pattern need to be investigated further, primarily in a larger sample. Still, the results from this work indicate the possibility that an impaired innate immune response plays a central role to uncovering why children with PV go on to have infection-associated symptoms after three years of age [194, 195].

Virus-induced wheeze episodes have been shown to fulfil all main characteristics of asthma which strongly suggests the existence of a childhood asthma phenotype that develops from persistent viral wheeze [196]. Indeed, the phenotype *persistent viral* wheeze has been described as predominantly unrelated to atopy and with relatively normal lung function, with the majority of children outgrowing their symptoms during early school years [186, 197]. In other studies, however, *persistent viral* wheeze has been described as a risk factor for a rapid decline in lung function in adult patients even in the absence of asthma. An impaired development of the airways, preventing these patients from reaching full lung function, could be a possible explanation for these opposing results. As lung function peaks at age 25, a low performance might not immediately be visible, explaining the relatively normal lung function at school age [38, 198].
Supporting the concept of *persistent viral* wheeze as a distinct phenotype, an atopy-independent pathway for asthma genesis that begins as early as in utero has been proposed as profiles of antiviral cytokines from non-asthmatic mothers during pregnancy relate at least partly to the child's asthma risk [199]. This suggests a distinct pathophysiologic mechanism through which viral infection leads to wheeze rather than the effect being mediated through the development of atopy, clearly

differentiating viral from multiple-trigger wheeze [200]. As visible in fig. 39 and as already mentioned above, this work shows a distinct gene expression signature for persistent wheeze that is clearly distinguishable from the other wheeze subgroups that were analysed, especially from persistent multitrigger wheeze. While these results, despite their high significance levels, need to be interpreted with caution due to sample size, they too point in the direction of a distinct childhood asthma phenotype that develops from persistent viral wheeze.

However, the definition of multiple-trigger wheeze also includes wheeze during viral infection. This, at first glance, seems contradictory, however recent research proposed an explanation through the type of virus infection. The two most common viral infections in childhood are rhinovirus (RV) infection, which has been linked to multitrigger wheeze, and respiratory syncytical virus (RSV) infection, linked to persistent viral wheeze. Both have been shown to interfere with innate immune responses [193]. Rhinovirus infection has been linked to multiple trigger wheeze, as infection under the age of three has been shown to be a risk factor for wheeze development. Additionally, it shows a strong link to subsequent childhood asthma, with one mechanism possibly linked to the susceptibility locus 17q21 in an interesting gene-environment-interaction proposition, and another to allergic sensitization [42, 201, 202]. As to persistent viral wheeze, studies have shown that while maternal atopic asthma is a risk factor for rhinovirus induced wheeze, it did not influence RSV induced wheeze [202, 203]. This fits with the emerging role of respiratory syncytical virus for the pathogenesis of *persistent viral* wheeze. Early life RSV infection, especially when so severe it requires hospitalization have been associated with recurrent wheezing in several studies and has already been discussed above [204].

As of yet, there is no specific treatment for rhinovirus infection, and whether corticosteroids can positively influence the resulting airway inflammation is unclear [83]. This, however, is different for respiratory syncytical virus infection.
A possible treatment that may even prevent the development of persistent viral wheeze, might lie in reducing RSV-related lower respiratory tract infections. In continuation of this hypothesis, this would in turn lead to reduced frequency of wheeze episodes in the first years of life, and has already been shown to be effective in reducing the need for asthma medications [170, 193, 202].

As especially RSV infections occur in the first few months of life, identifying children at risk already at birth could prove very valuable for these patients. Even if the progression to childhood asthma cannot be stopped, early identification could for example lead to closer clinical monitoring and maybe intermittent ICS therapy to alleviate morbidity for patients with persistent viral wheeze [205].
In summary, results from this work showed evidence for a transcriptional signature of *persistent viral* wheeze with low expression of genes important for antiviral immunity as well as calcium signaling that is closely related to the functions of innate immunity, directly visible at birth. These results indicate that further investigation of gene expression patterns at birth, for example in a larger cohort with additional follow-up information regarding RSV infection, could add to early identification of these patients in order to improve clinical management.

5.1.3 Main findings for *multitrigger* wheeze

In children presenting *multitrigger* wheeze, the majority of the candidate genes, whether associated with the immunological pathways of calcium signalling or innate antiviral immunity, are significantly upregulated in comparison to healthy controls and other wheeze subgroups. Significantly or trend-wise upregulated genes include ITPR2, CALM2, ORAI1, STIM2, ORMDL3, S100A9, IPS-1 and LY75. This upregulation was partly detectable in unstimulated cells and mostly in cells after stimulation with PHA, with some effects also showing after stimulation of the innate immune system with LpA.
Multiple-trigger wheeze in preschool children has consistently been proposed to be a phenotype at high risk for progression to allergic asthma [206]. Accordingly, it has been linked to lower lung function and a higher risk for atopy [207, 208].
Children with atopic asthma have been shown to have continuous airway inflammation while the same was not found in children with virally-induced wheeze. This suggests the existence of an inflammatory mechanism in MT wheeze that is permanently present, for example through a genetic predisposition for atopy that leads to allergic airway inflammation [209].
However, therapeutically targeting the known mechanisms of allergic airway inflammation, for example through specific antibodies, has so far fallen short of expectations. As it has already been explained in detail in the introduction (see chapter 1.4), this challenge has been one of the incentives to pursue endotyping as

the differences in clinical presentation most likely result from different pathogenetic mechanisms [13].

With this in mind, the *multitrigger* group was divided in the three subtypes *late-onset multitrigger* (LOM), *viral to multitrigger* (VM) and *persistent multitrigger* wheeze (see fig.9) for a second analysis. *Viral to multitrigger* wheeze was included in this analysis because in general, wheeze persistence does not necessarily mean the same pathophysiological reason remains. In this example of a longitudinal pattern, children wheeze because of impaired antiviral immunity early in life and then later because of atopic sensitization [210]. However, for the latter there was not enough power for reliable analysis due to small sample size, so the following paragraphs will focus on *persistent* and *late-onset multitrigger* wheeze.

This already illustrates the main challenge of this in-depth analysis, as due to the limited size of the multitrigger group (n=14), case numbers for this in-depth analysis were also small (n=2 for VM, n=4 for PM and n=8 for LOM), meaning the following discussion of the findings need to be interpreted with this limitation in mind. Even so, results from this work show significant results that point to the existence of separate endotypes for multitrigger wheeze that are clearly distinguishable through their longitudinal pattern as well as their gene expression signature.

5.1.3.1 Findings for persistent multitrigger wheeze

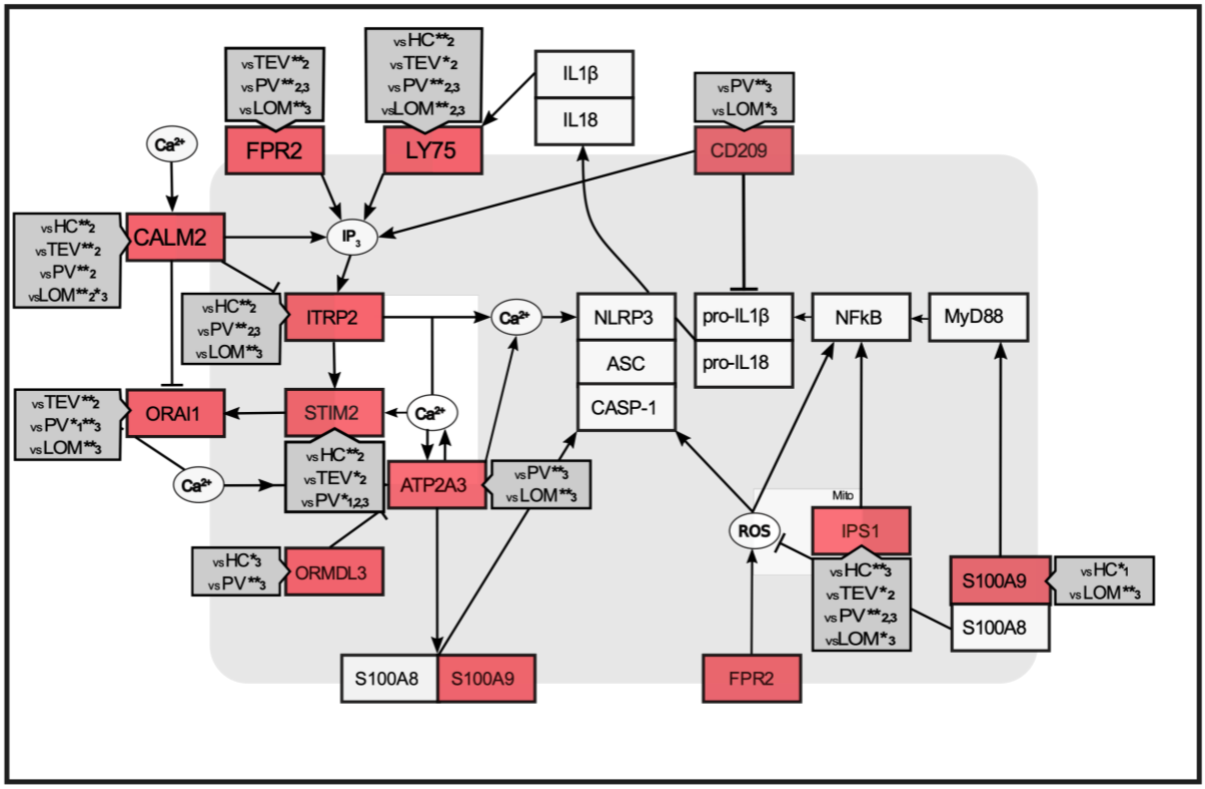

Figure 40: Overview of the significant findings for persistent viral wheeze, based on differences in ΔC_t values. HC healthy controls, TEV transient early viral wheeze, PV persistent viral wheeze, PM persistent multitrigger, LOM late-onset multitrigger, MT multitrigger. Red background marks higher expression, green background marks lower expression. All subgroups indicated in the grey boxes have been compared to persistent multitrigger wheeze. * trend like results (p ≤ 0,1); ** significant results (p ≤ 0,05). Stimulating conditions can be seen next to the level of significance: 1 unstimulated, 2 phytohemagglutinin, 3 lipid A. For a detailed legend see chapter 9.5 in the appendix.

Within the *multitrigger wheeze* group as well as the whole cohort selected for this work, the group with *persistent multitrigger* wheeze showed the most distinct gene signature with a high level of expression of the candidate genes, visible for ITPR2, CALM2, ORAI1, STIM2, ATP2A3, ORMDL3, IPS1, LY75, CD209 and FPR2. Despite the small sample size of n=4, the findings were highly significant, emphasizing their importance. This pattern, as illustrated in fig. 40, was predominantly visible after stimulation and for the majority of the candidate genes, both those associated to the immunological pathways of calcium signalling and to innate immunity, in different subgroup comparisons. LY75 as well as IPS1 are of special interest because the elevated gene expression pattern was significant or trend-wise significant in comparison to all other subgroups, although after different stimulating conditions. The most interesting effects are visible for CALM2, which shows significantly elevated expression in contrast with all other subgroups after PHA stimulation.

Children with multitrigger wheeze already at one to three years of age have been shown to have a high level of IgE during the first year of life. This sets atopy down as a strong predictor for persistent wheezing and subsequent asthma development, as wheeze patterns and lung function that are established at age six are relatively stable until young adulthood [170, 172]. Among high IgE levels, other atopic manifestations like atopic dermatitis and food allergy have been shown to be predictors for wheeze persistence [211]. The presence of atopy in asthma has been connected to severe asthma, with studies suggesting that atopy associated pathways additionally increase the severity of viral exacerbations [212, 213]. Children with severe asthma have been described as severely atopic with multiple aeroallergen sensitization [214]. Taken together with findings that asthma severity is established early in life, this adds to the hypothesis that persistent multitrigger wheeze could be the precursor of a severe asthma phenotype later in life [45].

However, eosinophilic inflammation that traditionally is considered as hallmark feature of allergy is only found in roughly half the patients with severe asthma, indicating the presence of another pathogenetic mechanism, such as for example neutrophilic airway inflammation, or mixed airway inflammation in which eosinophilic and neutrophilic inflammation are present [12, 187, 215-217].
In general, the results from this work support a genetic influence visible in upregulated expression of the candidate genes as important factor in the pathogenesis of *persistent multitrigger* wheeze, as well as the role of the IL-1β-pathway and its link to neutrophilic airway inflammation [218].

The finding of such a distinct gene expression signature for persistent multitrigger wheeze in this work suggests the possible existence of a distinct endotype for this group even though the small sample size limits the reliability of these data.
However, the theory of PM wheeze as a distinct endotype, potentially driven by neutrophilic inflammation, with a strong genetic signature is supported for example by Yeh et al. who demonstrated the existence of a distinct gene expression signature in PBMCs of children that were classified as neutrophilic severe asthmatics [219]. Other studies found evidence of transcriptional signatures that distinguished inflammatory phenotypes (eosinophilic and neutrophilic vs. less severe) in induced sputum from adult asthmatics, or controlled from severe asthma in schoolchildren. However, in

terms of early identification for predictive purposes, induced sputum and airway epithelial brushings, used in these studies, are impractical especially in younger children, highlighting the importance of gene expression profiling [216]. Consistently, the candidate genes chosen for this work relate to neutrophilic inflammation mainly through the influence of calcium on the NLRP3-Inflammasome and subsequently, the IL-1β-pathway. Upregulated expression of candidate genes such as CALM2, ITPR2, ORAI1, STIM2 all lead to an increased level of intracellular calcium and thus inflammasome activation [140]. Additionally, differential gene expressions also included influences on the NFκB pathway for example through elevated expression of IPS1, responsible for the translocation of NFκB to the nucleus and thus controlling its activity [144]. Both pathways have also previously been connected to innate activation in asthma patients with neutrophilic airway inflammation [220-223].

Even though the candidate genes predominantly show a functional relation to neutrophilic inflammation as opposed to eosinophilic inflammation, persistent wheeze in general has repeatedly and strongly been associated with parental allergy [12, 180, 224]. Additionally, a family predisposition for asthma has been shown in several studies, often linked to maternal asthma. Studies also shown that asthma increases for children who have two parents with asthma [225]. A large study by Ferreira et al. investigated this overlap of asthma and allergy and found shared genetic variations between both with possible functional relevance [226].
Even so, by far not all atopic children go on to develop asthma. An intriguing model explaining this sees the impact of allergic sensitization on asthma development as not binary but quantitative, with relevant influences being the time of sensitization and number of sensitized aeroallergens [227]. Early allergic sensitization to multiple aeroallergens that persists during childhood has been repeatedly identified as important risk factor for wheeze persistence and asthma inception as well as loss of lung function [171, 186, 187, 197, 228]. Together with recent results that show that allergic sensitization assessment can predict exacerbation rates and response to ICS therapy in children with persistent, recurring wheeze, reinforces the involvement of atopy in the development of multitrigger wheeze [229]. Consistently, the phenotype definition of multitrigger wheeze used for this work included parent-reported or doctor-diagnosed allergic comorbidities as well as positive allergy testing.

This conflicting information about the known characteristics of persistent multitrigger wheeze from other studies pose a main challenge in the interpretation of the findings from this work. On the one hand, persistent multitrigger wheeze is consistently closely connected to atopy and allergic, eosinophilic inflammation. On the other hand, the expression pattern of the candidate genes and other phenotypes with equally distinct gene expression signatures suggest it at least has features of neutrophilic inflammation.

It is possible that the answer is a combination of both with a pathogenetic involvement of both innate and adaptive immunity. Traditionally, atopy has always been assigned to adaptive immunity. However, since the discovery of the farm effect that links innate immune stimulation to reduced incidence of allergic disease, this has changed [5]. Recent studies highlight the importance of innate immunity in atopy through of ILC2 that produce type 2 cytokines and have been shown to drive airway inflammation in response to allergic sensitization, especially in paediatric patients with severe asthma [187, 230-232]. A study by Stadhouders et al. showed that ILCs also express genes known for their role in the pathogenesis of especially allergic asthma such as ORMDL3, highlighting their pathogenetic role and thus linking the innate immune system to allergic disease [233]. Consistently, data for PM wheeze from this work show an elevated expression of ORMDL3, for example in comparison to *persistent viral* wheeze.

The gene expression signature for persistent multitrigger wheeze that was found in this work includes candidate genes from calcium signalling as well as from innate immunity. Calcium signalling is important in adaptive immunity, for example through signal transduction after the stimulation of the TCR, as well as in innate immunity as shown through its influence on the IL-1β-pathway. The innate immunity receptors analysed in this work also have points of interaction to adaptive immunity and allergy, as for example CD209 and LY75 that have tolerance-inducing capacities that, if disrupted, lead to an increased risk of allergy.

With all this in mind, it seems possible that persistent multitrigger wheeze could be the result of a combination of atopy and predominantly neutrophilic inflammation as two main pathogenetic pathways. However, this is purely hypothetical as the sample size in this work was too small to allow any ultimate conclusions.

Currently most children with preschool multitrigger wheeze or subsequent (allergic) childhood asthma are treated with different forms of corticosteroids. However, even early initiation of ICS treatment does not prevent progression from preschool wheeze to childhood asthma. [187, 234]. This could be explained by the fact that ICS treatment is often started only after the occurrence of symptoms whereas the loss of lung function and genetic signature are already visible much earlier, i.e. at birth, as it was shown in this work. Additionally, the linkage of atopy to the innate immune system as proposed above could provide further explanation for this problem, as the innate immune system is intrinsically non-reacting to steroids. This underlines the importance of more diversely targeted therapeutic strategies [234, 235]. For example, studies have tried to target the avoidance of allergen sensitization with different levels of success, at the moment, the most promising approach seems to be early allergen immunotherapy [170, 236, 237].
Even where targeted therapies already exist, the next obstacle is to bring them to the right patients, a goal described as precision medicine [187, 238, 239]. The results from this work, after careful validation, could for example one day support precision medicine through a score supported by transcriptional signatures in cord blood with the goal of early identification of children that are at risk for *persistent multitrigger* wheeze. The importance of early identification is highlighted by findings that approximately 40% of lung function loss in children who show persistent wheeze at age 7 was already present at birth, and that this group benefits from therapy to maximize lung function [234, 240].

In summary, the results presented in this work support the existence of a high-risk transcriptional endotype that leads to the phenotype of persistent multitrigger wheeze that is already identifiable at birth. Data from this work also indicate that PM might be established through several pathogenetic pathways, with possible pathways in atopy as well as neutrophilic inflammation. These findings could, after replication and validation, contribute to early identification of these patients and thus early initiation of causal therapeutic approaches such as allergen immunotherapy.

5.1.3.2 Findings for late-onset multitrigger wheeze

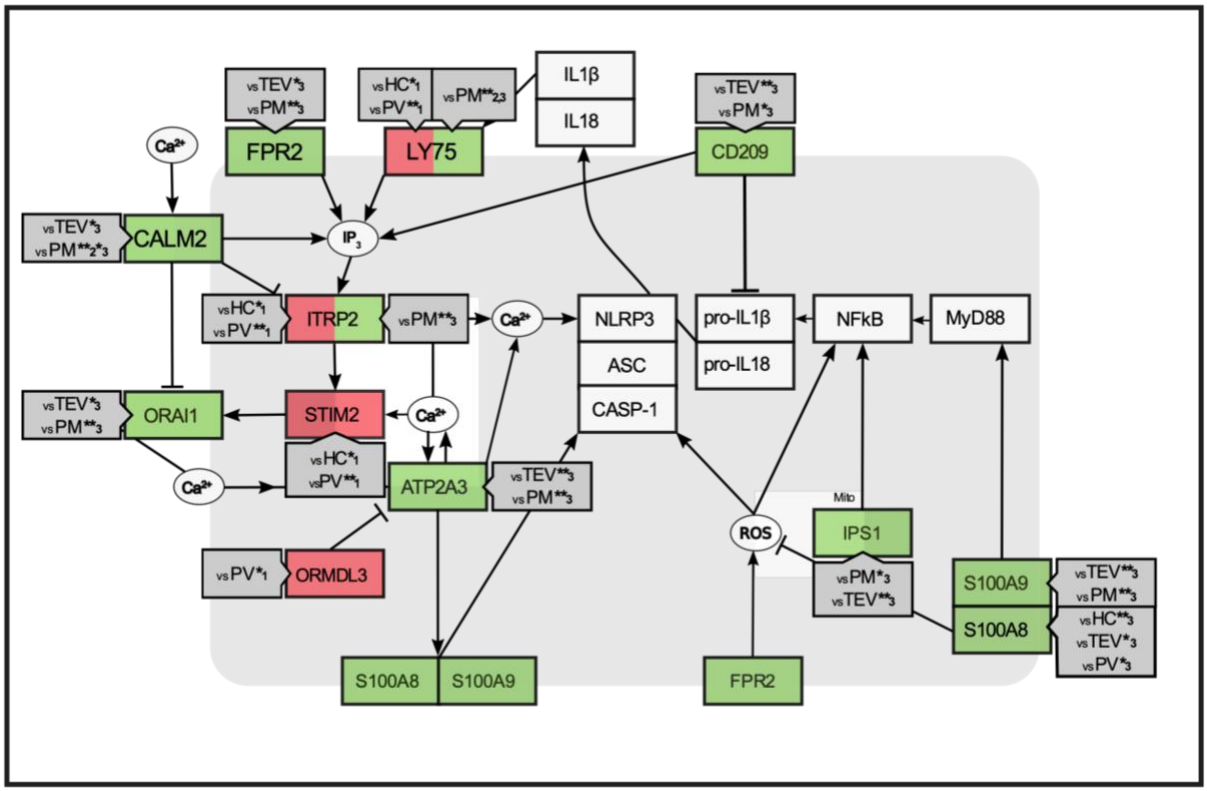

Figure 41: Overview of the significant findings for persistent viral wheeze, based on differences in ΔC_t values. HC healthy controls, TEV transient early viral wheeze, PV persistent viral wheeze, PM persistent multitrigger, LOM late-onset multitrigger, MT multitrigger. Red background marks higher expression, green background marks lower expression. All subgroups indicated in the grey boxes have been compared to late-onset multitrigger wheeze. * trend like results (p ≤ 0,1); ** significant results (p ≤ 0,05). Stimulating conditions can be seen next to the level of significance: 1 unstimulated, 2 phytohemagglutinin, 3 lipid A. For a detailed legend see chapter 9.5 in the appendix.

In this in-depth analysis, *late-onset multitrigger* wheeze, in contrast to *persistent multitrigger* wheeze, only begins after the age of three but shares the classical clinical characteristics of *multitrigger* wheeze, like the occurring of wheeze after multiple triggers including but not limited to viral infection. Parallel to in-depth analysis of PM wheeze discussed in the previous paragraphs, the main limitation for the interpretation of the results for LOM wheeze lies in the small sample size of n=8. Nevertheless, a main finding from this work is that instead of sharing a gene expression signature with PM wheeze, *late-onset multitrigger* wheeze shares strikingly similar patterns of gene expression with *persistent viral* wheeze.
All effects differentiating LOM wheeze from PM wheeze were of special interest. LOM wheeze showed lower expression of CALM2, ITPR2, ORAI1, ATP2A3, S100A9, IPS1, CD209 and LY75. This was mainly observed after microbial stimulation with LpA, but some effects were also visible after mitogen stimulation with PHA such as for LY75 and CALM2.

Despite the similarities through lower gene expression levels than PM wheeze, several trends and significant effects indicate differences between the *late-onset multitrigger* and the *persistent viral* wheeze group mainly through higher expression of the respective genes in LOM wheeze. This includes ITPR2, STIM2, ORMDL3 and LY75. On the other hand, LOM vs. PV showed a trend for lowered expression of the potentially anti-inflammatory S100A8 after LpA stimulation in the *late-onset multitrigger* group. Effects visible for LOM in comparison to PV wheeze are mimicked in effects for *late-onset multitrigger* wheeze in comparison to healthy controls, visible for ITPR2, STIM2, LY75 and S100A8. As both the differences from persistent viral wheeze and from healthy controls were found in unstimulated cells, this could potentially imply a higher baseline expression of these candidate genes as contributing factor to the phenotypic differences.

The phenotype of late-onset wheeze has been connected to a phenotype of recurrent, unremitting multitrigger wheeze, matching the definition of LOM in this work. This group was characterised by atopy, impaired lung function and varying association with smoke exposure in utero [17, 171, 180]. Late-onset multitrigger wheeze patients are distinguishable through lung function and prognosis from children who start to wheeze before age three, supporting existence of a distinct phenotype with separate pathophysiological mechanisms [172]. Findings from this work, while to be interpreted with caution, point in the same direction through several differences in gene expression of the candidate genes between *persistent* and *late-onset multitrigger* wheeze as summarized above.

The rapid rise in asthma prevalence over the last century highlights the importance of environmental influences such as viral infection [188]. Recent studies propose gene-environment interactions as important for the pathogenesis of childhood asthma, especially interesting for LOM wheeze in a two-hit hypothesis. A first hit, respiratory viral infection, and a second hit, atopic sensitization, interact to create airway inflammation through activation of innate and adaptive immunity and subsequent wheeze and childhood asthma [187, 227, 241]. As the *late-onset multitrigger* wheeze subgroup in this work had a genetic signature similar to that of *persistent viral* wheeze, it seems plausible they might also share the susceptibility to viral infection, especially as many similarities can be found antiviral innate immunity genes such as

IPS1, CD209 and FPR2. Multitrigger wheeze in general is strongly linked to atopy as it has been discussed above, and late-onset multitrigger wheeze is no exception, rendering atopic sensitization as a second hit likely. Children with both hits have been shown to have a very high risk for persistent asthma and have even been labelled as “true asthmatics” in the Tucson birth cohort, a landmark study [12, 186, 192].

The mechanisms through which viral respiratory infections can contribute to wheeze pathogenesis have already been discussed above for *persistent viral* wheeze and could also apply to *late-onset multitrigger* wheeze. Additionally, it has recently been proposed that RV infection as well as allergic inflammation lead to the production of IL33, a cytokine important in innate immunity that leads to a Th_2 shift. In children with difficult to control steroid resistant asthma, an upregulation in the IL33-pathway has been identified, while IL33 polymorphisms have been associated with late-onset wheeze, in an interesting interface between viral infection and allergic sensitization [227, 242, 243]. While these findings see respiratory infection contributing to allergic sensitization, allergic sensitization has been shown to impair antiviral (and antibacterial) defenses which could lead into a possible vicious circle [244]. Especially patients with allergic asthma suffer from impaired innate immunity that leaves them prone to viral infections, shown through impaired response to rhinovirus in PBMCs of allergic asthmatics [245]. The exact mechanisms of interaction between viral infection and aeroallergen sensitization are yet to be completely understood, but hypotheses include a cumulative airway damage as well as a higher immunopathogenic capacity for pre-existing aeroallergen sensitization in children with higher susceptibility for viral infection [186]. Some studies go as far as to suggest a causal relationship between rhinovirus induced wheeze and allergy development [246].

However, *persistent multitrigger* wheeze is also connected to atopy and aeroallergen sensitization, yet this phenotype is clearly different from LOM, through longitudinal pattern as well as in transcriptional signature as illustrated in fig. 39 and 41. Explaining this in a way that is compatible with the other results from this work, studies that suggest a redefinition of the term atopy, moving on from a yes/no dichotomy to a five-class approach. This sees both PM and LOM as atopy related,

with the difference in transcriptional signatures due to different kinds of atopy [241]. This approach stresses the importance of the pattern of atopic response (meaning age at development as well as type and number of specific allergens) over the simple dichotomy. As already discussed, multiple early sensitization has been proposed to predict persistent asthma, while for LOM an association with grass pollen sensitization has been shown, suggesting the influence of a seasonal allergen as possible atopic class for this phenotype [247, 248]. In this study, an analysis of the pattern of atopic response was not possible due to limited sample size. However, this would be desirable information when validating these data in a larger cohort.

Differentiating late-onset multitrigger wheeze patients from patients with persistent multitrigger wheeze as early as possible could result in therapeutic consequences. Multiple studies have investigated the reasons for poor response to ICS therapy in children with asthma, agreeing that different underlying pathogenetic mechanism are a main problem. The gene expression signature for *late-onset multitrigger* wheeze found in this work indicates that this could indeed be a distinct endotype with potential pathogenetic similarities to *persistent viral* wheeze. Thus, after further investigation of this theory and careful validation, the results of this work could contribute to improving disease management. Given the transcriptional signature, children with LOM wheeze are likely to benefit from intermittent ICS therapy that has been proposed to alleviate viral wheeze rather than the continuous ICS treatment that is currently used for patients with multitrigger wheeze [205, 249-251]. Additionally, using non-pharmacological treatment options like trigger avoidance and prevention of viral infection that are often underestimated could be very valuable if the pathogenetic mechanisms of LOM indeed prove to be more similar to *persistent viral* wheeze [169, 252].

In summary, results from this work indicate *late-onset multitrigger* wheeze as a separate endotype through the existence of a transcriptional phenotype distinctly different from persistent multitrigger wheeze despite similar clinical presentation and significant despite low case numbers, with a possibility of an equally distinct treatment response. A possible pathogenetic hypothesis fitting these results is a two-hit hypothesis that sees LOM wheeze as the result of environmental influences such as viral infection and atopic sensitization [253-256].

5.1.3.3 Schematic summary of the main findings

The data from this work show distinct gene signatures, already visible in cord blood at birth, for the wheeze phenotypes of *persistent multitrigger* wheeze, *late-onset multitrigger* wheeze and *persistent viral* wheeze. However, despite the significant findings, the results from this study need to be interpreted with the relatively small sample size in mind. Taken together, these findings add to the growing evidence that the heterogenous group childhood asthma actually consists of several endotypes with their respective set of pathogenetic properties.

In the following paragraphs, several hypothetical possibilities regarding the pathogenetic properties of the wheeze subgroups analysed in this work will be discussed.

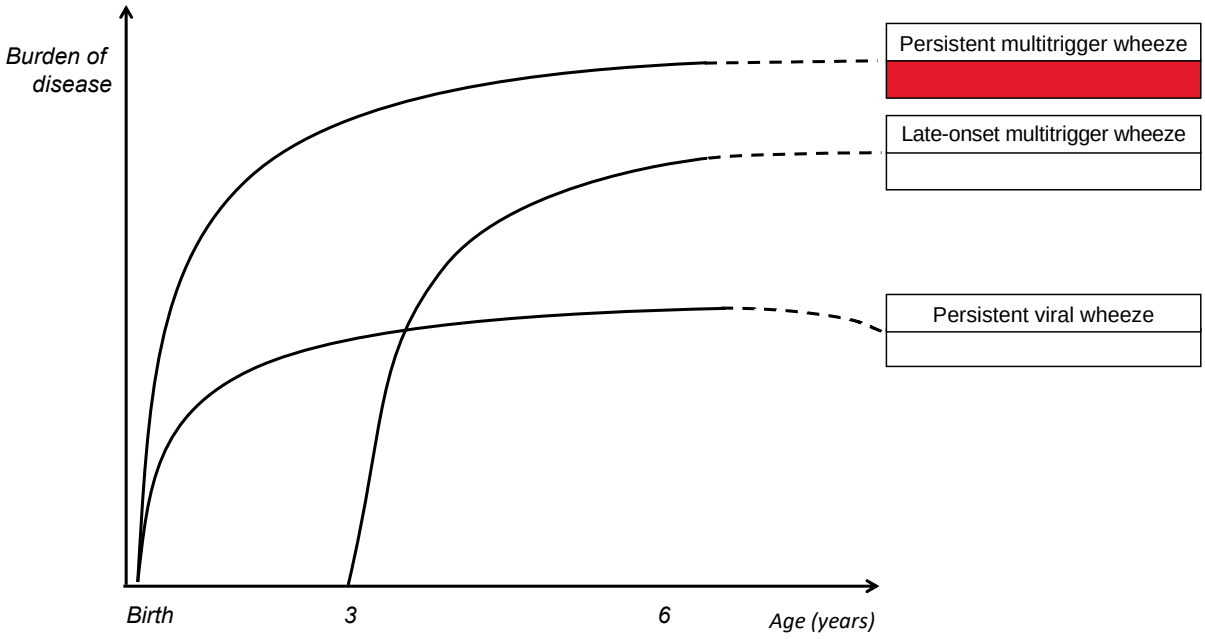

Figure 42: Schematic summary of figures 39 to 41. Continuous line represents longitudinal course used for this work. Dotted line represents hypothetical continuation after the age of six. Boxes indicate phenotype as defined for this work next to the respective trajectory. Gene expression levels as measured at birth, as shown in fig. 39-41, summarized in coloured boxes below phenotype identification. Upregulated, high gene expression represented in red, downregulated, low gene expression represented in green.

The pathogenetic pathway predominantly involved in *persistent multitrigger* wheeze seems to be a genetic susceptibility that favors the development of airway inflammation through different factors. The findings from this work are consistent with this theory as they show a distinct transcriptional signature with elevated expression of pro-inflammatory pathways in cord blood, for example in upregulated expression of the candidate genes ultimately influencing the activation of the NLRP3-

Inflammasome. Favouring the hypothesis that PM wheeze is heavily dependent on genetic influences, the CAPPS cohort that followed a high-risk birth cohort of children with a family history of asthma and atopy found prevalence rates of up to 21% for early persistent (multitrigger) wheeze, two times the prevalence in an average population [15, 176]. Additionally, a positive family history of asthma has been consistently associated with a higher risk for early onset persistent asthma, a definition that is very similar to PM wheeze, as opposed to other wheeze phenotypes [257]. However, as genetic susceptibility has been the subject of a multitude of studies, it becomes increasingly clear that while it might, for some endotypes such as persistent multitrigger wheeze, be the predominant pathogenetic factor, it cannot be the only driving pathogenetic mechanism – a theory summarized as “missing heritability” [258]. Proposed pathogenetic mechanisms involved include early life smoke exposure, however in this study, smoking and smoke exposure could not be identified as a confounder [257].
For persistent viral wheeze, altered postnatal maturation of the lungs through a lower respiratory tract infection with for example rhinovirus is proposed as a possible scenario for pathogenetic mechanisms. Additionally, results from this work show a transcriptional signature of downregulated expression of the candidate genes including antiviral innate immunity and calcium signalling, whose relevance for the IL-1β-pathway has already been discussed above. Thus they seem to be consistent with a hypothesis proposed by others that children with persistent viral wheeze have a higher susceptibility to viral infection [193].

Other results from our group show increased expression of ATP2A3 and ORMDL3 in asthmatic children vs. healthy controls, independent of their atopic status. However, results from this work indicate different expression between PV, PM and LOM. A possible explanation could be that atopy plays a role in all these children albeit with different stress on the role of atopy for each phenotype [77]. This exemplarily shows that while multitrigger and viral wheeze are phenotypes that are clear-cut at the extremes of their longitudinal manifestations, as for example in this work, their persistent forms, but apart from that, they show a considerable overlap. The findings for late-onset multitrigger wheeze presented in this work could be a first step towards further understanding of this overlap [14].

A possible theory for late-onset multitrigger wheeze pathogenesis could be a two-hit hypothesis as previously discussed [192]. In summary, this hypothesis sees the pathogenesis of late-onset multitrigger wheeze as an overlap between atopy and viral infection, with gene-environment interactions as potential mechanism [38, 253]. The results from this study show LOM wheeze as an overlap as well, with shared clinical characteristics of PM wheeze on one hand, and a similar gene expression signature to PV wheeze on the other. While this needs to be cautiously interpreted because of the small sample size, it is strengthened by the fact that others already found a lacking production of Th_1-cytokines, already visible in cord blood, for children with late-onset wheeze [186, 259, 260].

Despite the evidence that some pathogenetic pathways are involved in several wheeze phenotypes, there are points where they can be distinguished.
Both persistent and late-onset (multitrigger) wheeze were associated with atopy. However, lung function loss was more pronounced in children that persistent, early allergic sensitization as opposed to later onset of atopy. Additionally, (early) atopic sensitization was found to increase the airways' susceptibility to non-allergenic stimuli like house dust [261, 262]. Both findings imply that PM and LOM could for example be distinguished through disease severity [172, 248, 263].
As to the differentiation between *late-onset multitrigger* and *persistent viral* wheeze, a study investigating severe bronchiolitis found that while children that were additionally atopic were more likely to develop recurrent wheeze, children who only had severe bronchiolitis also had an increased risk for recurrent wheeze. This also supports the existence of distinct pathways leading to (late-onset) multitrigger wheeze and (persistent) viral wheeze respectively [191].
Possibilities include pathways such as IL13, as it was shown to be associated with both atopic and non-atopic asthmatics suggesting a distinct influence maybe through direct effect on the airways. Other pathogenetic pathways that have a direct influence on the airways separate of atopy might contribute to the clinical differences between LOM and PV, or the differences in severity between PM and LOM [264].

A possible hypothesis that emerges from summarizing all of the above, features two of the main known pathogenetic pathways. One of those pathways is genetic susceptibility, for example to atopic sensitization and airway inflammation, the other

is an altered lung development and function through viral respiratory infection. However, recent asthma research, including the results of this work, indicate that a simple dichotomy does not account for the multiple wheeze phenotypes that are clearly distinguishable through several characteristics, and as shown in this thesis, also on a transcriptional level. Instead, a graded model for the influence of these pathways seems to do asthma pathogenesis more justice. Applied to the wheeze phenotypes used in this work, *persistent multitrigger* wheeze would seem to be predominantly caused by the former, while for *persistent viral* wheeze, the latter is more visible, and *late-onset multitrigger* wheeze is influenced by both.
However, this is heavily speculative and further investigation is needed to test this hypothesis, for example in a larger cohort.

5.2 Overview of additional findings

5.2.1 ORMDL3 and 17q21

As previously discussed, especially *persistent* wheeze is characterized through high association to the known asthma susceptibility locus 17q21, which codes for ORDML3 [17, 265]. In another study cohort from our group investigating asthma manifestation in children at school age, ORMDL3 as well as ATP2A3 were upregulated, despite ORMDL3 physiologically inhibiting ATP2A3. The same was visible in this work for *persistent multitrigger* wheeze. A possible explanation for this might be that ATP2A3 is upregulated to overcome the upregulated inhibition via ORDML3 [77].

However, findings for ORMDL3 in this work were not as strong as one might have expected knowing these results and other studies [35]. Having said that, ORMDL3 results were inconsistent across different studies, being associated with TEV wheeze in some populations but with persistent and intermediate-onset wheeze in other which suggests a variety of gene-environment interactions in different populations [184, 265, 266]. Another explanation might be that the impact of ORMDL3 has been identified as mediating airway hyperreagibility in response to allergic sensitization especially in airway epithelium cells. As this work examined gene expression levels in cord blood, the true effect of ORMDL3 might only reveal itself through GEI that had not yet taken place. Fitting this theory as well as the results from this study, Lluis et al. found that ORMDL3 expression was lower in CMBCs than in PBMCs of older children, which they hypothesized could be due to ORMDL3 expression being dependent on immune maturation [267].

Of course, the findings in this work could also be due to genotype influence. In general, genotype data were assessed in our laboratory. However, this was not the focus of this thesis as a stratified analysis was not possible due to small case numbers and resulting power limitations.

Even so, *persistent multitrigger* wheeze showed a distinct pattern of elevated gene expression, including ORMDL3. This might indicate present, but not predominate function of ORMDL3 in neonatal immunity.

5.2.2 Arachidonic acid cascade

Candidate genes influencing different aspects of the arachidonic acid cascade were significantly differently expressed between the wheeze subgroups, including FPR2, both S100A8 and S100A9, and CD209.

A pattern that emerged from the in-depth group comparisons was an elevated expression of associated genes for *persistent multitrigger* wheeze (visible for FPR2, S100A9, and CD209) and lowered expression for *late onset multitrigger* wheeze (visible for S100A8, S100A9, FPR2 and CD209), in comparison to the other wheeze subgroups respectively as it can be seen in the overview figures 39 - 41.

Recently, the intriguing concept of an arachidonic acid mediated calcium influx as an alternative to SOCE has been proposed [268]. As the pattern for SOCE-associated calcium signalling and the genes associated with arachidonic acid metabolisms show the same expression patterns, the results from this study would support this theory However, this can only partially explain the effects observed. A possible explanation for the elevated expression in persistent multitrigger wheeze might be the induction of S100A8 and S100A9 via IL1β. For *late onset multitrigger* wheeze, it could be possible that lowered expression leads to a dysregulation in the arachidonic acid cascade, impairing resolution of airway inflammation after viral infection. In accordance, it has been found that therapy with the leukotriene antagonist montelukast shows a benefit for children with virally induced wheeze [269]. Epidemiological studies show these effects could be influenced by nutrition (namely, fish oil and omega-3 fatty acids), potentially already prenatally [270].

5.3 Discussion of limitations and strength

5.3.1 Study design

The PAULINA/PAULCHEN study is a birth cohort recruited in the metropolitan area of Munich and in rural southern Germany, respectively. Due to the natural prevalence of wheeze, and especially multitrigger wheeze, case numbers were limited to n=14 despite the relatively large cohort of n=200 [15]. Even so, results were strongly significant in spite of comparatively low case numbers, indicating powerful effects. From today's point of view, additional information from the questionnaires, for example more detailed information about viral infections, or a larger volume of cord blood available for additional analyses such as western blotting would have been desirable. However, conducting such a large birth cohort with in depth immune characterization beyond the performed functional studies was logistically not possible [68, 168].

It has been proposed that the time frame in which the individual risk for wheeze and asthma is set is very short, ranging from the prenatal period to just the first few years of life. As it is additionally becoming increasingly clear just how much prenatal events can shape the immune system and thus influence the development of childhood wheeze and asthma, birth cohorts are a well-established setting to study its pathogenesis [82, 253]. Early biomarkers are an important key to reduce asthma morbidity as harmful influences early in life that track through the whole life of the patient affected. Only an early biomarker, prenatal or at birth, can contribute minimizing these early in life harmful influences, so we studies transcriptional signatures in cord blood at birth [5].
This particular birth cohort's strengths include the strict employment of inclusion and exclusion criteria (see chapter 3.2), limiting the influence of possibly distorting factors to a minimum. The major strength is the detailed longitudinal characterization of the study population detailed questionnaires and high follow-up rates.

5.3.2 Wheeze definition

The questionnaires used provided detailed information about wheeze symptoms, other atopy-related diseases (e.g. allergic rhinoconjuctivitis, atopic dermatitis, etc.), medication, and general health status of the child as well as a socioeconomic

anamnesis. The use of nearly identical questionnaires in both PAULINA and the PAULCHEN enabled a joint analysis of the cohorts.
Using only a parent-dependent, questionnaire-based follow-up might risk incomplete or incorrect reporting of the children's symptoms. As means of compensation, the questionnaire included questions about a doctor's diagnosis of asthma- or atopy-related symptoms. A major advantage of the questionnaire follow-up was the high follow-up rates[10].
A challenge the epidemiological and the clinical approach of phenotyping share is dealing with changes in the clinical picture over time, as the assignment of a clinical phenotype tends to change, especially in children [38, 271]. One way to deal with this challenge is to view the change as a part of the phenotype, thus defining a trajectory for this kind of wheeze, increasing both stability and reliability of the phenotypes. The other way is that a change in clinical picture automatically leads to reassignment to another, more fitting phenotype, which comes at the cost of little to no stability of phenotypes [272]. In the PAULINA/PAULCHEN cohort, a combination of clinical wheeze phenotypes (multitrigger and viral) and transition trajectories of the well-established longitudinal Tucson cohort phenotypes (transient early, persistent and late onset) was used, thus increasing both stability and reliability of the results for the resulting subgroups investigated [14, 82]. This approach is supported by other studies with different methods that validated the wheeze phenotypes used in this work as comprehensively review by Belgrave et al. [17, 36, 53, 188, 273].

5.3.3 Stimulation of cord blood mononuclear cells

CBMC stimulation with PHA as well as LpA is a method commonly used to mimic the effects of natural stimulation of the immune system. Phytohemagglutinin (PHA) is a protein from the lectin family, and is a powerful stimulus triggering mitosis specifically in T-cells by binding to glykoproteins on the cell's surface, leading to agglutination and proliferation [274]. In contrast, LpA (Lipid A) is the biologically active component of bacterial LPS and triggers different effects within the innate immune systems as it is a ligand for TLR4 [60, 275]. The effect of stimulation was assessed by fold change, the comparison to expression of the candidate genes in unstimulated cells. Non-significant results indicate that gene expression was not significantly up- or

[10] Shown in the appendix (9.7).

downregulated after stimulation but do not concern the significance of the following comparison of the subgroup's differences in ΔC_t.
For the investigation of the objectives in this study, these two stimulating conditions and the additional information about the baseline expression are ideally suited as they representatively model effects in both the adaptive and the innate immune system respectively.

5.3.4 Cytokine measurements

The candidate genes chosen for this work join in the IL1β-pathway as final common path[11]. A logical follow-up question is whether the patterns of gene expression levels also continues to cytokine levels. The analysis of cytokine levels is currently conducted in additional replication studies by the laboratory and thus not the focus of this thesis.
Even so, Rothers et al. investigated cytokine levels in cord blood directly at birth and found that cytokine production in cord blood samples did not show any significant relation to asthma outcome. However, other results from their study imply that the predictive value of cytokine levels is not fully determined at birth but only develops during the first year of life, which underlines the importance of the gene expression signatures found in this work [264].

11 For overview of the candidate genes see a graphical illustration in the appendix.

5.4 Implications

This study supports a graded model for the pathogenesis of childhood wheeze and asthma through the findings for *persistent multitrigger*, *late-onset multitrigger* and *persistent viral* wheeze. Especially of interest are the strong findings for *late-onset multitrigger* wheeze despite the limited case numbers hinting that LOM wheeze could be the result of pathophysiological mechanisms that are clearly distinguishable from those causing *persistent multitrigger* wheeze despite their similar clinical appearance. Steps in this direction showed the longitudinal pattern of late-onset and persistent wheeze together with AHR and low lung function in childhood as predictors for asthma [276]. Findings that differences in lung function levels at age six track to adulthood reinforce the importance of early childhood for asthma pathogenesis and disease severity [172, 177]. Consistently, a loss of lung function in children that go on to have persistent wheeze disorders has been described for the immediate post-natal period [187, 277]. This highlights the fact that in clinical practice, the key to reducing overall asthma morbidity will be to improve the prediction of wheeze outcomes (see chapter 1.6.) in order to identify children at risk for different types of wheeze as early as possible [278].

However, patients will only truly benefit from early identification if therapeutic options are available, which provides for another challenge.

As previously stated, the main therapeutic strategy currently used is ICS therapy in different regimens which, however, cannot prevent progression of early to persistent wheeze [279]. Findings like this show ICS therapy is symptomatic rather than causal and thus should not unthinkingly be used for prevention in preschool children with wheeze. While a higher, earlier and more consistent therapy with ICS might prove beneficial to some high-risk patients, this is ethically not feasible as long as this high-risk group cannot be identified properly [278]. Results from this work differentiating persistent multitrigger wheeze as a possible high-risk group could provide means to test this therapeutic strategy.

Early and multiple aeroallergen sensitization seems to be pivotal in the pathogenesis of *persistent multitrigger* wheeze in particular. With allergen immunotherapy as a possible, causal therapeutic option, early identification of children at risk for PM wheeze could lead to an earlier treatment start, limiting consequential damages. Aeroallergen sensitization is also important for the development of LOM wheeze, and

early identification of these children could improve asthma prevention through for example advising parents to avoid allergens in sensitized children [227, 262, 280]. Supportingly but not un-controversially discussed, Owara et al. found that the wheeze trajectory of early wheeze could be modified by prenatal intervention such as the avoidance of house dust, pets, and environmental tobacco smoke and encouragement of exclusive breastfeeding [176].

Supporting this work's approach to early identification, a recent study by Howrylak et al. showed a correlation between clinically assigned phenotype and gene expression of atopy-related genes, albeit in a cohort of older children. Even so, these findings are parallel to what was shown in this work supporting the existence of transcriptional signatures for these particular phenotypes [281].
In order to be suitable for screening purposes, a candidate gene would have to show elevated expression in comparison to ideally all other possible groups, especially healthy controls. Based on the results showing that *persistent multitrigger* wheeze is a distinct entity with a specific pattern of gene expression within *multitrigger* wheeze, several candidate genes could be proposed as candidates for further investigation through replication and validation with the goal of identifying possible biomarkers for *persistent multitrigger* wheeze. The most promising of these results were seen for CALM2 after PHA stimulation. To date, high expression of CALM2 in CBMCs has not yet been associated to any other disease than childhood wheeze and asthma.
It has been shown that a biomarker alone cannot predict a complex disease like asthma, but combining different biomarkers largely increases the reliability of such predictions [75, 282]. Hence, eventually adding the measurement of CALM2, after further testing and validation, to a predictive model that already exists could largely increase the predictive value of the tool in question [283]. In addition, a biomarker from cord blood would have many advantages as it can be gained non-invasively and would predict the risk for wheeze development at the earliest point possible.

5.5 Outlook

Currently, our research group is working on the analysis of further follow-up to both the PAULINA and the PAULCHEN cohort, which will provide valuable information about the further development of the study population, as for example diagnoses of childhood asthma. Naturally, extending the subgroup definition to include the phenotypes at 10 years and then again comparing gene expression for the candidate genes would be a first validation of the results of this thesis and will be done in due course. In order to solve the puzzle that is the pathogenesis of childhood asthma, data from cohorts of older children and other groups should be brought together with data from birth cohorts so they can complement and complete each other [210]. While in this work this was already partly realized in the choosing of the candidate genes, our group will continue to do so as new insights are gained.

Additional findings from this work warrant further investigation concerning the role of the arachidonic acid cascade in asthma pathogenesis that would most likely contribute to a further understanding of the pathophysiology of steroid-resistant asthma.

For further validation a birth cohort with adjusted design, as well as a higher number of cases, should be recruited. The new design might consider including more extensive functional investigation of different subsets of CBMCs. Also, protein assessment e.g. by western blot to prove that elevated gene expression also leads to elevated protein counts (or lowered, respectively) would be of high interest. Ultimately, obtaining blood samples in addition to questionnaires at each follow-up would provide the opportunity to study changes in the expression of the candidate genes as the immune system matures.

5.6 Conclusion

In recent research, it has become clear that asthma is a complex, heterogeneous disease with a clear hereditary component. Many pathophysiological pathways have been identified. While findings of varied expression in single genes often only marginally impacts the asthma phenotype, this study detected a consistent pattern of varied gene expression for the calcium-signalling pathway revolving around store-operated calcium entry (SOCE) in children with different subsets of multitrigger wheeze. In particular, findings for late-onset multitrigger wheeze, while keeping in mind the small case numbers in this study, reinforce the diversity of mechanisms involved in asthma pathogenesis and the need for further endotyping.

6 Summary

Asthma remains one of the most common chronic diseases in childhood, developing already early in life. In the first year of life, children start to present with wheeze, which is currently classified as multi-trigger or viral-wheeze based on specific triggers and their long-term time course. An early prediction of children with wheeze and at risk for subsequent asthma is currently difficult and underlying immune mechanisms of distinct wheeze phenotypes are unknown.

As early priming of the immune system occurs already prenatally, we aim to identify predictive markers for children at risk for subsequent development of childhood wheeze and asthma. From previous work of our research group, innate immune regulation, in particular antiviral immunity (LY75, CD209, IPS1) and Ca^{2+}-signalling pathways, e.g. CALM2 and genes related to store-operated calcium entry (ITPR2, STIM2, ORAI1, ORMDL3 and ATP2A3) have been shown to be differentially expressed between asthmatics and healthy children during disease manifestation at school-age. Here, we aimed to assess these genes at the earliest time point, namely at birth. We investigated gene regulation as potential predictive markers for the development of subsequent wheeze phenotypes.

The mRNA-expression of selected candidate genes of Ca^{2+}-signalling (CALM2, ITPR2, ORAI1, STIM2, ORMDL3, ATP2A3) and innate immunity (CD209, LY75, FPR2, IPS1) was measured in CBMCs of children with subsequent wheeze phenotypes compared to healthy children (HC). Children were selected from the PAULINA/PAULCHEN cohort (n=200). Phenotypic classification in multitrigger, persistent viral or early viral wheeze and healthy control was based on clinical information from questionnaires answered by the parents at age 3 and/or 6 years. Previously, cord blood was taken from healthy neonates with strict inclusion criteria, and CBMCs were isolated within 24 hours, kept unstimulated or were stimulated with PHA or LpA for 72h. Gene expression at mRNA level was assessed via qRT-PCR, and analysed with the Wilcoxon rank sum test.

The following results were obtained and are presented in this thesis:

1. Expression of all candidate genes of calcium signalling and viral innate immunity was detectable and varied significantly or trend-wise between the different wheeze subgroups and healthy controls (mainly ITPR2, CALM2, ORAI1, STIM2, FPR2, IPS1, LY75).
2. Children with persistent viral wheeze showed a lower expression of the candidate

genes (ITPR2, CALM2, ORAI1, STIM2, ATP2A3, IPS1, LY75, CD209) than the other wheeze subgroups.

3. Children with persistent multitrigger wheeze showed a distinct pattern of elevated expression for the candidate genes (ITPR2, CALM2, ORAI1, STIM2, ORMDL3, ATP2A3, S100A9 FPR2, IPS1, LY75, CD209). This was visible for the whole calcium-signalling pathway, supporting the view of persistent multitrigger wheeze as distinct entity and suggests a pathophysiological relevance of calcium signalling.
4. Despite parallels in clinical presentation, late-onset multitrigger wheeze share gene expression patterns with persistent viral rather than persistent multitrigger wheeze, suggesting a different underlying pathophysiology.

Taken together, these results affirm the emerging view of childhood asthma as a complex disease and show that differences in immune regulation are already visible at birth. Importantly, especially the findings for *persistent multitrigger* wheeze could eventually, after suitable validation for example in a larger cohort with higher case numbers, help in the development of specific biomarkers before disease manifestation, which may contribute to closer clinical monitoring and in the long-term potentially to a change in therapy for patients with distinct phenotypes of childhood asthma.

7 Zusammenfassung

Asthma ist eine der häufigsten chronischen Erkrankungen im Kindesalter und entwickelt sich bereits in frühen Lebensjahren. Bereits im ersten Lebensjahr zeigen die Kinder *wheeze* (engl. die typische Asthmasymptomatik Pfeifen und Giemen), das zurzeit in Abhängigkeit der jeweiligen Auslöser sowie des longitudinalen Verlaufs entweder als *multi-trigger wheeze* oder viral bedingt klassifiziert wird. Eine frühe Identifikation von Kindern mit *wheeze* und einem erhöhten Risiko, ein daraus hervorgehendes Asthma zu entwickeln, ist im Moment schwierig. Die der Heterogenität der *wheeze*-Phänotypen zugrundeliegenden pathophysiologischen Mechanismen des Immunsystems sind nur unzureichend bekannt.

Da das frühe Priming des Immunsytems bereits pränatal stattfindet, war das Ziel dieser Arbeit, prädiktive Marker für Kinder mit einem erhöhten Risiko für *wheeze* und Asthma im Kindesalter zu identifizieren. Aus früheren Arbeiten der Arbeitsgruppe ist bereits bekannt, dass Gene der Regulation des angeborenen Immunsystems, vor allem im Bereich der antiviralen Abwehr (z.B. LY75, CD209, IPS-1) und des Calcium-Signalweges (z.B. CALM2) sowie der SOCE (store-operated calcium entry) (z.B. ITPR2, STIM2, ORAI1, ORMDL3 und ATP2A3) bei asthmatischen und gesunden Kindern während der Manifestationsphase der Erkrankung unterschiedlich hoch exprimiert werden. Im Folgenden soll die Expression dieser Gene zum frühestmöglichen Zeitpunkt, also bei Geburt, analysiert werden. Die Genexpression soll im Hinblick auf eine mögliche Verwendung als prädiktive Marker für die Entwicklung bestimmter *wheeze*-Phänotypen untersucht werden.

Die Expression von mRNA der ausgewählten Kandidatengene des Calciu-Signalweges (CALM2, ITPR2, ORAI1, STIM2, ORMDL3, ATP2A3) und des angeborenen Immunsystems (CD209, LY75, FPR2, IPS1) wurde in CBMCs gemessen. Im Anschluss wurde die Genexpression von Kindern mit durch Follow-Up Untersuchungen bekanntem *wheeze* mit der Genexpression von pulmonal gesunden Kindern (healthy controls, HC) verglichen. Die Kinder wurden aus der PAULINA/PAULCHEN-Geburtskohorte (n=200) ausgewählt. Von den in die Geburtskohorte eingeschlossen, gesunden Neugeborenen wurde Nabelschnurblut gewonnen. Daraus wurden innerhalb von 24h CBMCs (cord blood mononuclear cells) isoliert, die dann entweder unstimuliert belassen oder mit PHA bzw. LpA stimuliert wurden. Die Einteilung in die unterschiedlichen *wheeze*-Phänotypen *„multitrigger wheeze“* (durch mehrere Faktoren ausgelöst), *„persistent viral wheeze“*

(persistierende Symptome, die durch virale Infektionen ausgelöst werden) und *„transient early wheeze“* (frühe Symptome, die durch virale Infektionen ausgelöst werden und vorübergehend sind) sowie in die gesunde Kontrollgruppe erfolgte anhand der klinischen Informationen aus Fragebögen, die im Alter von 3 und/oder 6 Jahren von den Eltern ausgefüllt wurden. Die Genexpression wurde auf mRNA-Level durch qRT-PCR untersucht und mit dem „Wilcoxon rank sum“-Test statistisch analysiert.

Die folgenden Ergebnisse wurden erzielt und in dieser Doktorarbeit vorgestellt:

1. Die Expression von Kandidatengenen des Calciumsignalweges und der angeborenen, antiviralen Immunität war nachweisbar und zeigte sich signifikant oder als Trend unterschiedlich zwischen den unterschiedlichen *wheeze*-Gruppen sowie im Vergleich mit den gesunden Kontrollen (vor allem für ITPR2, CALM2, ORAI1, STIM2, FPR2, IPS1 und LY75).
2. Kinder mit *„persistent viral wheeze“* zeigten eine niedrigere Expression der Kandidatengene (ITPR2, CALM2, ORAI1, STIM2, ATP2A3, IPS1, LY75, CD209) als die anderen *Wheeze*-Untergruppen, besonders nach Stimulation mit LpA.
3. Kinder mit *„persistent multitrigger wheeze“* (durchgängig durch mehrere Mechanismen ausgelöst) zeigten ein abgegrenztes, charakteristisches Muster einer erhöhten Genexpression der Kandidatengene (ITPR2, CALM2, ORAI1, STIM2, ATP2A3, S100A9, FPR2, IPS1, LY75, CD209), vor allem nach Stimulation der CBMCs mit PHA. Dieses Muster war für den gesamten Calciumsignalweg sichtbar. Dies stützt die Theorie, nach der *„persistent multitrigger wheeze“* eine eigene, scharf abgegrenzte Entität darstellt, möglicherweise pathophysiologisch vermittelt durch Veränderungen im Calciumsignalweg.
4. Trotz der klinischen Zuordnung beider Gruppen als *„multitrigger wheeze“* zeigt *„late onset multitrigger wheeze“* (spät einsetzend, durch mehrere Faktoren ausgelöst) in der Genexpression der Kandidatengene kaum Ähnlichkeiten mit *„persistent multitrigger wheeze“*. Stattdessen finden sich auffällige Parallelen zu den Genexpressionsmustern von *„persistent viral wheeze“*, was einen unterschiedlichen pathogenetischen Mechanismus für die beiden Untergruppen von *„multitrigger wheeze“* nahelegt.

Zusammen bekräftigen diese Ergebnisse, dass Asthma als eine komplexe Krankheit anzusehen ist und dass Veränderungen in der Immunregulation bereits bei Geburt sichtbar sind. Besonders die Erkenntnisse über *„persistent multitrigger wheeze“* könnten eventuell, nach entsprechender Validierung z.B. in einer Kohorte mit größeren Fallzahlen, für das Fernziel der Entwicklung spezifischer Biomarker, die bereits vor der Manifestation der Krankheit verwendbar sind, von Interesse sein. Ein Vorteil, der sich daraus für Kinder mit Asthma ergeben könnte, wäre eine engmaschigere klinische Kontrolle und vielleicht als Fernziel eine Anpassung der Therapiestrategien an den jeweiligen Phänotyp.

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9 Appendix

9.1 Abbreviations

AHR	Airway hyper responsiveness	GWAS	Genome wide association studies	PAULCHEN	Prospective Cord Blood Study in Rural Southern Germany
(m)API	(Modified) asthma predictive index	HC	Healthy control	PAULINA	Pediatric Alliance For Unselected Longitudinal Investigation of Neonates for Allergy
(qRT-)PCR	Quantitative Real-Time Polymerase Chain Reaction	HDM	house-dust mite	PBMC	peripheral blood mononuclear cells
$[Ca^{2+}]_e$	Concentration of extracellular calcium	ICS	Inhalative corticosteroid	PG	prostaglandine
$[Ca^{2+}]_i$	Concentration of intracellular calcium	IFN	Interferon	PGE2	prostaglandin E2
AERD	Aspirin-exacerbated respiratory disease	IgE	Immunoglobulin E	PHA	phytohemagglutinin
APC	antigen presenting cells	IL	interleukin	PLC	phospholipase C
ASC	Apoptosis-associated speck-like protein containing a CARD	IP_3	inositoltriphosphate	PM	Persistent multitrigger wheeze
ATP	Adenosine triphosphate	IPS1	Synonym for Mitochondrial antiviral-signalling protein	PRR	Pattern Recognition Receptor
ATP2A3	ATPase Sarcoplasmic/Endoplasmic Reticulum Ca2+ Transporting 3	ITPR2	Inositol 1,4,5-trisphosphate receptor, type 2	PV	Persistent viral wheeze
Ca^{2+}	Calcium	LCA	Latent Class Analysis	RAST	radio-allergo-sorbent test
CALM2	Calmodulin 2	LOM	Late-onset multitrigger wheeze	RLR	RIG-I-like receptors
CALMKII	calmodulin-dependent kinase II	LpA	LipidA	ROS	Reactive oxygen species
cAMP	cyclic adenosine monophosphate	LT	leukotriene	S100A8	S100 calcium binding protein A8
Casp1	Caspase 1	LY75	Lymphocyte antigen 75	S100A9	S100 calcium binding protein A9

CBMC	Cord blood mononuclear cells	MAPK	mitogen-activated protein kinase	SERCA	sarco-endoplasmatic reticulum Ca^{2+} -ATPase
CD209	Cluster of Differentiation 209	MHC	Major Histocompatibility Complex	SNP	single nucleotide polymorphisms
CLR	C-Type Lectin receptors	miRNA	Micro-RNA	SOCE	Store-operated calcium entry
COPD	Chronic obstructive pulmonary disease	mRNA	Messenger-RNA	STIM1	Stromal interaction molecule 1
CRAC	Calcium release-activated channels	MT	Multitrigger wheeze	STIM2	Stromal interaction molecule 2
C_t	Cycle of threshold	NFAT	nuclear factor of activated T-cells	TCR	T-cell receptor
DAMP	damage-associated molecular patterns	NFκB	nuclear factor 'kappa-light-chain-enhancer'	TEV	Transient early viral wheeze
DC	Dendritic cells	NK	Natural killer cells	Th-cells	T-helper-cells
DDX58	DEXD/H-Box Helicase 58	NLR	Node-like receptors	TLR	Toll-Like receptors
DHX58	DEXH-Box Helicase 58	NLRP3	NACHT, LRR and PYD domains-containing protein 3	T_{reg}	Regulatory T-cells
EC	Epithelial cells	ORAI1	Calcium release-activated calcium channel protein 1	UFR	Unfolded protein response
ER	Endoplasmatic reticulum	ORMDL3	orosomucoid 1-like protein 3	VM	Viral to multitrigger wheeze
FPR2	N-formyl peptide receptor 2	PAMP	pathogen associated molecular patterns		

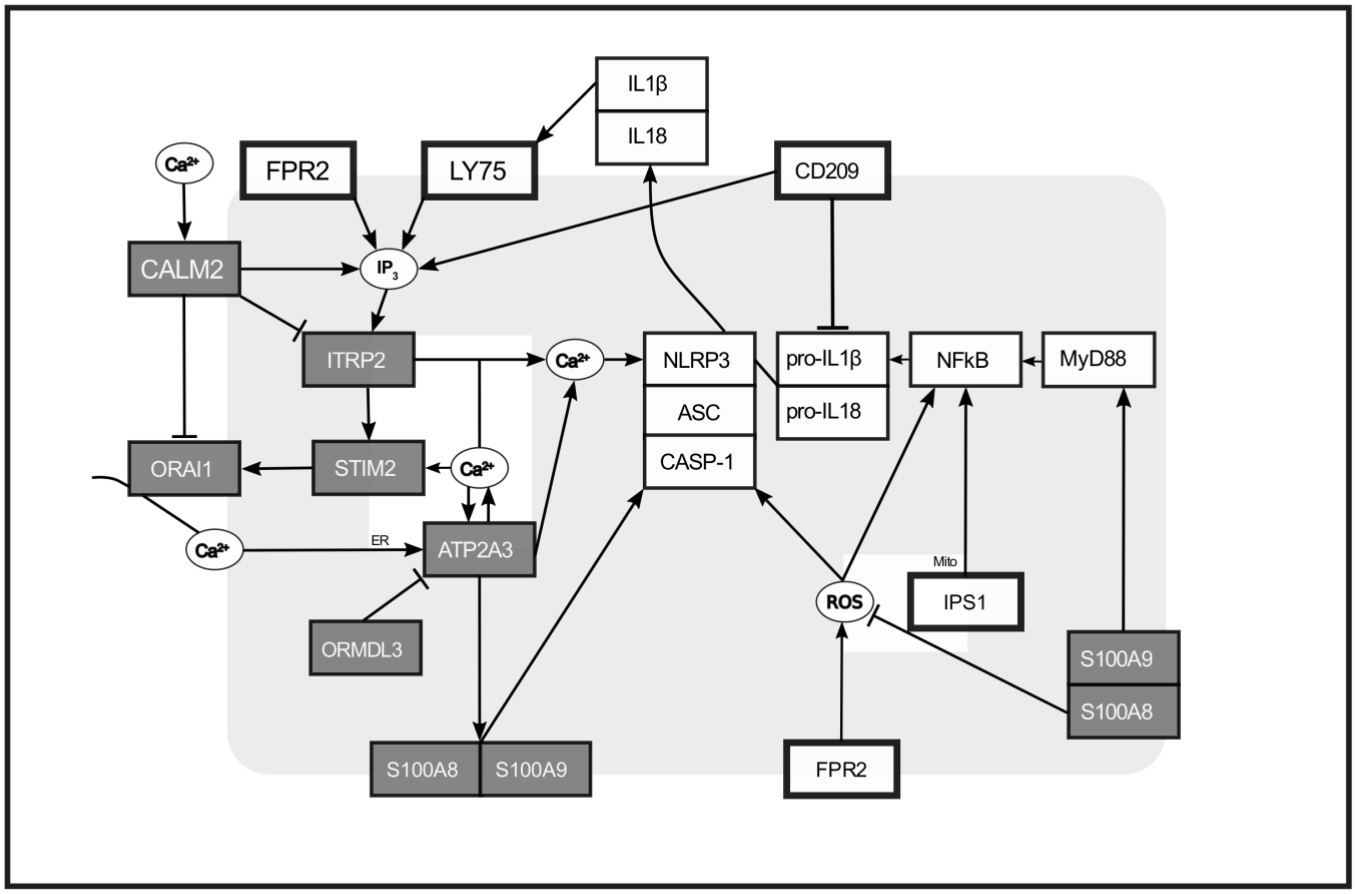
9.2 Register of figures

9.3 Register of tables

9.4 Primer sequences

Candidate gene	***Forward sequence***	***Reverse sequence (complementary)***
ATP2A3	GCTGGCTCTTCTTCCGATACCTG	TTTCAATGGTCACGAGCACGG
CALM2	GATGGCAAGAAAAATGAAAGACAC	ACCATCACCATCAATATCTGCTTC
CD209	GCTCGTCGTAATCAAAAGTGCTG	CATTTGTCGTCGTTCCAGCC
FPR2	CTCCACTCCTCTGAATGAATATGAAG	ATGGAGACAATGAGGAATGGTAATG
IPS1	GCAGAGAGAAGGAGCCAAGTTACCC	TTCTGTGTCCTGCTCCTGATGCC
ITPR2	TCGGTCAACGGCTTCATCAG	GGTTCCCTTGTTTGGCTTGC
LY75	GAAGAAGCATCCCCTAAGCCTG	AAACCAATCCACAGCCAATGC
ORAI1	GCACAATCTCAACTCGGTCAAGG	GTGGACGGCGAAGACGATAAAG
ORMDL3	GGACCAGGGCAAGGCGAG	CACGCTCATCAGGGACACGG
S100A8	GGGATGACCTGAAGAAATTGCTAG	CTACTCTTTGTGGCTTTCTTCATGG
S100A9	GAACGCAACATAGAGACCATCATC	GCATGATGAACTCCTCGAAGC
STIM2	GCCTGACCTCTTCCCTTTATTCTG	GGCTGCTGCTTCTGGCTAATG
18S	AGTCCCTGCCCTTTGTACACA	GATCCGAGGGCCTCACTAAAC

9.5 Gene overview map



Schematic overview of a cell (light grey background).			
	Genes associated to calcium signalling		Inhibiting influence
	Genes associated to innate signalling		Gene or respective protein
	Activating influence		Second messenger

9.6 Questionnaires

9.6.1 PAULINA at inclusion

Kinderklinik und Poliklinik im
Dr. von Haunerschen Kinderspital
Klinikum der Universität München
Direktor: Prof. Dr. Dietrich Reinhardt

Ludwig-Maximilians-Universität München

Datum:_____ Studiennummer:_____

Nabelschnurblutstudie PAULINA
Fragebogen für die Mutter

Wir freuen uns, dass Sie an unserer Nabelschnurblutstudie teilnehmen. Bitte kreuzen Sie die folgenden Fragen an. Ihre Antworten werden vertraulich behandelt. Wenn Sie eine Frage nicht beantworten möchten, lassen Sie sie bitte aus.

1. Sind Sie in Deutschland geboren?

Ja

Nein, ich bin in _____ geboren.

2. Welche Staatsangehörigkeit haben Sie?

_____

Wir fragen nach der Staatsangehörigkeit, damit wir einschätzen können, welche Bevölkerungsgruppe wir untersucht haben.

3. Welche Staatsangehörigkeit hat der Vater des Kindes?

_____

4. Welche Schulausbildung haben Sie abgeschlossen?

Hauptschule

Realschule

Gymnasium

Universität

Andere: bitte angeben: _____

5. Hat ein Arzt jemals eine der folgenden Erkrankungen bei Ihnen diagnostiziert?

Asthma

Heuschnupfen

Neurodermitis

Autoimmunerkrankung, wie z.B. Diabetes, rheumatoide Arthritis.

Schilddrüsenerkrankung, bitte angeben welche _____

Darmerkrankung (M. Crohn, ulzerative Kolitis)

Weitere: _____

Nein

6. Waren Sie während der Schwangerschaft an einer der folgenden Erkrankungen erkrankt?

Asthma

Heuschnupfen

Neurodermitis

Autoimmunerkrankung, wie z.B. Diabetes, rheumatoide Arthritis.

Schilddrüsenerkrankung, bitte angeben welche: _____

Darmerkrankung (M. Crohn, ulzerative Kolitis)

Weitere: _____

Nein

7. Haben Sie dafür Medikamente eingenommen?

Ja, ich habe _____/Tag eingenommen.

Nein

8. Haben Sie während der Schwangerschaft Medikamente eingenommen?

Ja, ich habe _____/Tag von der _____SSW (Schwangerschaftswoche) bis zur _____SSW eingenommen.

Nein

9. Hat ein Arzt bei dem Vater des Kindes jemals eine der folgenden Erkrankungen diagnostiziert?

Asthma

Heuschnupfen

Neurodermitis

Nein

10. Leidet er aktuell an einer der folgenden Erkrankungen?

Asthma

Heuschnupfen

Neurodermitis

Nein

11. Nimmt er dafür derzeit Medikamente ein?

Ja, er nimmt _____

Nein

12. Welche der folgenden Beschreibungen trifft für Sie am ehesten zu?

Ich habe niemals Zigaretten geraucht.

Ich habe früher Zigaretten geraucht, und vor Beginn der Schwangerschaft aufgehört.

Ich habe aufgehört zu rauchen, seit ich weiß, dass ich schwanger bin.

Ich rauche derzeit durchschnittlich _____Zigaretten/Tag.

13. Ist dies Ihre erste Schwangerschaft?

Ja

Nein, ich habe bereits _____Kinder.

14. Haben Sie jemals eine Fehlgeburt während einer vorangegangenen Schwangerschaft gehabt?

Ja, in der _____Schwangerschaftswoche.

Nein

Bei Fragen können Sie sich jederzeit gerne an uns wenden.

Studienleitung:
Prof. Dr. med. Erika von Mutius

Studienärztin:
Dr. med. Bianca Schaub
(Tel: 5160-7856)

Vielen herzlichen Dank

für Ihre Zeit den Fragebogen auszufüllen!

9.6.2 PAULINA three year follow-up

ID: _____

PAULINA

Fragebogen zum **3. Lebensjahr**

Ihres Kindes

München, 12.01.2008

Befragten-ID:

_____

Interviewer-ID:

_____

Datum des Ausfüllens

___/___/_____

Tag/ Monat/ Jahr

Beginn des Interviews (Uhrzeit):

___/___

Std / Min

Mit wem wurde das Interview durchgeführt?

Mutter..... ☐
Vater..... ☐
Andere:_____ ☐

Wie wurde der Fragebogen ausgefüllt?

Persönlich..... ☐
Telefonisch..... ☐
Selbstausfüller..... ☐

2

☞ *Vor Beginn des Interviews bitte inhaltlich wiedergeben*

- ☐ *Begrüßung*
- ☐ *Dauer des Interviews (ca. 30 min) erläutern*
- ☐ *Erläuterung des Interviewablaufs*

  Die meisten Fragen können mit Ja/ Nein beantwortet werden

  Bei einigen Fragen gibt es andere Antwortmöglichkeiten

  Bitte Fragen erst beantworten, nachdem sie vollständig vorgelesen wurden

  Bei Antworten bitte immer den gesamten Wortlaut der zutreffenden Antwortkategorie vorlesen

  Bei Verständnisproblemen bitte reagieren

- ☐ *Hinweis, dass nun das Interview mit einigen Fragen zur Gesundheit des Kindes beginnt.*

Angaben zur Gesundheit des Kindes

Wir beginnen mit Fragen zur Gesundheit Ihres Kindes.
Die Fragen zur Gesundheit Ihres Kindes beziehen sich – wo nicht anders angegeben – auf die letzten 3 Jahre (± 3 Monate), d.h. die Zeit seit Geburt.

Es folgen Fragen zu pfeifenden und keuchenden Atemgeräuschen. Mit pfeifenden Atemgeräuschen meinen wir ein pfeifendes Geräusch, das aus dem Brustkorb kommt, aber nicht geräuschvolles Atmen durch die Nase.

1. **Wie häufig hatte Ihr Kind <u>in den letzten 3 Jahren</u> pfeifende oder keuchende Atemgeräusche?**

   Nie..... ☐ ⇒ weiter mit Frage 8!
   Seltener als einmal pro Monat..... X
   Einmal pro Monat..... ☐
   Mindestens zweimal pro Monat..... ☐

2. **Wie häufig hatte Ihr Kind in den letzten 3 Jahren pfeifende oder keuchende Atemgeräusche, <u>ohne dass es erkältet war</u>?**

   Nie..... X ⇒ weiter mit Frage 8!
   Seltener als einmal pro Monat..... ☐
   Einmal pro Monat..... ☐
   Mindestens zweimal pro Monat..... ☐

3. **Hat Ihr Kind in den letzten 3 Jahren jemals durch <u>Aufregung oder körperliche Aktivität</u> pfeifende oder keuchende Atemgeräusche bekommen, ohne dass es erkältet war?**

   Ja..... ☐
   Nein..... ☐

3

4. Wodurch wurden bei Ihrem Kind die pfeifenden / keuchenden Atemgeräusche ausgelöst?

	Ja	Nein
Anstrengung	☐	☐
Erkältung	☐	☐
Kontakt mit Tieren	☐	☐
Kontakt mit Hausstaub	☐	☐
Kontakt mit Gras	☐	☐
Sonstiges: _____	☐	☐

5. Hat Ihr Kind jemals <u>in den letzten 3 Jahren</u> von einem Arzt Medikamente gegen pfeifende oder keuchende Atemgeräusche verschrieben bekommen?
(Gemeint sind damit nicht nur Medikamente zum Schlucken, sondern auch Inhalationen oder Sprays)

Ja ☐
Nein ☐ ⇨ weiter mit Frage 8!

6. Welche Medikamente waren dies?
Bitte notieren Sie jeweils möglichst genau den Markennamen.

1. _____
2. _____
3. _____

7. Erhält Ihr Kind solche Medikamente gegen pfeifende oder keuchende Atemgeräusche
nur bei besonders schweren Phasen solcher Atemgeräusche? ☐
bei (fast) jeder Phase pfeifender oder keuchender Atemgeräusche? ☐
sowohl während akuter Phasen derartiger Geräusche als auch vorbeugend? ☐

8. Wurde bei Ihrem Kind jemals von einem Arzt ein Allergietest durchgeführt?

	Ja	Nein
Ein Hauttest	☐	☐
Ein Bluttest	☐	☐
Ein anderer Test, z.B. Bioresonanz	☐	☐

☞ *Achtung*

Wenn alle drei Test-Arten mit „Nein" beantwortet wurden, weiter mit Frage 10!

9. Welche Allergie wurde dabei festgestellt?

	Ja	Nein
Gegen Pollen	☐	☐
Gegen Hausstaub(milben)	☐	☐
Gegen Tiere	☐	☐
Gegen Nahrungsmittel	☐	☐
Andere: _____	☐	☐

Es folgen Fragen zu Hauterkrankungen

10. Hatte Ihr Kind jemals <u>in den letzten 3 Jahren</u> einen <u>juckenden Hautausschlag</u> mit Kratzen und Reiben der Haut?

Ja ☐
Nein ☐ ⇨ weiter mit Frage 15!

11. War der Hautausschlag in den letzten 3 Jahren je an einer der folgenden Stellen?

	Ja	Nein
Gesicht	☐	☐
Hals	☐	☐
Ellenbeugen / Kniekehlen	☐	☐
Hand- / Fußgelenke	☐	☐

12. Wenn Sie die Zeiten, in denen Ihr Kind diesen Hautausschlag hatte, zusammen zählen: Wie lange haben Sie <u>in den letzten 3 Jahren</u> diesen Hautausschlag jeweils pro Jahr beobachtet?

Für insgesamt weniger als 6 Wochen / Jahr ☐
Für insgesamt 6 Wochen bis 2 Monate/ Jahr ☐
Für insgesamt 3-5 Monate/ Jahr ☐
Für insgesamt 6-11 Monate/ Jahr ☐
Praktisch für die gesamten 12 Monate/ Jahr ☐

13. Ist der Hautausschlag wieder völlig verschwunden, oder „kommt und geht" der Hautausschlag?

Der Hautausschlag ist vollständig verschwunden ☐
Der Hautausschlag „kommt und geht" ☐ ⇨ weiter mit Frage 15!
Der Hautausschlag ist noch da ☐ ⇨ weiter mit Frage 15!

14. Wie alt war Ihr Kind, als der Hautausschlag wieder vollständig verschwunden ist?

_____ Monate

15. Wie häufig kam es <u>in den letzten 3 Jahren</u> vor, dass ihr Kind sich kratzt?

Nie ☐ ⇨ weiter mit Frage 19!
Seltener als einmal <u>pro Monat</u> ☐
1 bis 3 mal <u>pro Monat</u> ☐
4 bis 6 mal <u>pro Woche</u> ☐
Ein- oder mehrmals täglich ☐

16. Wie häufig kam es in den letzten 3 Jahren vor, dass Ihr Kind sich wegen eines starken Juckreizes <u>blutig</u> gekratzt hat?

Nie ☐
Seltener als einmal <u>pro Monat</u> ☐
1 bis 3 mal <u>pro Monat</u> ☐
4 bis 6 mal <u>pro Woche</u> ☐
Ein- oder mehrmals täglich ☐

17. Wie häufig ist Ihr Kind in den letzten 3 Jahren <u>nachts</u> wegen Juckreiz aufgewacht?

Seltener als einmal pro Monat ☐
Einmal pro Monat ☐
Mindestens zweimal pro Monat ☐

18. Haben Sie die Haut Ihres Kindes in den letzten 3 Jahren mit einer cortisonhaltigen Creme / Salbe oder einer Tacrolimus- bzw. Pimecrolimus-haltigen Salbe (Protopic, Elidel) behandelt?

Ja .....☐
Nein.....☐

Es folgen Fragen zu anderen Erkrankungen

19. Wurde bei Ihrem Kind in den letzten 3 Jahren von einem Arzt/einer Ärztin eine spastische Bronchitis, obstruktive Bronchitis oder asthmatische Bronchitis diagnostiziert?

Nein, nie.....☐
Ja, einmal.....☐
Ja, mehrmals.....☐

20. Wurde bei Ihrem Kind in den letzten 3 Jahren von einem Arzt/einer Ärztin eine der folgenden Diagnosen gestellt?

	Ja	Nein
Asthma	☐	☐
Neurodermitis, atopische Dermatitis oder endogenes Ekzem	☐	☐

Es folgen Fragen zu Nahrungsunverträglichkeiten oder -allergien

21. Reagiert Ihr Kind auf irgendwelche Nahrungsmittel mit Hautveränderungen? Wir meinen damit eine Nesselsucht oder das Auftreten bzw. die Verschlechterung einer Neurodermitis.

Ja.....☐
Nein.....☐ ⇨ weiter mit Frage 25!

22. Auf welche Nahrungsmittel reagiert Ihr Kind mit derartigen Hautveränderungen?

	Ja	Nein
Milch und Milchprodukte	☐	☐
Hühnereier	☐	☐
Fisch	☐	☐
Weizenmehl oder andere Getreideprodukte	☐	☐
Nüsse	☐	☐
Soja	☐	☐
Zitrusfrüchte	☐	☐
Anderes Obst oder Gemüse	☐	☐
Andere Nahrungsmittel	☐	☐

Welche? _____
_____

23. Wurde bei Ihrem Kind von einem Arzt / einer Ärztin in den letzten 3 Jahren eine Nahrungsmittelallergie diagnostiziert?

Ja .....☐
Nein.....☐ ⇨ weiter mit Frage 25!

24. Wurde diese Nahrungsmittelallergie durch einen Allergietest bestätigt?

	Ja	Nein
Durch einen Hauttest, einen Bluttest oder einen oralen Provokationstest	☐	☐
Durch einen anderen Test, z.B. Bioresonanz	☐	☐

Nun haben wir die Fragen zur Gesundheit Ihres Kindes abgeschlossen.

Es geht nun um seine Ernährung, dann um die Umgebung.

25. A) Haben Sie Ihr Kind gestillt?

Ja ☐ Wie lange haben Sie Ihr Kind gestillt?.....
Nein ☐

Angaben zur Wohnungs- und Lebenssituation

26. A) Wie viele jüngere Geschwister hat Ihr Kind?

Bitte auch Stiefgeschwister mitzählen, die in Ihrer Familie leben!

Schwestern.....Stiefschwestern.....
Brüder.....Stiefbrüder.....

B) Wie viele ältere Geschwister hat Ihr Kind?
Bitte auch Stiefgeschwister mitzählen, die in Ihrer Familie leben!

Schwestern.....Stiefschwestern.....
Brüder.....Stiefbrüder.....

27. Bitte notieren Sie Name und Geburtsdatum der Geschwister Ihres Kindes.
Bitte auch Stiefgeschwister mitzählen, die in Ihrer Familie leben!

Name	Mädchen	Junge	Geburtsdatum
_____	☐	☐	___/___/_____
_____	☐	☐	___/___/_____
_____	☐	☐	___/___/_____

28. Wird Ihr Kind regelmäßig zusammen mit anderen Kindern betreut (z.B. durch eine Tagesmutter, in einer Kinderkrippe oder bei den Großeltern)? Die eigenen Geschwister sind dabei nicht gemeint.

Ja,.....☐
Mit wie vielen anderen Kindern? ____

Nein .....☐

29. Welche der folgenden Haustiere haben/hatten Sie innerhalb der Wohnung ?
(Mehrere Antworten sind möglich)

	Zur Zeit	Im 1. oder 2. Lebensjahr
Hund	☐	☐
Katze	☐	☐
Hamster	☐	☐
Meerschweinchen	☐	☐
Kaninchen	☐	☐
Vögel	☐	☐
Aquarium (Fische)	☐	☐

A) Darf oder durfte sich eine Katze im Zimmer, in dem Ihr Kind schläft aufhalten?

Ja ☐
Nein ☐

B) Darf oder durfte sich eine Katze im Bett Ihres Kindes aufhalten?

Ja ☐
Nein ☐

C) Darf oder durfte sich ein Hund im Zimmer, in dem Ihr Kind schläft aufhalten?

Ja ☐
Nein ☐

D) Darf oder durfte sich ein Hund im Bett Ihres Kindes aufhalten?

Ja ☐
Nein ☐

30. Hat/hatte Ihr Kind sonst regelmäßig (ca. 1x/Woche) Kontakt zu folgenden Tieren (z.B. in der Wohnung von Freunden/ Verwandten, Käfig/Stall außerhalb der Wohnung)?

(Mehrere Antworten sind möglich)

	Zur Zeit	Im 1. oder 2. Lebensjahr
Hund	☐	☐
Katze	☐	☐
Pferde	☐	☐

Es folgen Fragen zum Rauchverhalten

31. Haben Sie und Ihre Familie <u>in den letzten 3 Jahren</u> mit dem Rauchen in der Wohnung aufgehört bzw. das Rauchen innerhalb der Wohnräume eingeschränkt?

Ja ☐
Nein ☐
Es wurde nie geraucht ☐ ⇨ weiter mit Frage 33!

32. Wie viele Zigaretten werden durchschnittlich am Tag <u>in Ihrer Wohnung</u> (damit meinen wir auch die Küche) geraucht? Zigaretten, die auf dem Balkon oder der Terrasse geraucht werden, brauchen nicht mitgezählt zu werden.

Wie viele davon von... (keine=0)

Mutter	_____	pro Tag
Partner	_____	pro Tag
Andere Personen	_____	pro Tag
Insgesamt	_____	pro Tag

33. Sind Sie <u>in den letzten 3 Jahren</u> (d.h. seit das Kind geboren wurde) umgezogen?

Ja ☐ ⇨ **Bitte neue Adresse notieren!**
Nein ☐

Haben Sie noch weitere Kommentare zum Fragebogen oder allgemein?

_____
_____
_____
_____
_____
_____
_____

Wir danken Ihnen herzlich für das Ausfüllen des Fragebogens!

9.6.3 PAULINA six year follow-up

LMU KLINIKUM DER UNIVERSITÄT MÜNCHEN | CAMPUS INNENSTADT | KLINIK UND POLIKLINIK IM DR. VON HAUNERSCHEN KINDERSPITAL

ID:

PAULINA

Fragebogen zum **6. Lebensjahr**

Ihres Kindes

München, 03.08.2010

Datum:_____ Studiennummer:_____

Fragebogen für die Eltern

Wir freuen uns, dass Sie bereit sind weiterhin an der Paulina Studie teilzunehmen. Bitte kreuzen Sie die folgenden Fragen an. Ihre Antworten werden vertraulich behandelt. Wenn Sie eine Frage nicht beantworten möchten, lassen Sie sie bitte aus.

Wir danken Ihnen herzlich für Ihre Mitarbeit!

Wir beginnen mit Fragen zu pfeifenden und keuchenden Atemgeräuschen. Mit pfeifenden Atemgeräuschen meinen wir ein pfeifendes Geräusch, das aus dem Brustkorb kommt, aber nicht geräuschvolles Atmen durch die Nase.

1. Hat Ihr Kind jemals pfeifende bzw. keuchende Atemgeräusche gehabt?

Ja ☐
Falls Ja,
wann sind diese zum ersten Mal aufgetreten:
Nein ☐ ⇒ weiter mit Frage 12

2. Hatte Ihr Kind in den letzten 3 Jahren pfeifende bzw. keuchende Atemgeräusche?

Ja ☐
Nein ☐ ⇒ weiter mit Frage 12

3. Wie oft hatte Ihr Kind in den letzten 12 Monaten pfeifende bzw. keuchende Atemgeräusche?

Gar nicht ☐
1-3 mal ☐
4-12mal ☐
Mehr als 12 mal ☐

4. Hatte Ihr Kind in den letzten 12 Monaten jemals Atemnot, als die pfeifenden/ keuchenden Atemgeräusche auftraten?

Ja ☐
Nein ☐

5. Wie häufig ist Ihr Kind in den letzten 12 Monaten nachts wegen pfeifender oder keuchender Atemgeräusche aufgewacht?

Seltener als einmal pro Monat ☐
Einmal pro Monat ☐
Mindestens zweimal pro Monat ☐

6. Wodurch wurden bei Ihrem Kind die pfeifenden / keuchenden Atemgeräusche ausgelöst?

	Ja	Nein
Anstrengung	☐	☐
Erkältung	☐	☐
Kontakt mit Tieren	☐	☐
Kontakt mit Hausstaub	☐	☐
Kontakt mit Gras	☐	☐
Sonstiges	☐	☐

_____

7. Wie häufig hatte Ihr Kind in den letzten 12 Monaten pfeifende oder keuchende Atemgeräusche, ohne dass es erkältet war?

Nie ☐
Seltener als einmal pro Monat ☐
Einmal pro Monat ☐
Mindestens zweimal pro Monat ☐

8. Ist das Kind zwischen diesen Episoden völlig beschwerdefrei?

Ja ☐ ⇒ weiter mit Frage 12
Nein ☐

9. Hat Ihr Kind zwischen diesen Episoden folgende Beschwerden bei Anstrengung?

	Ja	Nein
Husten	☐	☐
Pfeifende Atemgeräusche	☐	☐
Atemnot	☐	☐

Sonstiges:_____

Bei Temperaturwechsel/Nebel?

	Ja	Nein
Husten	☐	☐
Pfeifende Atemgeräusche	☐	☐
Atemnot	☐	☐

Sonstiges:_____

Nachts?

	Ja	Nein
Husten	☐	☐
Pfeifende Atemgeräusche	☐	☐
Atemnot	☐	☐

Sonstiges:_____

Sonstige Beschwerden?

:_____

10. Hat Ihr Kind jemals in den letzten 3 Jahren von einem Arzt Medikamente gegen pfeifende oder keuchende Atemgeräusche, oder Giemen oder Atemnot verschrieben bekommen?

(Gemeint sind damit nicht nur Medikamente zum Schlucken, sondern auch Inhalationen oder Sprays)

Ja ☐
Nein ☐ ⇒ weiter mit Frage 12

11. Welche Medikamente waren dies?

Bitte geben Sie den Markennamen möglichst genau an! Und sofern Sie es wissen die Dosis sowie den Zeitraum, in dem das Medikament eingenommen wurde.

1. _____
2. _____
3. _____

12. Wurde bei Ihrem Kind jemals von einem Arzt ein Allergietest durchgeführt?

	Ja	Nein
Ein Hauttest	☐	☐
Ein Bluttest	☐	☐
Ein anderer Test, z.B. Bioresonanz	☐	☐

13. Welche Allergie wurde dabei festgestellt?

	Ja	Nein
Gegen Pollen	☐	☐
Gegen Hausstaub(milben)	☐	☐
Gegen Tiere	☐	☐
Gegen Nahrungsmittel	☐	☐
Andere:_____	☐	☐

14. Hat Ihr Kind jemals in den letzten 3 Jahren von einem Arzt Medikamente aus einem anderen Grund verschrieben bekommen?
(Gemeint sind damit nicht nur Medikamente zum Schlucken, sondern auch Inhalationen oder Sprays)

Ja ☐
Nein ☐ ⇒ weiter mit Frage 16

15. Welche Medikamente waren dies?
Bitte geben Sie den Markennamen möglichst genau an! Und sofern Sie es wissen die Dosis sowie den Zeitraum in dem das Medikament eingenommen wurde.

1. _____
2. _____
3. _____

Es folgen Fragen zu Beschwerden der Nase und der Augen

16. Hat Ihr Kind jemals Niesanfälle oder eine laufende, verstopfte oder juckende Nase, obwohl es nicht erkältet war?

Ja ☐
Falls Ja, wann ist dies zum ersten Mal aufgetreten:

Nein ☐ ⇒ weiter mit Frage 21

17. Hatte Ihr Kind in den letzten 3 Jahren Niesanfälle oder eine laufende, verstopfte oder juckende Nase, obwohl es nicht erkältet war?

Ja ☐
Nein ☐ ⇒ weiter mit Frage 21

18. Hatte Ihr Kind in den letzen 12 Monaten gleichzeitig mit diesen Nasenbeschwerden juckende oder tränende Augen?

Ja ☐
Nein ☐

19. Wann in den letzen 12 Monaten traten diese Nasen-Beschwerden auf?
Mehrere Antworten sind möglich.

Januar ☐	Mai ☐	September ☐
Februar ☐	Juni ☐	Oktober ☐
März ☐	Juli ☐	November ☐
April ☐	August ☐	Dezember ☐

20. Ist von einem Arzt bei Ihrem Kind schon einmal Heuschnupfen oder eine allergische Rhinitis bzw. Rhinokonjunktivitis festgestellt worden?

Ja ☐
Nein ☐

Es folgen Fragen zu Hauterkrankungen

21. Hatte Ihr Kind jemals eine Neurodermitis/atopische Dermatitis/ atopisches Ekzem

Ja ☐
Falls Ja, wann ist diese zum ersten Mal aufgetreten: _____
Nein ☐ ⇒ weiter mit Frage 31

22. Wurde bei Ihrem Kind die Diagnose einer Neurodermitis/atopischen Dermatitis/ atopisches Ekzem von einem Arzt gestellt?

Ja ☐
Nein ☐

23. Hatte Ihr Kind in den letzten 3 Jahren eine Neurodermitis/atopische Dermatitis/ atopisches Ekzem

Ja ☐
Nein ☐

24. War der Hautausschlag je an einer der folgenden Stellen?

	Ja	Nein
Gesicht	☐	☐
Hals	☐	☐
Ellenbeugen / Kniekehlen	☐	☐
Hand- / Fußgelenke	☐	☐
Brust/Rücken	☐	☐

25. Hat sich die Lokalisation des Ausschlages im Laufe der Zeit geändert?

Ja ☐ Nein ☐

Falls Ja, wo war er zu Beginn? Wo befindet er sich heute?

Zu Beginn:

	Ja	Nein
Gesicht	☐	☐
Hals	☐	☐
Ellenbeugen / Kniekehlen	☐	☐
Hand- / Fußgelenke	☐	☐
Brust/Rücken	☐	☐

Heute:

	Ja	Nein
Gesicht	☐	☐
Hals	☐	☐
Ellenbeugen / Kniekehlen	☐	☐
Hand- / Fußgelenke	☐	☐
Brust/Rücken	☐	☐

26. Wenn Sie die Zeiten, in denen Ihr Kind diesen Hautausschlag hatte, zusammenzählen: Wie lange haben Sie diesen Hautausschlag insgesamt beobachtet?

Für insgesamt weniger als 3 Monate .... ☐
Für insgesamt 3-6 Monate ..... ☐
Für insgesamt 6-12 Monate ..... ☐
Für länger als 12 Monate ..... ☐

27. Ist der Hautausschlag wieder völlig verschwunden, oder „kommt und geht" der Hautausschlag?

Der Hautausschlag ist vollständig Verschwunden ..... ☐
Der Hautausschlag „kommt und geht" ..... ☐
Der Hautausschlag ist noch da ..... ☐

28. Wie alt war Ihr Kind, als der Hautausschlag vollständig verschwunden ist?

_____ Monate

29. Wie häufig ist Ihr Kind nachts wegen Juckreiz aufgewacht?

Seltener als einmal pro Monat oder nie ..... ☐
Einmal pro Monat ..... ☐
Mindestens zweimal pro Monat ..... ☐

30. Haben Sie die Haut Ihres Kindes in den letzten 12 Monaten mit einer cortisonhaltigen Creme / Salbe oder einer Tacrolimus- bzw. Pimecrolimus-haltigen Salbe (Protopic, Elidel) behandelt?

Ja ☐
Nein ☐

Es folgen Fragen zu Nahrungsunverträglichkeiten oder –allergien

31. Hat Ihr Kind eine Nahrungsmittelallergie?

Ja ☐
Nein ☐ ⇒ weiter mit Frage 34

32. Wie äußert sich diese Nahrungsmittelallergie?

Ausschlag/rote Flecken um den Mund herum ..... ☐
Ausschlag/rote Flecken an anderen Körperstellen..... ☐
Schwellung der Lippen ..... ☐
Juckreiz ..... ☐
Durchfall ..... ☐
Erbrechen ..... ☐
Verschlechterung der Neurodermitis ..... ☐
Pfeifende Atemgeräusche ..... ☐
Atemnot ..... ☐
Kreislaufreaktion/Blutdruckabfall..... ☐
Sonstiges:

33. Auf welche Nahrungsmittel reagiert Ihr Kind?

	Ja	Nein
Milch und Milchprodukte	☐	☐
Hühnereier	☐	☐
Fisch	☐	☐
Weizenmehl oder andere Getreideprodukte	☐	☐
Nüsse	☐	☐
Soja	☐	☐
Zitrusfrüchte	☐	☐
Anderes Obst oder Gemüse	☐	☐
Andere Nahrungsmittel	☐	☐

Welche? _____

Es folgen Fragen zu anderen Erkrankungen

34. Wurde bei Ihrem Kind jemals von einem Arzt/einer Ärztin eine spastische Bronchitis, obstruktive Bronchitis oder asthmatische Bronchitis diagnostiziert?

Nein, nie..... ☐
Ja, einmal ..... ☐
Ja, mehrmals..... ☐

35. Wurde bei Ihrem Kind in den letzten 12 Monaten von einem Arzt/einer Ärztin eine der folgenden Diagnosen gestellt?

	Ja	Nein
Asthma	☐	☐
Neurodermitis, atopische Dermatitis oder endogenes Ekzem	☐	☐
Allergische Rhinitis/Heuschnupfen	☐	☐

36. Hatte Ihr Kind bisher eine der folgenden Erkrankungen nach dem dritten Lebensjahr?

	Ja	Nein
Mittelohrentzündung	☐	☐
Pseudokrupp	☐	☐
Lungenentzündung	☐	☐
Bronchitis	☐	☐
Bronchiolitis	☐	☐
Keuchhusten	☐	☐
Andere Infektionen	☐	☐

Welche? _____

Waren stationäre Aufenthalte im Krankenhaus notwendig .... ☐ ..... ☐

Warum? _____

_____

Angaben zur Wohnungs- und Lebenssituation

37. A) Wie viele jüngere Geschwister hat Ihr Kind?

Bitte auch Stiefgeschwister mitzählen, die in Ihrer Familie leben!

Schwestern..... Brüder.....

B) Wie viele ältere Geschwister hat Ihr Kind?
Bitte auch Stiefgeschwister mitzählen, die in Ihrer Familie leben!

Schwestern.....Brüder.....

38. Bitte notieren Sie Name und Geburtsdatum der Geschwister Ihres Kindes. Bitte auch Stiefgeschwister mitzählen, die in Ihrer Familie leben!

Name	Mädchen	Junge	Geburtsdatum
_____	☐	☐	___/___/_____
_____	☐	☐	___/___/_____
_____	☐	☐	___/___/_____

39. Wird Ihr Kind regelmäßig zusammen mit anderen Kindern durch eine Tagesmutter oder bei den Großeltern betreut? Die eigenen Geschwister sind dabei nicht gemeint.

Ja, ... ☐
Mit wie vielen anderen Kindern: _____
Nein ..... ☐

40. Wird Ihr Kind regelmäßig zusammen mit anderen Kindern in einer Kinderkrippe oder im Kindergarten betreut? Die eigenen Geschwister sind dabei nicht gemeint.

Ja, ... ☐
Mit wie vielen anderen Kindern? _____
Nein ☐

41. Welche der folgenden Haustiere haben/hatten Sie innerhalb der Wohnung?
Mehrere Antworten sind möglich.

Keine ☐
Hund ☐
Katze ☐
Hamster ☐
Meerschweinchen ☐
Kaninchen ☐
Vögel ☐
Aquarium (Fische) ☐
Sonstige ☐

Welche: _____

A) Darf oder durfte sich eine Katze im Zimmer, in dem Ihr Kind schläft aufhalten?
Ja ☐
Nein ☐

B) Darf oder durfte sich eine Katze im Bett Ihres Kindes aufhalten?
Ja ☐
Nein ☐

C) Darf oder durfte sich ein Hund im Zimmer, in dem Ihr Kind schläft aufhalten?
Ja ☐
Nein ☐

D) Darf oder durfte sich ein Hund im Bett Ihres Kindes aufhalten?
Ja ☐
Nein ☐

42. Hat Ihr Kind sonst regelmäßig (ca. 1x/Woche) Kontakt zu Tieren (z.B. in der Wohnung von Freunden/ Verwandten)? ***Mehrere Antworten sind möglich.***

	Ja	Nein
Hund	☐	☐
Katze	☐	☐
Sonstige	☐	☐

Welche:_____

43. Gibt es in Ihrer Wohnung Feuchtigkeitsflecken bzw. Schimmelbefall an Wänden oder Decken?

Feuchtigkeitsflecken in Bad oder Küche sind dabei nicht gemeint, sondern nur in Räumen wie Wohnzimmer, Schlafzimmer oder Kinderzimmer.

	Ja	Nein
Feuchtigkeitsflecken, aber ohne Schimmelbefall	☐	☐
Feuchtigkeitsflecken mit Schimmelbefall	☐	☐

Es folgen Fragen zum Rauchverhalten

44. Rauchen Sie oder Ihre Familie in Ihrer Wohnung/Haus?

Ja ☐
Nein ☐

45. Haben Sie und Ihre Familie in den letzten 12 Monaten mit dem Rauchen in der Wohnung aufgehört bzw. das Rauchen innerhalb der Wohnräume eingeschränkt?

Ja ☐
Nein ☐
Es wurde nie geraucht ☐

46. Wie viele Zigaretten werden durchschnittlich am Tag in Ihrer Wohnung (damit meinen wir auch die Küche) geraucht? Zigaretten, die auf dem Balkon oder der Terrasse geraucht werden, brauchen nicht mitgezählt zu werden. Wie viele davon von… (keine=0)

Mutter	_____	pro Tag
Partner	_____	pro Tag
Andere Personen	_____	pro Tag
Insgesamt	_____	pro Tag

Haben Sie noch weitere Kommentare zum Fragebogen oder allgemein?

_____

_____

_____

_____

_____

_____

Wir danken Ihnen herzlich für das Ausfüllen des Fragebogens!

Bei Fragen können Sie sich jederzeit gerne an uns wenden.

Studienleitung:
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9.6.4 PAULCHEN at inclusion

Kinderklinik und Poliklinik im
Dr. von Haunerschen Kinderspital
Klinikum der Universität München
Direktor: Prof. Dr. Dietrich Reinhardt

LMU Ludwig-Maximilians-Universität München

Datum:_____ Studiennummer:_____

Nabelschnurblutstudie PAULCHEN
Fragebogen für die Mutter

Wir freuen uns, dass Sie an unserer Nabelschnurblutstudie teilnehmen.
Bitte kreuzen Sie die folgenden Fragen an. Ihre Antworten werden vertraulich behandelt. Das Ausfüllen des Fragebogens dürfte nicht mehr als 15 Minuten dauern. Die meisten Fragen können mit Ja/Nein beantwortet werden. Bei einigen Fragen gibt es Auswahlmöglichkeiten. Wenn Sie eine Frage nicht beantworten möchten, lassen Sie sie bitte aus. Wenn Ihnen etwas unklar ist, bitte auf dem Fragebogen notieren.

Die ersten Angaben sind allgemein zu Ihrer Person:

1. Wann wurden Sie geboren?

___/_____/___
Tag/ Monat/ Jahr

2. Wie groß sind Sie?

___ cm

3. Welches Körpergewicht hatten Sie vor Beginn der Schwangerschaft?

___ kg

4. Sind Sie in Deutschland geboren?

Ja

Nein, ich bin in _____ geboren.

5. Welche Staatsangehörigkeit haben Sie?

_____

Wir fragen nach der Staatsangehörigkeit, damit wir einschätzen können, welche Bevölkerungsgruppe wir untersucht haben.

6. Welche Staatsangehörigkeit hat der Vater des Kindes?

_____

7. Wie ist Ihr gegenwärtiger Familienstand? Sind Sie:

verheiratet
in einer festen Beziehung lebend, aber nicht verheiratet
geschieden oder getrennt lebend
alleinlebend
sonstiges (z.B. verwitwet)

8. Welche Schulausbildung haben Sie abgeschlossen?

Hauptschule
Realschule
Gymnasium
Universität/ Fachhochschule
Kein Schulabschluss
Andere: bitte angeben: _____

9. Welchen Beruf haben Sie vorwiegend in den letzten 5 Jahren ausgeübt?

_____

_____

Die nächsten Fragen beziehen sich auf Gesundheitsbeschwerden:

10. Hat ein Arzt jemals eine der folgenden Erkrankungen bei Ihnen diagnostiziert?

Asthma
Heuschnupfen
Neurodermitis (atopisches Ekzem, atopische Dermatitis)
Autoimmunerkrankung, wie z.B. Diabetes mellitus, rheumatoide Arthritis.
Schilddrüsenerkrankung, bitte angeben welche _____
Darmerkrankung (Morbus Crohn, ulzerative Kolitis)
Weitere: _____

Nein

11. Waren Sie während der Schwangerschaft an einer der folgenden Erkrankungen erkrankt?

Asthma
Heuschnupfen
Neurodermitis (atopisches Ekzem, atopische Dermatitis)
Autoimmunerkrankung, wie z.B. Diabetes mellitus, rheumatoide Arthritis.
Schilddrüsenerkrankung, bitte angeben welche: _____
Darmerkrankung (M. Crohn, ulzerative Kolitis)
Weitere: _____

Nein

12. Haben Sie dafür Medikamente eingenommen?

Ja, ich habe folgendes Medikament eingenommen: _____
Dosis: _____

Nein

13. Haben Sie während der Schwangerschaft Medikamente eingenommen?

Ja, ich habe folgende Medikamente eingenommen: _____
Dosis: _____
von der _____SSW (Schwangerschaftswoche) bis zur _____SSW.

Nein

14. Hat ein Arzt bei dem Vater des Kindes jemals eine der folgenden Erkrankungen diagnostiziert?

	Ja	Nein
Asthma		
Heuschnupfen		
Neurodermitis (atopisches Ekzem, atopische Dermatitis)		

15. Welche der folgenden Beschreibungen trifft für Sie am ehesten zu?

Ich habe niemals Zigaretten geraucht.
Ich habe früher Zigaretten geraucht, und vor Beginn der Schwangerschaft aufgehört.
Ich habe aufgehört zu rauchen, seit ich weiß, dass ich schwanger bin.
Ich rauche derzeit durchschnittlich _____Zigaretten/Tag.

16. Ist dies Ihre erste Schwangerschaft?

Ja
Nein, ich habe bereits _____Kinder.

17. Haben Sie jemals eine Fehlgeburt während einer vorangegangenen Schwangerschaft gehabt?

Ja, in der _____Schwangerschaftswoche.
Nein

18. Falls Sie weitere Kinder haben:
Leidet eines oder mehrere Kinder unter einer der folgenden Erkrankungen, die von einem Arzt diagnostiziert wurden:

	Ja	Nein
Asthma		
Heuschnupfen		
Neurodermitis (atopisches Ekzem, atopische Dermatitis)		

Die folgenden Fragen beziehen sich auf ihr Lebensumfeld:

19. Leben Sie auf einem Bauernhof, auf dem Vieh gehalten wird ?

Ja
Nein (bitte weiter mit Frage 26)

20. Bewirtschaftet Ihre Familie den Hof?

Ja
Nein (bitte weiter mit Frage 26)

21. Sind Sie selbst aktiv an der Bewirtschaftung des Hofes beteiligt?

Ja
Nein (bitte weiter mit Frage 26)

22. Seit wann und wie viele Jahre arbeiten Sie auf dem Hof?

Seit dem Jahre _____für _____ Jahre

23. Welche Nutztiere werden gehalten und in welcher Zahl?

Milchkühe	_____Anzahl
Schweine	_____Anzahl
Geflügel (Hühner, Puten, Enten, Gänse etc.)	_____Anzahl
Pferde (Ponys, Esel etc.)	_____Anzahl
Schafe/Ziegen	_____Anzahl
Hasen/Kaninchen	_____Anzahl

24. Welches Futter erhalten die Tiere?

Heu	Ja	Nein
Grassilage	Ja	Nein
Maissilage	Ja	Nein
Andere Silage	Ja	Nein
Grascops	Ja	Nein
Anderes Futter in pelletierter Form	Ja	Nein
Kraftfutter bzw. Milchleistungsfutter	Ja	Nein
Sonstiges:	_____	

25. Bei welcher der folgenden Tätigkeiten sind Sie normalerweise beteiligt oder bei der Durchführung anwesend?

Melken	Ja	Nein
Entmisten	Ja	Nein
Einstreuen	Ja	Nein
Waschen der Melkutensilien	Ja	Nein
Kälber tränken	Ja	Nein
Reinigen der Tiere	Ja	Nein
Umgang mit Heu	Ja	Nein
Umgang mit Silage	Ja	Nein
Umgang mit Kompost	Ja	Nein
Vieh überwachen, Medikamente verabreichen	Ja	Nein
Kehren des Bodens /	Ja	Nein
Reinigung von Ställen oder Scheunen		
Bewegen der Tiere innerhalb des Stalls	Ja	Nein
Tätigkeiten mit engem Kontakt zum Vieh (z.B. Zähne-Schneiden, Anbringen der Ohrmarken, Kastrierung)	Ja	Nein
Dreschen oder Getreidemahlen	Ja	Nein
Eier im Geflügelstall einsammeln	Ja	Nein
Reinigung des Hühnerstalles	Ja	Nein
Sonstiges:	_____	

26. Wie häufig im Durchschnitt haben Sie sich während dieser Schwangerschaft auf einem Bauernhof im Stall aufgehalten, dort ausgeholfen oder gearbeitet ?
Gemeint sind Ställe von Großvieh, d.h. Kühe, Schweine, Pferde, Schafe, Ziegen. Es können auch halbe Stunden (0,5 Std.) angegeben werden.

Im 1. bis 3. Schwangerschaftsmonat,
O gar nicht
O seltener als einmal pro Woche:
Durchschnittlich _____ Stunden pro Monat
O mindestens einmal pro Woche:
Durchschnittlich _____ Tage pro Woche
An diesen Tagen durchschnittlich _____ Stunden pro Tag

Im 4. bis 6. Schwangerschaftsmonat,
O gar nicht
O seltener als einmal pro Woche:
Durchschnittlich _____ Stunden pro Monat
O mindestens einmal pro Woche:
Durchschnittlich _____ Tage pro Woche
An diesen Tagen durchschnittlich _____ Stunden pro Tag

Im 7. bis 9. Schwangerschaftsmonat,
O gar nicht
O seltener als einmal pro Woche:
Durchschnittlich _____ Stunden pro Monat
O mindestens einmal pro Woche:
Durchschnittlich _____ Tage pro Woche
An diesen Tagen durchschnittlich _____ Stunden pro Tag

27. Wie häufig im Durchschnitt haben Sie sich während dieser Schwangerschaft auf einem Bauernhof in der Scheune aufgehalten, dort ausgeholfen oder gearbeitet?

Im 1. bis 3. Schwangerschaftsmonat,
O gar nicht
O seltener als einmal pro Woche:
Durchschnittlich _____ Stunden pro Monat
O mindestens einmal pro Woche:
Durchschnittlich _____ Tage pro Woche
An diesen Tagen durchschnittlich _____ Stunden pro Tag

Im 4. bis 6. Schwangerschaftsmonat,
O gar nicht
O seltener als einmal pro Woche:
Durchschnittlich _____ Stunden pro Monat
O mindestens einmal pro Woche:
Durchschnittlich _____ Tage pro Woche
An diesen Tagen durchschnittlich _____ Stunden pro Tag

Im 7. bis 9. Schwangerschaftsmonat,
O gar nicht
O seltener als einmal pro Woche:
Durchschnittlich _____ Stunden pro Monat
O mindestens einmal pro Woche:
Durchschnittlich _____ Tage pro Woche
An diesen Tagen durchschnittlich _____ Stunden pro Tag

28. Wie oft hatten Sie während dieser Schwangerschaft Kontakt zu den folgenden Nutztieren?

Im 1. bis 3. Schwangerschaftsmonat:

	nie oder ≤ 1/Monat	mehrmals pro Monat	mehrmals pro Woche	täglich
Pferde (Ponys, Esel etc.)				
Kühe				
Schweine				
Schafe / Ziegen				
Hasen / Kaninchen				
Geflügel (Hühner, Puten, etc.)				

Im 4. bis 6. Schwangerschaftsmonat:

	nie oder ≤ 1/Monat	mehrmals pro Monat	mehrmals pro Woche	täglich
Pferde (Ponys, Esel etc.)				
Kühe				
Schweine				
Schafe / Ziegen				
Hasen / Kaninchen				
Geflügel (Hühner, Puten, etc.)				

Im 7. bis 9. Schwangerschaftsmonat:

	Nie oder ≤ 1/Monat	mehrmals pro Monat	mehrmals pro Woche	täglich
Pferde (Ponys, Esel etc.)				
Kühe				
Schweine				
Schafe / Ziegen				
Hasen / Kaninchen				
Geflügel (Hühner, Puten, etc.)				

Nun folgen Fragen zum Lebensumfeld während Ihrer Kindheit:

29. Lebten Sie als Kind auf einem Bauernhof

Ja
Nein (dann weiter mit Frage 32)

30. Wurde auf diesem Hof Vieh gehalten?

Ja
Nein (dann weiter mit Frage 32)

31. Welche Tierarten wurden gehalten?

	Als hauptsächliche Tierart	**Zusätzlich**
Kühe, Rinder, Jungvieh		
Schweine		
Andere Tiere (z.B. Schafe, Ziegen, Geflügel, Hasen)		

32. Haben Sie als Kind beim Heuen geholfen?

	Ja	**Nein**
Bis zum 10. Lebensjahr		
Zwischen 10. und 18. Lebensjahr		
Nach dem 18. Lebensjahr		

33. Wie viele Personen leben zur Zeit ständig in Ihrem Haushalt, Sie selbst mit eingerechnet?

____Personen

34. Wie viele Personen sind davon unter 18 Jahre?

___ Kinder und Jugendliche

35. Haben Sie Haustiere?

Ja
Nein (dann weiter mit Frage 38)

36. Wie viele Tiere der folgenden Tierarten haben Sie?

Katzen _____ Anzahl
Hunde _____ Anzahl
Vögel _____ Anzahl

Sonstige (bitte angeben): _____ _____ Anzahl

37. Halten Sich die Tiere innerhalb der Schlafräume auf?

Ja
Nein

Es folgen nun noch einige Fragen zur Ernährung:

38. Haben Sie während der Schwangerschaft Frischmilch direkt vom Bauernhof getrunken?

Ja
Nein

39. Kochen Sie diese Milch normalerweise vor dem Trinken ab?

Ja, aber nur während der Sommermonate
Ja, immer
Nein

40. Wie viele Gläser Frischmilch vom Bauernhof haben Sie durchschnittlich pro Tag während dieser Schwangerschaft getrunken?
Ein Glas entspricht etwa 0,2 Liter.

Im 1. bis 3. Schwangerschaftsmonat
Wie viele Gläser pro Tag? _____

Im 4. bis 6. Schwangerschaftsmonat
Wie viele Gläser pro Tag? _____

Im 7. bis 9. Schwangerschaftsmonat
Wie viele Gläser pro Tag? _____

Vielen herzlichen Dank

für Ihre Zeit den Fragebogen auszufüllen!

Bei Fragen können Sie sich jederzeit gerne an uns wenden.

Studienleitung:
Prof. Dr. med. Erika von Mutius

Studienärztin:
Dr. med. Bianca Schaub

Dr. von Haunersches Kinderspital
Lindwurmstr. 4
80337 München
(Tel: 089/5160-7856 oder -7783)

9.6.5 PAULCHEN three-year follow-up

ID: _____

PAULCHEN

Fragebogen zum **3. Lebensjahr**

Ihres Kindes

München, 01.09.2008

Von wem wurde der Fragebogen ausgefüllt?
Mutter ☐, Vater ☐, Andere: _____ ☐

Datum des Ausfüllens: ____/____/_____(Tag/ Monat/ Jahr)

Allgemeine Angaben zu Ihrem Kind:

Wann wurde das Kind geboren? _____ / _____ / _____ (Tag / Monat / Jahr)

Wie heißt das Kind? _____
Wie schwer ist Ihr Kind jetzt? _____ kg
Wie groß ist Ihr Kind jetzt? _____ cm

Angaben zur Gesundheit des Kindes

Wir beginnen mit Fragen zur Gesundheit Ihres Kindes.

Die Fragen zur Gesundheit Ihres Kindes beziehen sich – wo nicht anders angegeben – auf die letzten 3 Jahre (± 3 Monate), d.h. die Zeit seit Geburt.

Es folgen Fragen zu pfeifenden und keuchenden Atemgeräuschen. Mit pfeifenden Atemgeräuschen meinen wir ein pfeifendes Geräusch, das aus dem Brustkorb kommt, aber nicht geräuschvolles Atmen durch die Nase.

1. Wie häufig hatte Ihr Kind in den letzten 3 Jahren pfeifende oder keuchende Atemgeräusche?

Nie ..... ☐ ⇒ weiter mit Frage 81
Seltener als einmal pro Monat..... ☐
Einmal pro Monat ..... ☐
Mindestens zweimal pro Monat..... ☐

2. Wie häufig hatte Ihr Kind in den letzten 3 Jahren pfeifende oder keuchende Atemgeräusche, ohne dass es erkältet war?

Nie ..... ☐ ⇒ weiter mit Frage 81
Seltener als einmal pro Monat..... ☐
Einmal pro Monat ..... ☐
Mindestens zweimal pro Monat..... ☐

3. Hat Ihr Kind in den letzten 3 Jahren jemals durch Aufregung oder körperliche Aktivität pfeifende oder keuchende Atemgeräusche bekommen, ohne dass es erkältet war?

Ja ☐ Nein ☐

4. Wodurch wurden bei Ihrem Kind die pfeifenden / keuchenden Atemgeräusche ausgelöst?

	Ja	Nein
Anstrengung	☐	☐
Erkältung	☐	☐
Kontakt mit Tieren	☐	☐
Kontakt mit Hausstaub	☐	☐
Kontakt mit Gras	☐	☐
Sonstiges:		
_____	☐	☐

5. Hat Ihr Kind jemals in den letzten 3 Jahren von einem Arzt Medikamente gegen pfeifende oder keuchende Atemgeräusche verschrieben bekommen? ***(Gemeint sind damit nicht nur Medikamente zum Schlucken, sondern auch Inhalationen oder Sprays)***

Ja ☐

Nein ☐ ⇒ weiter mit Frage 8!

6. Welche Medikamente waren dies?

Bitte notieren Sie jeweils möglichst genau den Markennamen.

1. _____
2. _____
3. _____

7. Erhält Ihr Kind solche Medikamente gegen pfeifende oder keuchende Atemgeräusche

nur bei besonders schweren Phasen solcher Atemgeräusche? ☐

bei (fast) jeder Phase pfeifender oder keuchender Atemgeräusche? ☐

sowohl während akuter Phasen derartiger Geräusche als auch vorbeugend? ☐

8. Wurde bei Ihrem Kind jemals von einem Arzt ein Allergietest durchgeführt?

	Ja	Nein
Ein Hauttest	☐	☐
Ein Bluttest	☐	☐
Ein anderer Test, z.B. Bioresonanz	☐	☐

☞ ***Achtung: wenn alle drei Test-Arten mit „Nein" beantwortet wurden, weiter mit Frage 10!***

9. Welche Allergie wurde dabei festgestellt?

	Ja	Nein
Gegen Pollen	☐	☐
Gegen Hausstaub(milben)	☐	☐
Gegen Tiere	☐	☐
Gegen Nahrungsmittel	☐	☐
Andere:		
_____	☐	☐

Es folgen Fragen zu Hauterkrankungen

10. Hatte Ihr Kind jemals in den letzten 3 Jahren einen juckenden Hautausschlag mit Kratzen und Reiben der Haut?

Ja ☐ Nein ☐ ⇒ weiter mit Frage 15!

11. War der Hautausschlag in den letzten 3 Jahren je an einer der folgenden Stellen?

	Ja	Nein
Gesicht	☐	☐
Hals	☐	☐
Ellenbeugen / Kniekehlen	☐	☐
Hand- / Fußgelenke	☐	☐

12. Wenn Sie die Zeiten, in denen Ihr Kind diesen Hautausschlag hatte, zusammen zählen: Wie lange haben Sie in den letzten 3 Jahren diesen Hautausschlag jeweils pro Jahr beobachtet?

Für insgesamt weniger als 6 Wochen / Jahr ☐

Für insgesamt 6 Wochen bis 2 Monate/ Jahr ☐

Für insgesamt 3-5 Monate/ Jahr ☐

Für insgesamt 6-11 Monate/ Jahr ☐

Praktisch für die gesamten 12 Monate/ Jahr ☐

13. Ist der Hautausschlag wieder völlig verschwunden, oder „kommt und geht" der Hautausschlag?

Der Hautausschlag ist vollständig verschwunden ☐

Der Hautausschlag „kommt und geht" ☐ ⇒ weiter mit Frage 15!

Der Hautausschlag ist noch da ☐ ⇒ weiter mit Frage 15!

14. Wie alt war Ihr Kind, als der Hautausschlag wieder vollständig verschwunden ist? _____ Monate

15. Wie häufig kam es in den letzten 3 Jahren vor, dass ihr Kind sich kratzt?

Nie ☐ ⇒ weiter mit Frage 19!

Seltener als einmal pro Monat ☐

1 bis 3 mal pro Monat ☐

4 bis 6 mal pro Woche ☐

Ein- oder mehrmals täglich ☐

16. Wie häufig kam es in den letzten 3 Jahren vor, dass Ihr Kind sich wegen eines starken Juckreizes blutig gekratzt hat?

Nie ☐

Seltener als einmal pro Monat ☐

1 bis 3 mal pro Monat ☐

4 bis 6 mal pro Woche ☐

Ein- oder mehrmals täglich ☐

17. Wie häufig ist Ihr Kind in den letzten 3 Jahren nachts wegen Juckreiz aufgewacht?

Seltener als einmal pro Monat ☐

Einmal pro Monat ☐

Mindestens zweimal pro Monat ☐

18. Haben Sie die Haut Ihres Kindes in den letzten 3 Jahren mit einer cortisonhaltigen Creme / Salbe oder einer Tacrolimus- bzw. Pimecrolimus-haltigen Salbe (Protopic, Elidel) behandelt?

Ja ☐ Nein ☐

Es folgen Fragen zu Nahrungsunverträglichkeiten oder -allergien

19. Reagiert Ihr Kind auf irgendwelche Nahrungsmittel mit Hautveränderungen? Wir meinen damit eine Nesselsucht oder das Auftreten bzw. die Verschlechterung einer Neurodermitis.

Ja ☐ Nein ☐ ⇒ weiter mit Frage 23!

20. Auf welche Nahrungsmittel reagiert Ihr Kind mit derartigen Hautveränderungen?

	Ja	Nein
Milch und Milchprodukte	☐	☐
Hühnereier	☐	☐
Fisch	☐	☐
Weizenmehl oder andere Getreideprodukte	☐	☐
Nüsse	☐	☐
Soja	☐	☐
Zitrusfrüchte	☐	☐
Anderes Obst oder Gemüse	☐	☐
Andere Nahrungsmittel	☐	☐

Welche? _____

21. Wurde bei Ihrem Kind von einem Arzt / einer Ärztin in den letzten 3 Jahren eine Nahrungsmittelallergie diagnostiziert?

Ja ☐ Nein ☐ ⇨ weiter mit Frage 23!

22. Wurde diese Nahrungsmittelallergie durch einen Allergietest bestätigt?

	Ja	Nein
Durch einen Hauttest, einen Bluttest oder einen oralen Provokationstest	☐	☐
Durch einen anderen Test, z.B. Bioresonanz	☐	☐

23. Haben Sie Ihr Kind gestillt?

Ja ☐ Wie lange haben Sie Ihr Kind gestillt? _____

Nein ☐

24 . Hat Ihr Kind in den letzten 3 Jahren Frischmilch direkt vom Bauernhof getrunken?

Ja ☐ Nein ☐ ⇨ weiter mit Frage 27!

25. Kochen Sie diese Milch normalerweise vor dem Trinken ab?

Ja, aber nur während der Sommermonate ☐

Ja, immer ☐

Nein ☐

26. Wie viele Gläser Frischmilch vom Bauernhof hat Ihr Kind in den letzten 3 Jahren durchschnittlich pro Tag getrunken? (Ein Glas entspricht etwa 0,2 Liter)

Im 1. bis 12. Lebensmonat
Wie viele Gläser pro Tag? _____

Im 12. bis 24. Lebensmonat
Wie viele Gläser pro Tag? _____

Im 24. bis 36. Lebensmonat
Wie viele Gläser pro Tag? _____

7

Es folgen Fragen zu anderen Erkrankungen

27. Wurde bei Ihrem Kind in den letzten 3 Jahren von einem Arzt/einer Ärztin eine spastische Bronchitis, obstruktive Bronchitis oder asthmatische Bronchitis diagnostiziert?

Nein, nie ☐

Ja, einmal ☐

Ja, mehrmals ☐

28. Wurde bei Ihrem Kind in den letzten 3 Jahren von einem Arzt/einer Ärztin eine der folgenden Diagnosen gestellt?

	Ja	Nein
Asthma	☐	☐
Neurodermitis, atopische Dermatitis oder endogenes Ekzem	☐	☐

Angaben zur Wohnungs- und Lebenssituation

29. Hat Ihre Familie in den letzten 3 Jahren auf einem Bauernhof gelebt, auf dem Vieh gehalten wird? Ja ☐ Nein ☐

30. Bewirtschaftete Ihre Familie den Hof in den letzten 3 Jahren?

Ja ☐ Nein ☐

31. Welche Nutztiere werden gehalten und in welcher Zahl?

Milchkühe	_____Anzahl
Schweine	_____Anzahl
Geflügel (Hühner, Puten, Enten, Gänse etc.)	_____Anzahl
Pferde (Ponys, Esel etc.)	_____Anzahl
Schafe/Ziegen	_____Anzahl
Hasen/Kaninchen	_____Anzahl

8

32. Welches Futter erhalten die Tiere?

	Ja	nein
Heu	☐	☐
Grassilage	☐	☐
Maissilage	☐	☐
Andere Silage	☐	☐
Grascops	☐	☐
Anderes Futter in pelletierter Form	☐	☐
Kraftfutter bzw. Milchleistungsfutter	☐	☐

Sonstiges: _____

33. Wie häufig wurde Ihr Kind im ersten Lebensjahr mit in den Stall genommen? (*Gemeint sind Ställe von Großvieh, d.h. Kühe, Schweine, Pferde, Schafe, Ziegen).*

gar nicht ☐

seltener als einmal pro Woche: ☐

Durchschnittlich _____ Stunden pro Monat

mindestens einmal pro Woche: ☐

Durchschnittlich _____ Tage pro Woche

An diesen Tagen durchschnittlich _____ Stunden pro Tag

34. Wie häufig wurde Ihr Kind im zweiten Lebensjahr mit in den Stall genommen?

gar nicht ☐

seltener als einmal pro Woche: ☐

Durchschnittlich _____ Stunden pro Monat

mindestens einmal pro Woche: ☐

Durchschnittlich _____ Tage pro Woche

An diesen Tagen durchschnittlich _____ Stunden pro Tag

35. Wie häufig wurde Ihr Kind im dritten Lebensjahr mit in den Stall genommen?

gar nicht ☐

seltener als einmal pro Woche: ☐

Durchschnittlich _____ Stunden pro Monat

mindestens einmal pro Woche: ☐

Durchschnittlich _____ Tage pro Woche

An diesen Tagen durchschnittlich _____ Stunden pro Tag

36. Wie häufig wurde Ihr Kind im ersten Lebensjahr mit in die Scheune genommen?

gar nicht ☐

seltener als einmal pro Woche: ☐

Durchschnittlich _____ Stunden pro Monat

mindestens einmal pro Woche: ☐

Durchschnittlich _____ Tage pro Woche

An diesen Tagen durchschnittlich _____ Stunden pro Tag

9

37. Wie häufig wurde Ihr Kind im zweiten Lebensjahr mit in die Scheune genommen?

gar nicht ☐

seltener als einmal pro Woche: ☐

Durchschnittlich _____ Stunden pro Monat

mindestens einmal pro Woche: ☐

Durchschnittlich _____ Tage pro Woche

An diesen Tagen durchschnittlich _____ Stunden pro Tag

38. Wie häufig wurde Ihr Kind im dritten Lebensjahr mit in die Scheune genommen?

gar nicht ☐

seltener als einmal pro Woche: ☐

Durchschnittlich _____ Stunden pro Monat

mindestens einmal pro Woche: ☐

Durchschnittlich _____ Tage pro Woche

An diesen Tagen durchschnittlich _____ Stunden pro Tag

39. Hatte Ihr Kind im ersten Lebensjahr regelmäßig direkten Kontakt zu Heu, beispielsweise in dem es (z.B. auf einer Decke) aufs Heu gelegt wurde / wird? (*Regelmäßig bedeutet mindestens einmal pro Woche).*

ja ☐ nein ☐

40. In welchem Alter wurde Ihr Kind erstmals mit zur Heuernte genommen?

bereits im ersten Lebensjahr ☐

erst nach dem ersten Geburtstag, nämlich mit _____ Jahren ☐

noch nie ☐

10

41. Wie häufig im Durchschnitt halte Ihr Kind in letzten 3 Jahren Kontakt zu den folgenden Nutztieren?

Im 0. bis 12. Lebensmonat,

	nie oder ≤ 1/Monat	mehrmals pro Monat	mehrmals pro Woche	täglich
Pferde (Ponys, Esel etc.)	☐	☐	☐	☐
Kühe	☐	☐	☐	☐
Schweine	☐	☐	☐	☐
Schafe / Ziegen	☐	☐	☐	☐
Hasen / Kaninchen	☐	☐	☐	☐
Geflügel (Hühner, Puten, etc.)	☐	☐	☐	☐

Im 12. bis 24. Lebensmonat,

	nie oder ≤ 1/Monat	mehrmals pro Monat	mehrmals pro Woche	täglich
Pferde (Ponys, Esel etc.)	☐	☐	☐	☐
Kühe	☐	☐	☐	☐
Schweine	☐	☐	☐	☐
Schafe / Ziegen	☐	☐	☐	☐
Hasen / Kaninchen	☐	☐	☐	☐
Geflügel (Hühner, Puten, etc.)	☐	☐	☐	☐

Im 24. bis 36. Lebensmonat,

	Nie oder ≤ 1/Monat	mehrmals pro Monat	mehrmals pro Woche	täglich
Pferde (Ponys, Esel etc.)	☐	☐	☐	☐
Kühe	☐	☐	☐	☐
Schweine	☐	☐	☐	☐
Schafe / Ziegen	☐	☐	☐	☐
Hasen / Kaninchen	☐	☐	☐	☐
Geflügel (Hühner, Puten, etc.)	☐	☐	☐	☐

11

42. Welche der folgenden Haustiere haben/hatten Sie innerhalb der Wohnung ?
(Mehrere Antworten sind möglich)

	Zur Zeit	Im 1. oder 2. Lebensjahr
Hund	☐	☐
Katze	☐	☐
Hamster	☐	☐
Meerschweinchen	☐	☐
Kaninchen	☐	☐
Vögel	☐	☐
Aquarium (Fische)	☐	☐

A) Darf oder durfte sich eine Katze im Zimmer, in dem Ihr Kind schläft aufhalten? Ja ☐ Nein ☐

B) Darf oder durfte sich eine Katze im Bett Ihres Kindes aufhalten? Ja ☐ Nein ☐

C) Darf oder durfte sich ein Hund im Zimmer, in dem Ihr Kind schläft aufhalten? Ja ☐ Nein ☐

D) Darf oder durfte sich ein Hund im Bett Ihres Kindes aufhalten? Ja ☐ Nein ☐

43. Hat Ihr Kind sonst regelmäßig (ca. 1x/Woche) Kontakt zu folgenden Tieren (z.B. in der Wohnung von Freunden/ Verwandten, Käfig/Stall außerhalb der Wohnung)?
(Mehrere Antworten sind möglich)

	Zur Zeit	Im 1. oder 2. Lebensjahr
Hund	☐	☐
Katze	☐	☐
Pferde	☐	☐

12

Es folgen Fragen zum Rauchverhalten

44. Haben Sie und Ihre Familie in den letzten 3 Jahren mit dem Rauchen in der Wohnung aufgehört bzw. das Rauchen innerhalb der Wohnräume eingeschränkt?

Ja ☐
Nein ☐
Es wurde nie geraucht ☐ ⇒ weiter mit Frage 46!

45. Wie viele Zigaretten werden durchschnittlich am Tag in Ihrer Wohnung (damit meinen wir auch die Küche) geraucht? Zigaretten, die auf dem Balkon oder der Terrasse geraucht werden, brauchen nicht mitgezählt zu werden.

Wie viele davon von... (keine=0)

Mutter	_____	pro Tag
Partner	_____	pro Tag
Andere Personen	_____	pro Tag
Insgesamt	_____	pro Tag

46. A) Wie viele jüngere Geschwister hat Ihr Kind?
Bitte auch Stiefgeschwister mitzählen, die in Ihrer Familie leben!

Schwestern.....Stiefschwestern.....
Brüder.....Stiefbrüder.....

B) Wie viele ältere Geschwister hat Ihr Kind?
Bitte auch Stiefgeschwister mitzählen, die in Ihrer Familie leben!

Schwestern.....Stiefschwestern.....
Brüder.....Stiefbrüder.....

47. Bitte notieren Sie Name und Geburtsdatum der Geschwister Ihres Kindes.
Bitte auch Stiefgeschwister mitzählen, die in Ihrer Familie leben!

Name	Mädchen	Junge	Geburtsdatum
_____	☐	☐	___/___/_____
_____	☐	☐	___/___/_____
_____	☐	☐	___/___/_____

13

48. Wird Ihr Kind regelmäßig zusammen mit anderen Kindern betreut (z.B. durch eine Tagesmutter, in einer Kinderkrippe oder bei den Großeltern)? Die eigenen Geschwister sind dabei nicht gemeint.

Ja ☐ Mit wie vielen anderen Kindern? _____
Nein ☐

49. Falls Sie andere Kinder haben:
Leidet eines oder mehrere Kinder unter einer der folgenden Erkrankungen, die von einem Arzt diagnostiziert wurden:

	Ja	Nein
Asthma	☐	☐
Heuschnupfen	☐	☐
Neurodermitis(atopisches Ekzem, atopische Dermatitis)	☐	☐

50. Sind Sie in den letzten 3 Jahren (d.h. seit das Kind geboren wurde) umgezogen?

Ja ☐ ⇒ **Bitte neue Adresse mitteilen!**
Nein ☐

14

Bei Fragen können Sie sich jederzeit gerne an uns wenden.

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Tel: 089/5160-7856 (Büro)
089/5160-7781 (Labor)

Haben Sie noch weitere Kommentare zum Fragebogen oder allgemein?

Wir danken Ihnen herzlich für das Ausfüllen des Fragebogens!

9.6.6 PAULCHEN six-year follow-up

LMU KLINIKUM DER UNIVERSITÄT MÜNCHEN | CAMPUS INNENSTADT | KLINIK UND POLIKLINIK IM DR. VON HAUNERSCHEN KINDERSPITAL

ID:

PAULCHEN

Fragebogen zum **6. Lebensjahr**

Ihres Kindes

München, 03.08.2010

Datum:_____ Studiennummer:_____

Fragebogen für die Eltern

Wir freuen uns, dass Sie bereit sind weiterhin an der Paulchen Studie teilzunehmen. Bitte kreuzen Sie die folgenden Fragen an. Ihre Antworten werden vertraulich behandelt. Wenn Sie eine Frage nicht beantworten möchten, lassen Sie sie bitte aus.

Wir danken Ihnen herzlich für Ihre Mitarbeit!

Wir beginnen mit Fragen zu pfeifenden und keuchenden Atemgeräuschen. Mit pfeifenden Atemgeräuschen meinen wir ein pfeifendes Geräusch, das aus dem Brustkorb kommt, aber nicht geräuschvolles Atmen durch die Nase.

1. Hat Ihr Kind jemals pfeifende bzw. keuchende Atemgeräusche gehabt?

Ja ..... ☐
Falls Ja,
wann sind diese zum ersten Mal aufgetreten:
Nein ..... ☐.... ⇒ weiter mit Frage 12

2. Hatte Ihr Kind in den letzten 3 Jahren pfeifende bzw. keuchende Atemgeräusche?

Ja..... ☐
Nein..... ☐ ⇒ weiter mit Frage 12

3. Wie oft hatte Ihr Kind in den letzten 12 Monaten pfeifende bzw. keuchende Atemgeräusche?

Gar nicht ..... ☐
1-3 mal ..... ☐
4-12mal ..... ☐
Mehr als 12 mal ..... ☐

4. Hatte Ihr Kind in den letzten 12 Monaten jemals Atemnot, als die pfeifenden/ keuchenden Atemgeräusche auftraten?

Ja ..... ☐
Nein ..... ☐

5. Wie häufig ist Ihr Kind in den letzten 12 Monaten nachts wegen pfeife nder oder keuchender Atemgeräusche aufgewacht?

Seltener als einmal pro Monat ..... ☐
Einmal pro Monat ..... ☐
Mindestens zweimal pro Monat ..... ☐

6. Wodurch wurden bei Ihrem Kind die pfeifenden / keuchenden Atemgeräusche ausgelöst?

	Ja	Nein
Anstrengung	☐	☐
Erkältung	☐	☐
Kontakt mit Tieren	☐	☐
Kontakt mit Hausstaub	☐	☐
Kontakt mit Gras	☐	☐
Sonstiges	☐	☐

_____

7. Wie häufig hatte Ihr Kind in den letzten 12 Monaten pfeifende oder keuchende Atemgeräusche, ohne dass es erkältet war?

Nie ☐
Seltener als einmal pro Monat ☐
Einmal pro Monat ☐
Mindestens zweimal pro Monat ☐

8. Ist das Kind zwischen diesen Episoden völlig beschwerdefrei?

Ja ☐ ⇒ weiter mit Frage 12
Nein ☐

9. Hat Ihr Kind zwischen diesen Episoden folgende Beschwerden bei Anstrengung?

	Ja	Nein
Husten	☐	☐
Pfeifende Atemgeräusche	☐	☐
Atemnot	☐	☐

Sonstiges:_____

Bei Temperaturwechsel/Nebel?

	Ja	Nein
Husten	☐	☐
Pfeifende Atemgeräusche	☐	☐
Atemnot	☐	☐

Sonstiges:_____

Nachts?

	Ja	Nein
Husten	☐	☐
Pfeifende Atemgeräusche	☐	☐
Atemnot	☐	☐

Sonstiges:_____

Sonstige Beschwerden?

:_____

10. Hat Ihr Kind jemals in den letzten 3 Jahren von einem Arzt Medikamente gegen pfeifende oder keuchende Atemgeräusche, oder Giemen oder Atemnot verschrieben bekommen?
(Gemeint sind damit nicht nur Medikamente zum Schlucken, sondern auch Inhalationen oder Sprays)

Ja ☐
Nein ☐ ⇒ weiter mit Frage 12

11. Welche Medikamente waren dies?
Bitte geben Sie den Markennamen möglichst genau an! Und sofern Sie es wissen die Dosis sowie den Zeitraum, in dem das Medikament eingenommen wurde.

1. _____
2. _____
3. _____

12. Wurde bei Ihrem Kind jemals von einem Arzt ein Allergietest durchgeführt?

	Ja	Nein
Ein Hauttest	☐	☐
Ein Bluttest	☐	☐
Ein anderer Test, z.B. Bioresonanz	☐	☐

13. Welche Allergie wurde dabei festgestellt?

	Ja	Nein
Gegen Pollen	☐	☐
Gegen Hausstaub(milben)	☐	☐
Gegen Tiere	☐	☐
Gegen Nahrungsmittel	☐	☐
Andere:_____	☐	☐

14. Hat Ihr Kind jemals in den letzten 3 Jahren von einem Arzt Medikamente aus einem anderen Grund verschrieben bekommen?
(Gemeint sind damit nicht nur Medikamente zum Schlucken, sondern auch Inhalationen oder Sprays)

Ja ☐
Nein ☐ ⇒ weiter mit Frage 16

15. Welche Medikamente waren dies?
Bitte geben Sie den Markennamen möglichst genau an! Und sofern Sie es wissen die Dosis sowie den Zeitraum in dem das Medikament eingenommen wurde.

1. _____
2. _____
3. _____

Es folgen Fragen zu Beschwerden der Nase und der Augen

16. Hat Ihr Kind jemals Niesanfälle oder eine laufende, verstopfte oder juckende Nase, obwohl es nicht erkältet war?

Ja ☐
Falls Ja, wann ist dies zum ersten Mal aufgetreten:

Nein ☐ ⇒ weiter mit Frage 21

17. Hatte Ihr Kind in den letzten 3 Jahren Niesanfälle oder eine laufende, verstopfte oder juckende Nase, obwohl es nicht erkältet war?

Ja ☐
Nein ☐ ⇒ weiter mit Frage 21

18. Hatte Ihr Kind in den letzen 12 Monaten gleichzeitig mit diesen Nasenbeschwerden juckende oder tränende Augen?

Ja ☐
Nein ☐

19. Wann in den letzen 12 Monaten traten diese Nasen-Beschwerden auf?
Mehrere Antworten sind möglich.

Januar ☐	Mai ☐	September ☐
Februar ☐	Juni ☐	Oktober ☐
März ☐	Juli ☐	November ☐
April ☐	August ☐	Dezember ☐

20. Ist von einem Arzt bei Ihrem Kind schon einmal Heuschnupfen oder eine allergische Rhinitis bzw. Rhinokonjunktivitis festgestellt worden?

Ja ☐
Nein ☐

Es folgen Fragen zu Hauterkrankungen

21. ..... Hatte Ihr Kind jemals eine Neurodermitis/atopische Dermatitis/ atopisches Ekzem

Ja ☐
Falls Ja, wann ist diese zum ersten Mal aufgetreten: _____
Nein ☐ ⇒ weiter mit Frage 31

22. Wurde bei Ihrem Kind die Diagnose einer Neurodermitis/atopischen Dermatitis/ atopisches Ekzem von einem Arzt gestellt?

Ja ☐
Nein ☐

23. Hatte Ihr Kind in den letzten 3 Jahren eine Neurodermitis/atopische Dermatitis/ atopisches Ekzem

Ja ☐
Nein ☐

24. War der Hautausschlag je an einer der folgenden Stellen?

	Ja	Nein
Gesicht	☐	☐
Hals	☐	☐
Ellenbeugen / Kniekehlen	☐	☐
Hand- / Fußgelenke	☐	☐
Brust/Rücken	☐	☐

25. Hat sich die Lokalisation des Ausschlages im Laufe der Zeit geändert?

Ja ☐ Nein ☐

Falls Ja, wo war er zu Beginn? Wo befindet er sich heute?

Zu Beginn:

	Ja	Nein
Gesicht	☐	☐
Hals	☐	☐
Ellenbeugen / Kniekehlen	☐	☐
Hand- / Fußgelenke	☐	☐
Brust/Rücken	☐	☐

Heute:

	Ja	Nein
Gesicht	☐	☐
Hals	☐	☐
Ellenbeugen / Kniekehlen	☐	☐
Hand- / Fußgelenke	☐	☐
Brust/Rücken	☐	☐

26. Wenn Sie die Zeiten, in denen Ihr Kind diesen Hautausschlag hatte, zusammenzählen: Wie lange haben Sie diesen Hautausschlag insgesamt beobachtet?

Für insgesamt weniger als 3 Monate .... ☐
Für insgesamt 3-6 Monate ..... ☐
Für insgesamt 6-12 Monate ..... ☐
Für länger als 12 Monate ..... ☐

27. Ist der Hautausschlag wieder völlig verschwunden, oder „kommt und geht" der Hautausschlag?

Der Hautausschlag ist vollständig Verschwunden ..... ☐
Der Hautausschlag „kommt und geht".. ☐
Der Hautausschlag ist noch da..... ☐

28. Wie alt war Ihr Kind, als der Hautausschlag vollständig verschwunden ist?

_____ Monate

29. Wie häufig ist Ihr Kind nachts wegen Juckreiz aufgewacht?

Seltener als einmal pro Monat oder nie ..... ☐
Einmal pro Monat ..... ☐
Mindestens zweimal pro Monat ..... ☐

30. Haben Sie die Haut Ihres Kindes in den letzten 12 Monaten mit einer cortisonhaltigen Creme / Salbe oder einer Tacrolimus- bzw. Pimecrolimus-haltigen Salbe (Protopic, Elidel) behandelt?

Ja ☐
Nein ☐

Es folgen Fragen zu Nahrungsunverträglichkeiten oder –allergien

31. Hat Ihr Kind eine Nahrungsmittelallergie?

Ja ☐
Nein ☐ ⇨ weiter mit Frage 34

32. Wie äußert sich diese Nahrungsmittelallergie?

Ausschlag/rote Flecken um den Mund herum ..... ☐
Ausschlag/rote Flecken an anderen Körperstellen..... ☐
Schwellung der Lippen ..... ☐
Juckreiz ..... ☐
Durchfall ..... ☐
Erbrechen ..... ☐
Verschlechterung der Neurodermitis..... ☐
Pfeifende Atemgeräusche ..... ☐
Atemnot ..... ☐
Kreislaufreaktion/Blutdruckabfall ..... ☐
Sonstiges:

33. Auf welche Nahrungsmittel reagiert Ihr Kind?

	Ja	Nein
Milch und Milchprodukte	☐	☐
Hühnereier	☐	☐
Fisch	☐	☐
Weizenmehl oder andere Getreideprodukte	☐	☐
Nüsse	☐	☐
Soja	☐	☐
Zitrusfrüchte	☐	☐
Anderes Obst oder Gemüse	☐	☐
Andere Nahrungsmittel	☐	☐

Welche? _____

Es folgen Fragen zu anderen Erkrankungen

34. Wurde bei Ihrem Kind jemals von einem Arzt/einer Ärztin eine spastische Bronchitis, obstruktive Bronchitis oder asthmatische Bronchitis diagnostiziert?

Nein, nie..... ☐
Ja, einmal..... ☐
Ja, mehrmals..... ☐

35. Wurde bei Ihrem Kind in den letzten 12 Monaten von einem Arzt/einer Ärztin eine der folgenden Diagnosen gestellt?

	Ja	Nein
Asthma	☐	☐
Neurodermitis, atopische Dermatitis oder endogenes Ekzem	☐	☐
Allergische Rhinitis/Heuschnupfen	☐	☐

36. Hatte Ihr Kind bisher eine der folgenden Erkrankungen nach dem dritten Lebensjahr?

	Ja	Nein
Mittelohrentzündung	☐	☐
Pseudokrupp	☐	☐
Lungenentzündung	☐	☐
Bronchitis	☐	☐
Bronchiolitis	☐	☐
Keuchhusten	☐	☐
Andere Infektionen	☐	☐

Welche? _____

Waren stationäre Aufenthalte im Krankenhaus notwendig.... ☐ ☐

Warum? _____

_____

Angaben zur Wohnungs- und Lebenssituation

37. **A) Wie viele jüngere Geschwister hat Ihr Kind?**

Bitte auch Stiefgeschwister mitzählen, die in Ihrer Familie leben!

Schwestern..... Brüder.....

B) Wie viele ältere Geschwister hat Ihr Kind?
Bitte auch Stiefgeschwister mitzählen, die in Ihrer Familie leben!

Schwestern.....Brüder.....

38. **Bitte notieren Sie Name und Geburtsdatum der Geschwister Ihres Kindes. Bitte auch Stiefgeschwister mitzählen, die in Ihrer Familie leben!**

Name	Mädchen	Junge	Geburtsdatum
_____	☐	☐	___/___/_____
_____	☐	☐	___/___/_____
_____	☐	☐	___/___/_____

39. **Wird Ihr Kind regelmäßig zusammen mit anderen Kindern durch eine Tagesmutter oder bei den Großeltern betreut? Die eigenen Geschwister sind dabei nicht gemeint.**

Ja, ...☐
Mit wie vielen anderen Kindern: _____
Nein .....☐

40. **Wird Ihr Kind regelmäßig zusammen mit anderen Kindern in einer Kinderkrippe oder im Kindergarten betreut? Die eigenen Geschwister sind dabei nicht gemeint.**

Ja, ...☐
Mit wie vielen anderen Kindern? _____
Nein ☐

41. **Hat Ihr Kind in den letzten 3 Jahren Frischmilch direkt vom Bauernhof getrunken?**

Ja ☐
Nein ☐ ⇨ weiter mit Frage 44

42. **Kochen Sie Ihre Milch normalerweise vor dem Trinken ab?**

Ja, aber nur während der Sommermonate ☐
Ja, immer ☐
Nein ☐

43. **Wie viele Gläser Frischmilch hat Ihr Kind durchschnittlich in den letzten 12 Monaten getrunken? (Ein Glas entspricht etwa 0,2 Liter)**

Gläser pro Tag: _____

44. **Hat Ihre Familie in den letzen 3 Jahren auf einem Bauerhof gelebt auf dem Vieh gehalten wird?**

Ja ☐
Nein ☐ ⇨ weiter mit Frage 53

45. **Bewirtschaftete Ihre Familie den Hof in den letzten 3 Jahren?**

Ja ☐
Nein ☐ ⇨ weiter mit Frage 53

46. **Welche Nutztiere werden gehalten und in welcher Zahl?**

Milchkühe	_____(Anzahl)
Schweine	_____(Anzahl)
Geflügel (Hühner, Puten, Enten, Gänse, etc.)	_____(Anzahl)
Pferde (Ponys, Esel)	_____(Anzahl)
Schafe/Ziegen	_____(Anzahl)
Hasen/Kaninchen	_____(Anzahl)

47. **Welches Futter erhalten die Tiere?**

	Ja	Nein
Heu	☐	☐
Grassilage	☐	☐
Maissilage	☐	☐
Andere Silage	☐	☐
Grascops	☐	☐

Anderes Futter in pelletierter Form ..... ☐.....☐
Kraftfutter bzw. Milchleistungsfutter ..... ☐.....☐
Sonstiges: _____

48. **Wie häufig war Ihr Kind in den letzten 12 Monaten im Stall? (Gemeint sind Ställe von Großvieh, d.h. Kühe, Schweine, Pferde, Schafe, Ziegen).**

Gar nicht..... ☐
Seltener als einmal pro Woche ..... ☐
Durchschnitt _____ Stunden pro Monat
Mindestens einmal pro Woche ..... ☐
Durchschnitt _____ Tage pro Monat
An diesen Tagen durchschnittlich _____ Stunden pro Tag

49. **Wie häufig war Ihr Kind in den letzten 12 Monaten in der Scheune?**

Gar nicht..... ☐
Seltener als einmal pro Woche ..... ☐
Durchschnitt _____ Stunden pro Monat
Mindestens einmal pro Woche ..... ☐
Durchschnitt _____ Tage pro Monat
An diesen Tagen durchschnittlich _____ Stunden pro Tag

50. **Hatte Ihr Kind in den letzten 12 Monaten regelmäßig direkten Kontakt zu Heu? (Regelmäßig bedeutet mindestens einmal pro Woche)**

Ja ☐
Nein ☐

51. **In welchem Alter wurde Ihr Kind zum ersten Mal mit zur Heuernte genommen?**

Mit _____ Jahren ☐
Noch nie

52. **Wie häufig hatte Ihr Kind im Durchschnitt in den letzten 12 Monaten Kontakt zu folgenden Nutztieren?**

	Nie oder ≤ 1/Monat	mehrmals pro Monat	mehrmals pro Woche	täglich
Pferde	☐	☐	☐	☐
Kühe	☐	☐	☐	☐
Schweine	☐	☐	☐	☐
Schafe/Ziegen	☐	☐	☐	☐
Hasen/Kaninchen	☐	☐	☐	☐
Geflügel	☐	☐	☐	☐

53. **Welche der folgenden Haustiere haben/hatten Sie innerhalb der Wohnung?**
Mehrere Antworten sind möglich.

Keine	☐	Kaninchen	☐
Hund	☐	Meerschweinchen	☐
Katze	☐	Vögel	☐
Hamster	☐	Aquarium (Fische)	☐
Sonstige ☐	Welche:		

A) Darf oder durfte sich eine Katze im Zimmer, in dem Ihr Kind schläft aufhalten?
Ja ☐
Nein ☐

B) Darf oder durfte sich eine Katze im Bett Ihres Kindes aufhalten?
Ja ☐
Nein ☐

C) Darf oder durfte sich ein Hund im Zimmer, in dem Ihr Kind schläft aufhalten?
Ja ☐
Nein ☐

D) Darf oder durfte sich ein Hund im Bett Ihres Kindes aufhalten?
Ja ☐
Nein ☐

54. Hat Ihr Kind sonst regelmäßig (ca. 1x/Woche) Kontakt zu Tieren (z.B. in der Wohnung von Freunden/ Verwandten)? *Mehrere Antworten sind möglich.*

	Ja	Nein
Hund	☐	☐
Katze	☐	☐
Sonstige	☐	☐

Welche:_____

55. Gibt es in Ihrer Wohnung Feuchtigkeitsflecken bzw. Schimmelbefall an Wänden oder Decken?

Feuchtigkeitsflecken in Bad oder Küche sind dabei nicht gemeint, sondern nur in Räumen wie Wohnzimmer, Schlafzimmer oder Kinderzimmer.

	Ja	Nein
Feuchtigkeitsflecken, aber ohne Schimmelbefall	☐	☐
Feuchtigkeitsflecken mit Schimmelbefall	☐	☐

Es folgen Fragen zum Rauchverhalten

56. Rauchen Sie oder Ihre Familie in Ihrer Wohnung/Haus?

Ja ☐
Nein ☐

57. Haben Sie und Ihre Familie in den letzten 12 Monaten mit dem Rauchen in der Wohnung aufgehört bzw. das Rauchen innerhalb der Wohnräume eingeschränkt?

Ja ☐
Nein ☐
Es wurde nie geraucht ☐

58. Wie viele Zigaretten werden durchschnittlich am Tag in Ihrer Wohnung (damit meinen wir auch die Küche) geraucht? Zigaretten, die auf dem Balkon oder der Terrasse geraucht werden, brauchen nicht mitgezählt zu werden. Wie viele davon von... (keine=0)

Mutter	_____	pro Tag
Partner	_____	pro Tag
Andere Personen	_____	pro Tag
Insgesamt	_____	pro Tag

Haben Sie noch weitere Kommentare zum Fragebogen oder allgemein?

_____
_____
_____
_____
_____
_____
_____

Wir danken Ihnen herzlich für das Ausfüllen des Fragebogens!

Bei Fragen können Sie sich jederzeit gerne an uns wenden.

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9.7 Follow-up rates

Cohort		
PAULINA	3 year follow-up	88.9%
PAULINA	3 year follow-up	84.4%
PAULCHEN	6 year follow-up	91.2%
PAULCHEN	6 year follow-up	89.01%

Danksagung

Es ist mir ein sehr großes Anliegen, mich an dieser Stelle bei allen zu bedanken, die das Gelingen dieser Arbeit erst möglich gemacht haben.

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LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Promotionsbüro
Medizinische Fakultät

MMRS

Eidesstattliche Versicherung

Anselm, Bettina

Name, Vorname

Ich erkläre hiermit an Eides statt,

dass ich die vorliegende Dissertation mit dem Titel

Early priming of the immune system: Identifying predictive markers of innate immunity and calcium signalling for the development of asthma.

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

Zürich, den 29.1.2020

Ort, Datum

Bettina Anselm

Unterschrift Doktorandin bzw. Doktorand