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PRESCHOOL ASTHMA: INFANT LUNG FUNCTION, INFLAMMATION AND SKIN BARRIER FUNCTION IN EARLY LIFE

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Preschool asthma: infant lung function, inflammation and skin barrier function in early life

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Martin Färdig

The thesis will be defended in public at Karolinska Institutet in Solna at Rockefeller auditorium, September 19, at 09.00.

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POPULAR SCIENCE SUMMARY OF THE THESIS

Asthma is one of the most common respiratory disorders in children, characterized by airway obstruction leading to symptoms such as wheeze, cough and shortness of breath, negatively affecting daily life of many children. While the cause of asthma remains largely unknown, the disorder likely begins early in life. In this project, we first investigated if early signs of airway obstruction by lower lung function was linked to asthma development at preschool age. Second, we explored if skin-related conditions in early life – such as the skin's ability to hold moist, or eczema with dry, itchy and red skin, or mutations in a gene essential for the skin barrier's normal function – were related to preschool asthma development. Third, we aimed to identify if early-life inflammation – such as higher levels of an inflammatory substance released by white blood cells (eosinophils), commonly increasing during allergic reactions – was connected to asthma development among preschoolers. Since lower lung function (1), skin-related conditions (2) and inflammation by higher levels of the inflammatory substance (3) previously have been associated with asthma among older children, adolescents and adults, the overall purpose was to explore if these three potential risk factors (1-3) in early life was linked to preschool asthma in almost 2400 boys and girls participating in a study in Norway and Sweden, followed from birth to preschool age.

1. With infant lung function measured both while awake and sleeping, we first determined whether lung function differed between the two arousal states. Infant lung function was lower while sleeping compared to the awake state, suggesting it is important to record lung function separately according to arousal state. In the awake state, lower infant lung function was linked to a five times higher likelihood of preschool asthma. However, separating boys and girls, lower infant lung function was only linked to asthma in boys, with an eight times higher likelihood.

2. In regard to skin-related conditions, neither eczema nor mutations in the skin barrier gene were overall associated with preschool asthma. However, in infancy, lower ability of the skin to hold moist was associated with a two times higher likelihood of developing preschool asthma, suggesting a possible link between skin barrier impairment and respiratory health. Interestingly, separating boys and girls, mutations in the skin barrier gene were related to a three times higher likelihood of preschool asthma in girls only.

3. Concerning the inflammatory substance released by white blood cells, higher levels measured in blood in late infancy and at preschool age were connected to preschool asthma, increasing the likelihood by two and almost five times at the two ages, respectively. When separating girls and boys, elevated levels of the same inflammatory substance in infancy increased the likelihood by four times in boys only, whereas at preschool age, elevated levels increased the likelihood in both sexes.

In summary, based on our findings we conclude that lower lung function, skin-related conditions as well as inflammation in early life overall may be linked to preschool asthma

development. However, the likelihood of preschool asthma differed between boys and girls, suggesting sex is an important factor.

ABSTRACT

Background

Asthma is among the most common obstructive respiratory disorders in children, characterized by airway inflammation with features such as wheeze, cough and shortness of breath. Though the etiology behind the disease partly remains unknown, the roots likely origin from early life. Lower lung function, skin barrier impairment, as well as eosinophil inflammation are factors related to asthma development, but are largely unexplored in early life. The overall aim of this doctoral thesis was to explore early risk factors for the development of preschool asthma, related to infant lung function, eosinophil inflammation and skin barrier function in early life.

Methods

All four sub studies (**I-IV**) were based on data prospectively collected from children actively participating in the Preventing Atopic Dermatitis and ALLergies in children (PreventADALL) mother-child cohort (n=2394) recruited from the general population in Sweden and Norway.

First, with infant lung function measured in both the awake and sleeping state at three months, we aimed to determine whether lung function by tidal flow-volume (TFV) loops differed according to arousal state (**sub study I**). Thereafter, we investigated the potential association between infant lung function while awake and preschool asthma at three years (**sub study IV**).

Second, we aimed to explore if infant skin barrier function by transepidermal water loss (TEWL), eczema at three months and mutations in the skin barrier filaggrin (*FLG*) gene were associated with infant lung function at three months (**sub study II**) and/or preschool asthma at three years (**sub study IV**).

Third, we aimed to identify if higher levels of inflammation by serum eosinophil-derived neurotoxin (EDN) at one and three years were associated with preschool asthma at three years (**sub study III**).

Lastly, we aimed to assess if the potential associations between infant lung- and skin barrier function as well as early-life eosinophil inflammation and preschool asthma differed by sex (**sub study III-IV**).

Results

Among 91 infants with paired lung function measurements, TFV loop parameters overall differed, with a higher ratio of time to peak tidal expiratory flow to expiratory time, t_{PTEF}/t_E, shorter t_E but similar t_{PTEF} while awake compared to the sleeping state. Studying 563 children with information on infant lung function while awake and preschool asthma, a five-

respectively three-fold higher odds ratio (OR) of asthma was observed among infants with lower t_{PTEF}/t_E (<0.25) and shorter t_{PTEF} (<0.17 s).

While the presence of eczema or *FLG* mutations were not associated with a shorter t_{PTEF} in 899 infants, a high TEWL was. Similarly, a two-fold higher OR of preschool asthma was observed in infants with high TEWL, but not eczema or *FLG* mutations among 1337 children.

Among 1233 children with available serum eosinophil-derived neurotoxin (EDN) levels, EDN levels ≥26.7 µg/L in late infancy and ≥20.5 µg/L at preschool age were associated with preschool asthma, increasing the OR by two- and almost five-fold at respective age.

Overall, boys had higher rates of preschool asthma compared to girls; ≥15% versus ≤9%. Similarly, infant lung function was lower in boys compared to girls; 0.38 versus 0.40 for t_{PTEF}/t_E and 0.20 versus 0.21 s for t_{PTEF}, whereas TEWL, eczema and *FLG* mutations were distributed equally across the sexes. Boys had higher EDN levels in late infancy and at preschool age (32.0 and 20.9 µg/L) compared to girls (24.5 and 19.0 µg/L). The associations to preschool asthma differed by sex, with an eight- and three-fold higher OR in boys with a lower t_{PTEF}/t_E respectively shorter t_{PTEF}, and a four-fold higher OR observed in girls with *FLG* mutations, only, whereas neither eczema nor high TEWL remained significant risk factors. Higher EDN levels in late infancy increased the OR of preschool asthma by four-fold in boys only, whereas at preschool age, higher levels increased the OR in both sexes.

Conclusion

Based on our findings, we conclude that lower lung function in the awake state, skin barrier impairment as well as eosinophil inflammation in early life overall may be linked to preschool asthma. However, as the associations to preschool asthma differed by sex, with a higher OR observed in boys with lower lung function, while *FLG* gene mutations increased the OR in girls, and in late infancy, higher EDN levels remained a significant risk factor in boys, only, our observations point to the importance of potential sex differences.

LIST OF SCIENTIFIC PAPERS AND MANUSCRIPTS INCLUDED IN THE THESIS

I. Bains KES*, **Färdig M***, Gudmundsdóttir HK, Almqvist C, Hedlin G, Nordhagen LS, Rehbinder EM, Skjerven HO, Söderhäll C, Vettukattil R, Nordlund B, Lødrup Carlsen KC. Infant tidal flow-volume parameters and arousal state. ERJ Open Res. 2022 Oct 17;8(4):00163-2022. doi: 10.1183/23120541.00163-2022. PMID: 36267897; PMCID: PMC9574559.
* Shared first authorship

II. **Färdig M**, Gudmundsdóttir HK, Hoyer A, Bains KES, Almqvist C, Monceyron Jonassen C, Rehbinder EM, Skjerven HO, Staff AC, Vettukattil R, Söderhäll C, Carlsen KCL, Nordlund B. Skin Barrier Function and Infant Tidal Flow-Volume Loops-A Population-Based Observational Study. Children (Basel). 2022 Dec 31;10(1):88. doi: 10.3390/children10010088. PMID: 36670639; PMCID: PMC9856825.

III. **Färdig M**, Lie A, Borres MP, Ekenkrantz T, Granum B, Haugen G, Jonassen CM, Movérare R, Rehbinder EM, Skjerven HO, Staff AC, Vettukattil R, Lødrup Carlsen KC, Söderhäll C, Nordlund B. Eosinophil derived-neurotoxin levels by sex and in relation to preschool asthma – a prospective observational study. [*under review*]

IV. **Färdig M**, Hoyer A, Almqvist C, Bains KES, Lødrup Carlsen KC, Gudmundsdóttir HK, Granum B, Haugen G, Hedlin G, Jonassen CM, Konradsen JR, Lie A, Rehbinder EM, Skjerven HO, Staff AC, Vettukattil R, Söderhäll C, Nordlund B. Infant lung function and early skin barrier impairment in the development of preschool asthma. [*under review*]

Scientific papers not included in the thesis:

I. Tedner SG, Söderhäll C, Konradsen JR, Bains KES, Borres MP, Carlsen KH, Carlsen KCL, **Färdig M**, Gerdin SW, Gudmundsdóttir HK, Haugen G, Hedlin G, Jonassen CM, Kreyberg I, Mägi CO, Nordhagen LS, Rehbinder EM, Rudi K, Skjerven HO, Staff AC, Vettukattil R, van Hage M, Nordlund B, Asarnoj A. Extract and molecular-based early infant sensitization and associated factors-A PreventADALL study. Allergy. 2021 Sep;76(9):2730-2739. doi: 10.1111/all.14805. Epub 2021 May 4. PMID: 33751598.

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III. Wärnberg Gerdin S, Lie A, Asarnoj A, Borres MP, Lødrup Carlsen KC, **Färdig M**, Konradsen JR, Monceyron Jonassen C, Olsson Mägi CA, Rehbinder EM, Rudi K, Skjerven HO, Staff AC, Söderhäll C, Tedner SG, van Hage M, Vettukattil R, Nordlund B. Impaired skin barrier and allergic sensitization in early infancy. Allergy. 2022 May;77(5):1464-1476. doi: 10.1111/all.15170. Epub 2021 Nov 12. PMID: 34738238.

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V. Bains KES, Gudmundsdóttir HK, **Färdig M**, Amnö E, Jonassen CM, Nordlund B, Rehbinder EM, Skjerven HO, Rueegg CS, Vettukattil R, Lødrup Carlsen KC. Infant lung function: criteria for selecting tidal flow-volume loops. ERJ Open Res. 2022 Oct 24;8(4):00165-2022. doi: 10.1183/23120541.00165-2022. PMID: 36299362; PMCID: PMC9589321.

VI. Gudmundsdóttir HK, Carlsen OCL, Bains KES, **Färdig M**, Haugen G, Jonassen CM, LeBlanc M, Nordlund B, Rehbinder EM, Skjerven HO, Staff AC, Vettukattil R, Lødrup Carlsen KC. Infant lung function and maternal physical activity in the first half of pregnancy. ERJ Open Res. 2022 Oct 31;8(4):00172-2022. doi: 10.1183/23120541.00172-2022. PMID: 36329799; PMCID: PMC9619250.

VII. Gudmundsdóttir HK, Hilde K, Bains KES, **Färdig M**, Haugen G, LeBlanc M, Nordhagen LS, Nordlund B, Rehbinder EM, Skjerven HO, Staff AC, Vettukattil R, Carlsen KCL. Fetal thoracic circumference in mid-pregnancy and infant lung function. Pediatr Pulmonol. 2023 Jan;58(1):35-45. doi: 10.1002/ppul.26153. Epub 2022 Oct 11. PMID: 36097818.

VIII. Hilde K, Gudmundsdóttir HK, Bains KES, Carlsen KCL, Färdig M, Jonassen CM, Kreyberg I, LeBlanc M, Nordlund B, Rehbinder EM, Roland MCP, Skjerven HO, Staff AC, Vettukattil R, Haugen G. Third trimester fetal lung volume, thoracic circumference, and early infant lung function. Pediatr Pulmonol. 2023 Jul;58(7):2050-2058. doi: 10.1002/ppul.26431. Epub 2023 Apr 26. PMID: 37098847.

IX. Hilde K, Gudmundsdóttir HK, Stensby Bains KE, **Färdig M**, Lødrup Carlsen KC, Jonassen CM, Kreyberg I, Nordlund B, Rehbinder EM, Paasche Roland MC, Skjerven HO, Staff AC, Vettukattil R, Haugen G. Fetal pulmonary artery Doppler blood flow velocity measures and early infant lung function. A prospective cohort study. J Matern Fetal Neonatal Med. 2023 Dec;36(1):2213796. doi: 10.1080/14767058.2023.2213796. PMID: 37197978

CONTENTS

LIST OF ABBREVIATIONS

AD	atopic dermatitis
AS	allergic sensitization
AUC	area under curve
β	beta
CI	confidence interval
DC	dendritic cell
ECP	eosinophil cationic protein
EDN	eosinophil-derived neurotoxin
EPO	eosinophil peroxidase
FLG	filaggrin
GA	gestational age
GDPR	general data protection regulation
$g/m^2/h$	grams per square meter per hour
IgE	immunoglobulin E
IL	interleukin
ILC2	innate lymphoid cells type 2
IQR	interquartile range
kU_A/L	kilounits of allergen specific IgE per liter
MBP	major basic protein
NPV	negative predictive value
OR	odds ratio
PPV	positive predictive value
PreventADALL	Preventing Atopic Dermatitis and ALLergies in children
rBO	recurrent bronchial obstruction
RR	respiratory rate
ROC	receiver operating characteristic
SD	standard deviation
SPT	skin prick test
t_E	expiratory time
TEWL	transepidermal water loss

TFV	tidal flow-volume
Th2	t-helper type 2
t_{PTEF}	time to peak tidal expiratory flow
TSLP	thymic stromal lymphopoietin
UKWP	United Kingdom Working Party
V_T	tidal volume

1 LITERATURE REVIEW

1.1 PRESCHOOL ASTHMA

Asthma is among the most common chronic obstructive respiratory disorders,[1] characterized by inflammation leading to hyperresponsiveness, reversible airflow obstruction and remodeling of the airway.[2] The prevalence of asthma among preschool children is around 11%,[3] and include common features such as wheeze, cough, chest rigidity and shortness of breath,[4] of which at least one symptom is present during an asthma attack.[5] At preschool age, exacerbations are predominately triggered by viral infections[6] rather than physical activity, allergens, irritants and pollutants,[4] often observed in older children.[6] Nonetheless, the morbidity of childhood asthma is high, associated with reduced quality of life, higher school-absenteeism[7-9] and hospitalization.[7,8]

1.1.1 Diagnosis of preschool asthma

Since many children experience recurrent infection-triggered symptoms of wheeze, the most commonly occurring and specific symptom of asthma in young children,[5] decisions regarding diagnosis and treatment can be difficult as not all wheezing indicates asthma.[10] In contrast to school-aged children, adolescents and adults, lung function testing supporting asthma diagnosis is not routine in infants and preschool children, due to the challenges accomplishing reproducible measurements. Instead, the diagnostic criteria of asthma in preschool children is mainly based on patterns of symptoms,[10] often reported by parents at examination[5] (such as persistent wheezing or coughing, nocturnal exacerbations, physical or social inactiveness), identified risk factors (such as history or heredity of allergic diseases), response to asthma treatment and exclusion of alternative diagnosis.[10] Although most children develop asthma in early life, many outgrow their asthma-like symptoms at preschool age. Therefore, many clinicians avoid labelling children with “asthma” at this age, while others promote using the term as an umbrella diagnosis gathering children with similar symptoms but with uncertain development.[11] Supporting the need for a disease label at preschool age, a recent birth cohort study reported a 20-150-fold increased risk of asthma at five years in children wheezing during first years of life.[12]

Treating asthma-like symptoms in preschool children aims to prevent exacerbations and improve control,[13] while reducing treatment delay and morbidity burden.[14] The standard treatment of asthma includes inhaled bronchodilators for symptom relief, and anti-inflammatory drugs such as corticosteroids,[15,16] and in serious cases the addition of immunomodulatory medication, for control.[17,18]

1.1.2 Phenotypes of wheeze and preschool asthma

Young children have considerably smaller airways compared to older children and adults, which in the presence of inflammation may lead to airway obstruction and wheeze. In many children, wheezing is the first episode of asthma. About one third of all wheezing preschool children have persisting symptoms and develop asthma by school age. Thus, the classification

of wheezing phenotypes is important to identify children at risk of asthma.[14] Based on symptoms, time of onset and/or atopic status,[19] there are at least three phenotypes of childhood wheeze; 1) Early transient wheeze; which is manifested in the first three years of life and resolves before six. 2) Persisting wheeze; in which episodes also begin before age three but persists after six. 3) Late-onset wheeze; with symptoms occurring between three and six years of age.[5] Yet, the stability and clinical usefulness of these phenotypes are debateable.[10] However, both persisting and late-onset wheezers are more likely to have persisting symptoms into adolescence and adulthood,[20] with an increasing risk of developing asthma for every year older they get,[5] while early onset wheezing is linked to comorbidity of other allergic diseases in childhood.[4]

1.1.3 Etiology of asthma and disease development in early life

Childhood asthma is heterogeneous, and the etiology behind the disease is partly still unknown.[7] Studies suggest that the roots of the disease origin from early life, even though it may become clinically evident first many years later.[1,7]

First, asthma is a known heritable disease estimated to account for a large part of the disease burden in childhood.[9,21] Though poorly understood, combinations of genetic susceptibility and environmental factors appear to be important determinants,[22] where exposures in prenatal and postnatal life may have lifelong effect on respiratory health[1,23] in which children are believed to be particularly sensitive, where timing of exposure seems critical.[24] Among early-life risk factors maternal nicotine use and diet during pregnancy, cesarean section delivery, preterm birth, the use of antibiotics and antipyretics, exposure to mold, mites, animal dander, and other allergens have been identified.[6] While some environmental exposures such as tobacco smoke are avoidable, contact to allergens or viruses are more difficult to avert.[1] As lifestyles become more westernized in low-income countries the prevalence of asthma also rapidly increases, a global pattern demonstrating the impact of environmental factors.[4] Linked to higher risk of childhood asthma among socially disadvantaged children,[25,26] a lack of socioeconomical and environmental stability may additionally potentiate the effect of environmental exposures (**Figure 1**).[27]

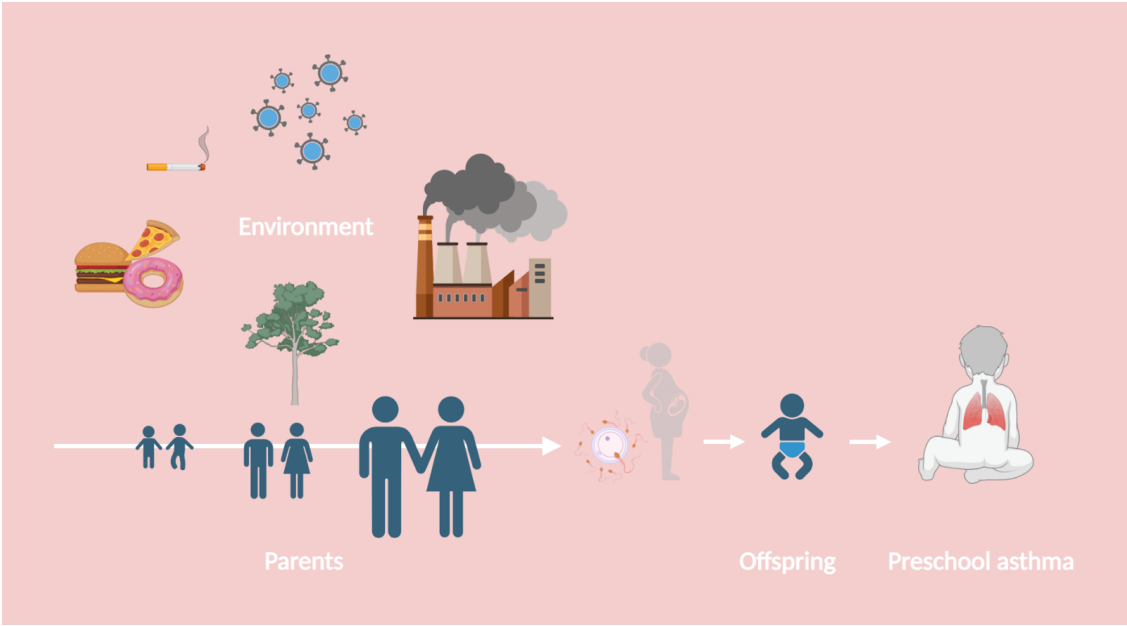

Figure 1. *The potential influence of genetics as well as pre- and postnatal environmental factors on respiratory health in offspring. The original illustration by Svanes et al. has been modified.*[23]

* The illustration was created with Biorender.com

Second, allergic comorbidity is common[28,29] and often develops in a time-based order,[30] supporting the theory that the conditions are closely connected to each other.[28,29] This temporal progression is commonly referred to as the *atopic march*, beginning in infancy with manifestations of atopic dermatitis (AD) and allergic sensitization (AS) to food, followed by asthma and allergic rhinitis at preschool- and school age (**Figure 2**).[30,31] However, the clinical characteristics of allergic diseases often differ between individuals and age.[32,33] While some allergic conditions resolve with age, others persist throughout childhood into adulthood.[30] In a recent review, the authors established that AD, asthma and allergic rhinitis often co-exist in childhood, but found no evidence of the hypothesized sequential development (atopic march) explaining the heterogeneity of allergic diseases, concluding that the relationship between the individual diseases ought to be interpreted from a multimorbidity framework, rather than a progressive or causal one.[34]

Atopic dermatitis is an allergic inflammatory condition characterized by red, inflamed and itchy skin,[35,36] with the first signs in infancy often occurring during the second postnatal month.[37]

Food allergy is defined as an adverse immune response after intake of food leading to a hypersensitivity reaction,[38] in Western countries affecting approximately 2.0% in infancy and 8.0% at preschool age,[39] in which specific immunoglobulin E (IgE) antibodies to common allergens such as cow's milk, egg, nuts, wheat, soy, fish and shell fish are often the cause.[38] By performing skin prick tests or analyzing serum for IgE antibodies to specific allergens, it is possible to detect and identify possible allergies.[2]

In contrast to AD and food allergies, *allergic rhinitis* often emerges later in childhood, affecting between 5.0-8.5% of all preschool children.[40] The disease is characterized by inflammation in the nasal mucosa due to allergic sensitization to airborne allergens such as pollens, mold and animal dander, with symptoms including sneezing, itching, runny and stuffy nose.[41]

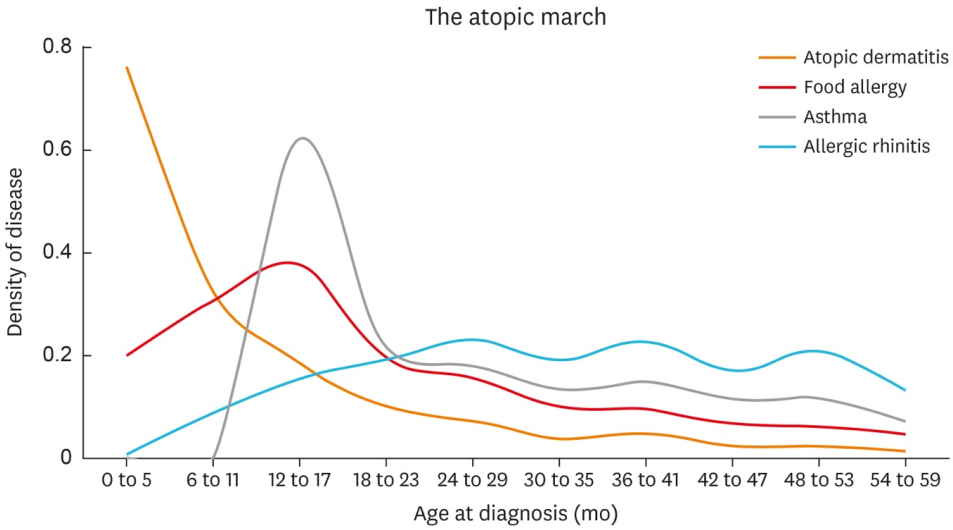

***Figure 2**. Illustration of the distribution of allergic diseases in the hypothesized atopic march, starting with atopic dermatitis and food allergy in infancy, followed by asthma and allergic rhinitis later in childhood.[42]*

mo: months

* Reproduced with permission from Allergy Asthma Immunol Res. 2019 Jan; 11(1): 4–15. doi: 10.4168/aair.2019.11.1.4

1.1.3.1 Infant lung function

Lower lung function in early life is a potential trajectory for future lung function[43] and respiratory disease,[7,44] and increases the overall mortality and morbidity risk.[45] The fetal development of the lung starts at five weeks gestational age (GA) and involves five different phases: the embryonic (<6 weeks GA), the pseudoglandular (6-16 weeks GA), the canicular

(16-24 weeks GA), the saccular (24-40 weeks GA) and the alveorization (mostly after birth). In the last phase, alveorization commences and continues to develop postnatally the first two to four years in life,[1] potentially throughout the entire childhood into adulthood.[1,44] Lung growth is rapid in childhood, developing proportionately after body size influenced by age, sex and ethnicity,[46] resulting in a 20-fold increase of lung function in early adolescence.[47] Lung function measured in volume reaches a peak in late adolescence and early adulthood, a plateau lasting for many years, before it progressively begins to decrease[43] due to lung elasticity loss.[48] This pattern of growth and decline is a determinant of future lung function.[43]

The lung's capacity for repair can be negatively affected by peri- and postnatal insults,[1,44] such as perinatal adverse events, environmental exposures and airway infections,[44] leaving the child more vulnerable to later respiratory disease. Airway epithelial cell damage is critical in lung development, potentially causing inflammation and cell death. If the subsequent cell regeneration is unsuccessful, remodeling with abnormal tissue structures may follow, characteristic for several respiratory diseases.[1] Children born with low lung function often retain a low lung function later in life. Duration, type and severity of insult influence the timing and outcome of abnormal lung development.[46]

Existing guidelines advise tailoring asthma treatment first handedly after asthmatic symptoms, and to a smaller extent, lung function. Nevertheless, objective lung function measurements are an important tool in diagnosing, monitoring and optimizing therapy of asthmatic children. Controversially, only a fraction of asthmatic children is offered lung function measurements.[49]

Reference values for lung function measurements are obtained from healthy children, aiming to present normal lung function, where body size and age are used as proxies for chest size and maturity.[48] With reliable reference values largely lacking[48] and objective lung function measurements being difficult to perform on young children, potential diagnoses are often based on symptoms reported by parents.[7] While it is possible to obtain lung function tests from older children actively participating, it has historically been challenging assessing lung function in infants and young children without sedation. Commonly, chloral hydrate has been used as a sedative[50] in in- and outpatient facilities;[51] a drug negatively affecting normal ventilation,[52] associated with several cases of overdosing, respiratory depression, cardiopulmonary arrest and fatal events.[51] Studies suggest future research ought to concentrate on lung function techniques, allowing repeated measurements in awake young children.[49]

Assessing lung function in awake young children, tidal breath flow-volume (TFV) loops can be analyzed [49,53], presenting compound measurements including size of airways, mechanical characteristics[54] and respiratory control[55] and may expose abnormal patterns of breathing and airway obstruction.[56] The technique allows lung function measurements regardless of age and ability to cooperate, providing important information in both research and clinical settings. To reduce intra-individual variation, measurements should be performed under same

circumstances on each occasion; awake children in a friendly setting[53] calmly breathing into a face mask (**Figure 3**).[57]

Figure 3*. Tidal flow-volume loop measurement in the awake state at three months.*

* Reproduced with parental consent.

With a lower ratio of time to peak tidal expiratory flow (t_{PTEF}) to total expiratory time (t_E), t_{PTEF}/t_E (**Figure 4**), previous studies using TFV loops in the awake state were able to identify infants with lower lung function[58,59] and older infants with airway obstruction,[60] distinguish asthmatic from non-asthmatic children,[61] as well as track lung function from birth to adolescence in asthmatic children with allergic comorbidities.[62] Lower t_{PTEF}/t_E is further associated with preschool wheeze[63,64] and asthma later in childhood.[59,65] In addition, infants and children with obstructive respiratory diseases generally reach t_{PTEF} earlier (and

consequently after a smaller expiration volume) during the expiratory phase.[66] Representing one of the two TFV loop indices comprising the more common t_{PTEF}/t_E ratio, lower t_{PTEF} values have been reported in infants and children with airway obstruction and asthma.[66-68] However, since few longitudinal studies yet have confirmed the usefulness of the method, evidence for routine monitoring with TFV loops is still lacking.[56] Moreover, existing reference values are largely based upon measurements performed in sleeping or sedated infants.[69] As merely one study from the 1990's compared TFV loops according to arousal state suggesting separate reference values for sleeping and awake infants may be required,[70] further research is needed.

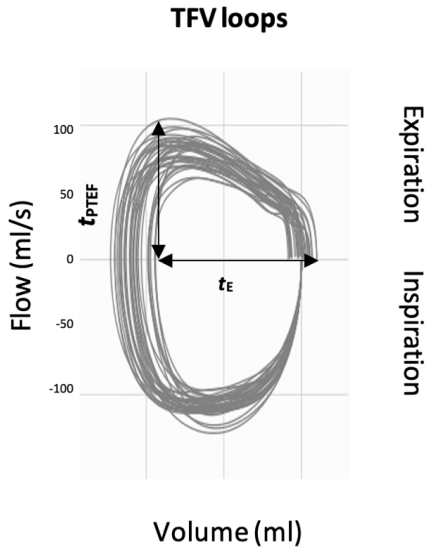

***Figure 4**. Illustration of tidal flow-volume (TFV) loops, including the TFV loop parameters of time to peak tidal expiratory flow (t_{PTEF}) and expiratory time (t_E) comprising the t_{PTEF}/t_E ratio.*

Knowledge gap

→ Does lung function by TFV loops differ while awake compared to the sleeping arousal state in early infancy, and is lower infant lung function in the awake state associated with the development of preschool asthma?

1.1.3.2 Infant skin barrier function

Upholding the homeostasis by balancing body temperature, hydration, endocrine and immune system,[71] the skin and airway epithelial constitute a natural protective barrier from allergens, microbes and environmental pollutants.[72] However, modern-day lifestyle factors such as frequent bathing, soap use, heating, air conditioners and dehumidifiers can negatively affect the epithelial barrier function.[73] Advances in research suggest skin barrier impairment may contribute to the development of inflammatory diseases, including allergic conditions of the skin and the lungs.[74,75]

Of all children, about one fifth suffer from AD.[35,36] In the development of AD, reduced epidermal barrier function and dysregulation of the immune system are important factors, on

which both genetic and environmental factors have influence.[76] Among genes associated with allergic diseases, Filaggrin (*FLG*) gene mutations are believed to contribute to the atopic march,[28] foremost linked to AD, but also AS[77] and food allergy, asthma and allergic rhinitis.[73] Filaggrin is a protein essential for the skin barrier function by creating a permeable barrier, producing natural emollients protecting from transepidermal water loss (TEWL) and maintaining the pH balance protecting the skin from harmful microbes.[71,73] *FLG* mutations increase the TEWL, change the structure of the epidermis and leave the skin dry.[71] Not only does elevated TEWL reflect a dysfunctional skin barrier function, as seen in AD, elevated TEWL can also precede clinical manifestations of the disease.[78] Since *FLG* is not expressed in the upper airways,[73] though this evidence seems conflicting,[79] it is likely that innate cells of the skin[80] and lungs[81] initiate a cascade of complex immunological responses triggered by allergens entered through *FLG*-deficient skin, leading to AS to specific allergens (IgE), involved in both AD and asthma development (**Figure 5**).[30,73] When encountering the same allergens later in life, perhaps through the airways, the body reacts and triggers a local inflammatory process in the lungs resulting in airway obstruction and asthma.[35,75] In support of this theory, studies have shown that *FLG* mutations are associated with AD, AS and asthma in children with previous history of AD.[82] As the first defense against allergens and other environmental irritants, reduced skin barrier may simply function as a facilitator for AD, leading the pathway from inflammation and sensitization to allergens via the skin to allergic expressions in the respiratory system.[35,71,83] However, the relationship between high TEWL, eczema and *FLG* mutations in early life and infant lung function as well as preschool asthma is largely unexplored.

Knowledge gap

→ Is skin barrier function by TEWL, eczema and *FLG* mutations in early infancy associated with infant lung function by TFV loops in the awake state, as well as the development of preschool asthma?

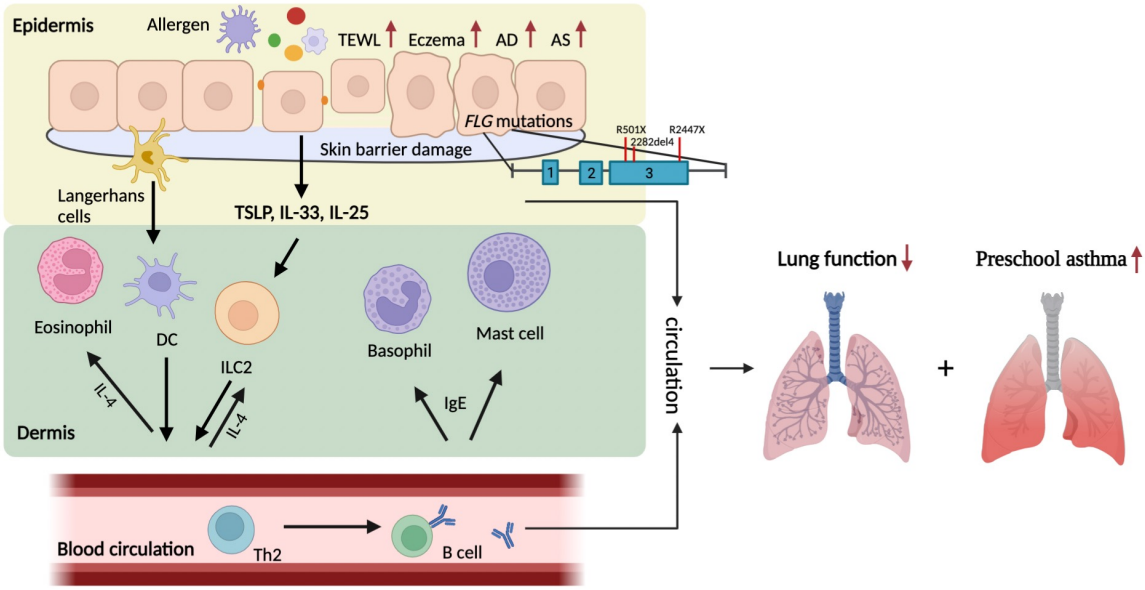

Figure 5. *Model of the possible contribution of skin barrier impairment through the type II (Th2) inflammation, a complex immune response characterized by the manifestation of Th2 cells and IgE,[2] present in most individuals suffering from asthma and other allergic diseases.[84] In the first step, food and inhalant allergens penetrate the skin due to barrier impairment caused by factors such as loss-of-function mutations (R501X, 2282del4, R2447X) in the FLG gene, high TEWL and/or the presence of eczema. Secondly, innate epithelial cells in the skin secrete cytokines (for instance TSLP, interleukin (IL)-25, and IL-33), stimulated by allergens. Further, innate immune cells (such as eosinophils, DC, ILC2, basophils and mast cells) are triggered, releasing cytokines (such as the IL-4) and create Th2 cells producing IgE in the lymphatic organs. The Th2 cells additionally release cytokines (IL-4) to stimulate more eosinophils and ILC2, whereas IgE have the ability to incite basophils and mast cells. Thirdly, this positive feedback loop of IgE to mast cells and basophils causes the phenotype of AD. TSLP, IL-25, IL-33, Th2 cells and IgE are thought to enter the lungs via the blood circulation and hereby lead to the development of asthma. The original figure by Yang et al. has been adapted.[30]*

TEWL: transepidermal water loss, FLG: filaggrin, TSLP: thymic stromal lymphopoietin, IL-33: interleukin-33, IL-25: interleukin-25, IL-4: interleukin-4, DC: dendritic cells, ILC2: innate lymphoid cells type 2 Th2: T-helper type 2 cells, IgE: immunoglobulin E, AD: atopic dermatitis, AS: allergic sensitization
* The illustration was created with Biorender.com

1.1.3.3 Early-life eosinophil inflammation

An inappropriate immune response to allergens is the shared characteristics of allergy, and may contribute to the development of allergic diseases, including asthma.[75] IgE reactions are common, but hypersensitivity responses are not seen in all with allergic diseases.[33] Though

childhood asthma is heterogeneous,[7] many children with asthma share immunological clinical features causing inflammation, hyperresponsiveness and obstruction of the airway,[85] in which eosinophils may play an important part.[86,87]

Eosinophils are a type of white blood cells of the innate immunity with a homeostatic function in the body's immune responses against parasites, bacteria and viruses, but commonly also play a pathologic role in inflammatory diseases, such as asthma.[88] Allergic eosinophil asthma, characterized by the type II inflammation in the airways, is accountable for approximately half of all asthma cases, and even higher in pediatric populations.[89] Stimulated by allergens and infections encountered in the epithelial mucosa,[90] activated eosinophils secrete pro-inflammatory degranulation products, such as the eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), and major basic protein (MBP), and are found in various body fluids, including nasal mucus, sputum, urine and blood.[91] These degranulation products hold cytotoxic and anti-infectious properties,[92] which in the lungs cause tissue damage and dysfunction, mucus hypersecretion, airway remodeling[93] and inflammation, characteristics also observed in children with asthma.[86]

During the past decades, ECP has previously been of particular interest as a promising biomarker in childhood asthma, today widely characterized and studied.[94] In preschool- and school children, higher ECP levels have been found in children with wheeze,[95] asthma,[96] allergic asthma[97] as well as AS,[97] and AD,[98] associated with wheeze, asthma,[96,99] asthma severity,[97,100] as well as AS[101], AD severity,[102] and change in lung function by tidal flow-volume (TFV) loops after bronchodilator treatment in wheezing children.[103]

Unlike ECP, serum EDN is less sticky showing negligible tube binding after collection, and may thus require smaller specimen amounts, a beneficial characteristic when sampling young children.[93] Furthermore, EDN lacks a clear biological rhythm, and appears to retain stable serum concentrations over time and at different temperatures.[104] Interestingly, higher EDN levels are detectable in tissues and body fluids long before asthmatic symptoms are evident.[86,90] Compared to healthy preschool- and school children, higher EDN levels have been reported in children with asthma,[101,105-111] linked to bronchial hyper-responsiveness,[107,108] higher symptom burden,[106,107,112] being without corticosteroid treatment,[101] as well as AS[113] and AD.[101] Similarly, higher EDN levels were found in wheezing children compared to asymptomatic children recuperating after respiratory tract infections[114] and related to the number of future episodes of wheeze.[115] Thus, EDN may have potential as a biomarker.[86] However, with reference values lacking,[93] and the relationship between early-life EDN levels and the risk of asthma in the general pediatric population remaining undetermined, its role in pediatric clinical practice is unknown.

Knowledge gap

→ Is early-life eosinophil inflammation by serum EDN levels associated with the development of preschool asthma?

1.1.3.4 Sex differences

The prevalence of wheeze and asthma is higher in young boys compared to girls.[4,20,116] One leading theory for this trend is the anatomical differences observed between the sexes. Already in early life, female fetuses to a larger extent display signs of lung maturation, such as mouth movements, in fetal life related to breathing and surfactant production.[117] Postnatally, growth of airways is proportional to lung size in girls,[117] whereas boys have smaller airways in relation to lung size.[4] Though likely multifactorial, this structural discrepancy may contribute to the higher asthma prevalence in young boys.[4] In adolescence this pattern reverses to a female predominance.[4,116] Although the underlying mechanism for this change in puberty remains undetermined,[116] the focus shifts to female sex hormones in combination with sex-specific differences in environmental exposures altering immune system responses.[20,116,118] Other possible explanations are genetic differences together with clinical characteristics and symptom severity varying by sex and with age, influencing diagnosis and treatment, ultimately leading to the discrepancy observed in reported asthma prevalence.[116]

Knowledge gap

→ Do the possible associations between infant lung function and skin barrier function as well as early-life eosinophil inflammation and the development of preschool asthma differ by sex?

2 RESEARCH AIMS

The overall aim of this doctoral thesis was to explore early risk factors for the development of preschool asthma, related to infant lung function, eosinophil inflammation and skin barrier function in early life (**Figure 6**).

Specific aims	***Sub study***
• To determine if infant lung function differed while awake compared to the sleeping state, and investigate the association between lung function in early infancy and the development of preschool asthma.	I, IV
• To explore the possible association between skin barrier function and lung function in early infancy as well as the development of preschool asthma.	II, IV
• To identify if early-life eosinophil inflammation is associated with the development of preschool asthma.	III
• To assess if the potential associations between infant lung-, and skin barrier function as well as early-life eosinophil inflammation and the development of preschool asthma differed by sex.	III, IV

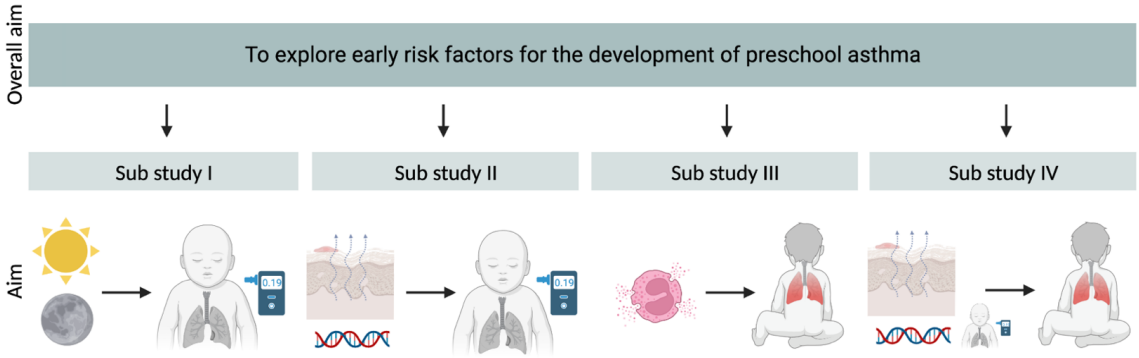

***Figure 6**. Illustration of the sub studies (**I-IV**) included in the thesis.*

* The illustration was created with Biorender.com

3 MATERIALS AND METHODS

3.1 STUDY DESIGN

The four sub studies (**I-IV**) included in this thesis were observational studies, including data prospectively collected in the Preventing Atopic Dermatitis and ALLergies in children (PreventADALL) birth cohort study.[119]

3.1.1 The PreventADALL study

The PreventADALL study is a Scandinavian, multicenter, population-based, prospective birth cohort study with two 2x2 factorially designed randomized controlled interventions; skin care and early food introduction, aiming to determine if primary prevention of allergic diseases is possible.[119]

All pregnant women attending the routine ultrasound screening at approximately 18 weeks GA in Norway (Oslo University Hospital and Østfold Hospital Trust) and Sweden (Karolinska University Hospital, Ultragyn AB, Ultraljudsbarnmorskorna, Solna barnmorskemottagning, Danderyds sjukhus, BB-Sophia, Mama Mia, Södersjukhuset, Södertälje sjukhus and BB-Stockholm) were invited to participate. Exclusion criteria were insufficient language skills (Norwegian or Swedish), planning to move away from study site within the first year postnatally, or pregnancy with more than two fetuses. From December 2014 to October 2016, 2697 women were recruited; 2149 in Norway and 552 in Sweden.[119]

The last infant was born in April 2017, rendering 2397 mother-child pairs, of which three later withdrew consent. Exclusion criteria were premature birth (<35 weeks GA) and severe malformations or disease. At birth, infants were randomly allocated (1:1:1:1) into one of four groups; “skin intervention” (oil-baths and facial cream at least four days per week, from two weeks to nine months), “food intervention” (complementary feeding of peanut, cow’s milk, wheat and egg, from three months), “combined skin and food intervention” or “no intervention” (**Figure 7**).[119] Neither the skin, nor the food intervention reduced the risk of AD in the first year of life.[120] However, early complementary food introduction reduced the risk of food allergy at preschool age.[121]

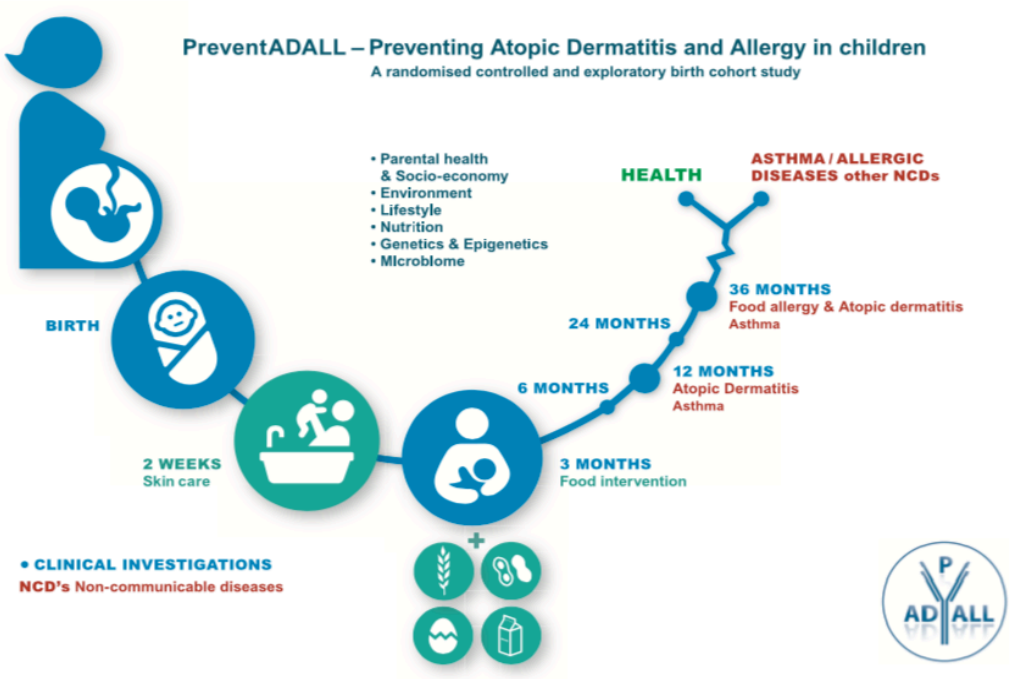

Figure 7*. Flowchart of the PreventADALL birth cohort study, illustrating the skin and food interventions, time points for data collection and outcomes.*

3.2 DATA COLLECTION

The four sub studies (**I-IV**) in this thesis focused on information gathered about the participating PreventADALL children, from early infancy to preschool age (**Figure 8**).

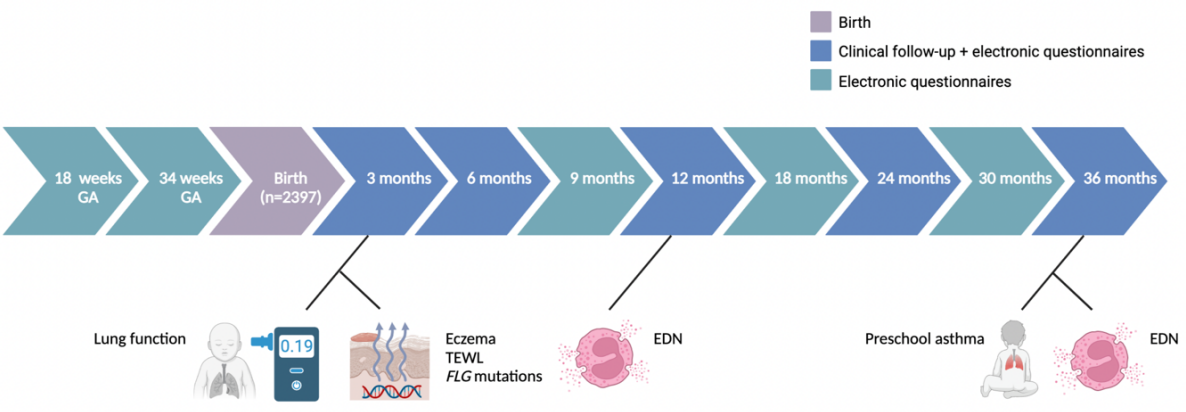

***Figure 8**. Timeline of the PreventADALL birth cohort study, including time points for data collection through clinical follow-ups and electronic questionnaires. The four sub studies (**I-IV**) in the current thesis explored the associations between infant lung function, TEWL, eczema at three months, FLG mutations, as well as EDN levels at one and three years and preschool asthma at three years.*

GA: gestational age, TEWL: transepidermal water loss, EDN: eosinophil-derived neurotoxin
* The illustration was created with Biorender.com

3.2.1 Follow-up and data collection

Shortly after enrollment, after informed consent was collected, mothers answered two electronic questionnaires at approximately 18 and 34 GA weeks, gathering information on maternal health, including allergic diseases, together with lifestyle and environmental factors and information about the father.

Within the first day after birth, after consent from the parent(s) was given, the infants were included and randomly allocated to one of the PreventADALL intervention groups by study personnel visiting the maternity wards. At the same occasion, birth data, anthropometrics, and biological samples were collected.

Comprehensive electronic questionnaires were sent every third month the first year starting from three months, thereafter twice annually. Clinical follow ups were performed at three, six and 12, 24 and 36 months. Data collection included measurements of weight, length, lung function, skin barrier function, allergic diseases, and biological sampling, all conducted by

trained study personnel following standard operating procedures and the PreventADALL study protocol.

3.2.1.1 Infant lung function

Lung function by TFV loops was measured at the three-, 12- and 36-month clinical follow-ups. In infancy, lung function measurements were overall performed prior to other investigations, in the arousal state of which the infants arrived at the study site to ensure infants were calm. If the first TFV measurement was conducted while the infant was asleep, a second test was attempted in the awake state. Infants were positioned in a supine position in the caregiver's lap or the cot, whichever was successful. Using the employed Exhalyzer® D (Eco Medics AG, Duernten, Switzerland), TFV loops were sampled through an ultrasound flow head and a dead-space reducer, with a carefully fitted face mask with an air-inflated rim, by a stable grip over the mouth and nose of the infant, using fingers to control for minimal leakage from the mask, according to existing guidelines.[57,122] When the infant breathed calmly and evenly into the mask, loop sampling started, aspiring to record at least ten consecutive breaths.

All TFV loop measurements were manually analyzed by three reviewers in Oslo (Norway) and Stockholm (Sweden), focusing on loop shape and reproducibility (**Figure 9**). Technically unacceptable loops were manually removed before approval. Further information about the standard operating procedure for TFV loop measurements and manual loop selection is described elsewhere.[123] At three months, 1183 infants had at least one acceptable TFV loop measurement; 899 in the awake state, 375 in the sleeping state, and 91 in both the awake and sleeping state.

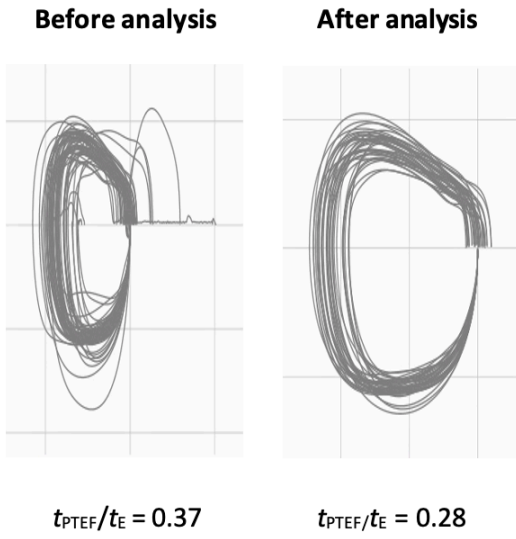

***Figure 9**. Visual description of tidal flow-volume (TFV) loops before and after the manual loop selection.*

t_{PTEF}/t_E: time to peak tidal expiratory flow to expiratory time

3.2.1.2 Clinical skin assessments

Clinical skin examinations were carried out by trained study personnel at three, six, 12, 24 and 36 months educated together at workshops to minimize inter-observer variability. In infancy, the parents were advised not to bathe their children in oils or apply skin emollients 24 hours prior to the clinical follow-ups.

Clinically observed eczematous skin lesions were confirmed by physicians excluding common differential diagnosis to AD, such as seborrheic and contact dermatitis.[124] AD was assessed using the United Kingdom Working Party (UKWP) criteria in the first year of life, UKWP and Hanifin and Rajka criteria at the subsequent clinical follow-ups.[125]

TEWL (g/m^2/h) was measured at three, six, 12 and 36 months on the left lateral upper arm with an open chamber DermaLab USB (Cortex, Hadsund, Denmark), at room temperature between 20-25 °C and within acceptable ranges of humidity, in line with previous findings.[126] After 15 minutes of acclimatization, with the children wearing only diapers or underpants, three consecutive measurements were performed in calm children, distanced from direct sunlight, while windows and doors were kept closed.

3.2.1.3 Serum eosinophil-derived neurotoxin and FLG mutations

Blood samples were collected from cord blood at birth and at clinical follow-ups at three, six, 12, 24 and 36 months. All samples were kept in room temperature for 60-90 minutes before centrifugation and separation of serum and plasma, thereafter, kept at -20 °C for five to seven days before being transferred for storage at -80 °C.

Serum EDN at 12 and 36 months was measured using an automated ImmunoCAP EDN research assay (Thermo Fisher Scientific, Uppsala, Sweden). Description and performance of the method is reported elsewhere.[109]

DNA was extracted from blood and genotyped for the most common European loss-of-function mutations in the *FLG* gene (R501X, 2282del4 and R2447X), using the TaqMan-based allelic discrimination assay, as described by Hoyer *et al*.[127]

3.2.1.4 Preschool asthma

Information on doctor-diagnosed asthma and wheeze was collected from questionnaires at three, six, nine, 12, 18, 24, 30 and 36 months and clinical follow-ups at 24 and 36 months. Asthma medication use was retrieved from questionnaires at 12, 18, 24, 30 and 36 months. To compensate for missing information on medication use in some participants, the children's medical records were reviewed for the prescription of asthma medication between nine months through three years.

Allergic sensitization

Skin prick tests (SPT) were performed at three years using standard allergen solutions for egg, cow's milk, peanut, wheat, soy, cod, birch, grass, dog, cat, and house dust mite (Soluprick ALK-Albelló, Hørsholm, Denmark). In addition, at the same time point analyses for specific serum immunoglobulin E (IgE) to egg, cow's milk, peanut, wheat, soy, cod, birch, grass, mugwort, dog, cat, horse, house dust mite, and mold (Cladosporium herbarium) were performed using the ImmunoCAP (Thermo Fisher Scientific).

3.3 STUDY POPULATIONS

The study populations of the included sub studies (**I-IV**) are shown in **Figure 10**.

Sub study I: 91 infants with available infant lung function measurements (TFV loops) in the awake and sleeping state at three months.

Sub study II: 899 infants with available infant lung function measurements (TFV loops) in the awake state as well as either TEWL, eczema at three months and/or *FLG* mutations.

Sub study III: 1233 children with available serum EDN levels at one and/or three years.

Sub study IV: 1337 children with available information related to the presence or absence of preschool asthma at three years, as well as either infant lung function measurements (TFV loops) in the awake state, TEWL, eczema at three months and/or *FLG* mutations.

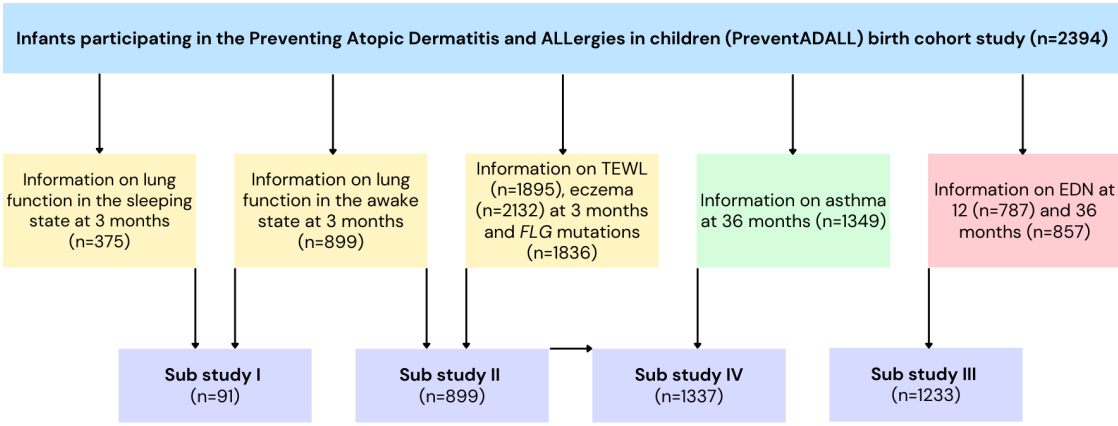

***Figure 10**. Flow chart of the study populations in the four sub studies (**I-IV**) included in this thesis.*

TEWL: transepidermal water loss, FLG: filaggrin, EDN: eosinophil-derived neurotoxin

3.4 DEFINITIONS

3.4.1 Exposures

Lower infant lung function

Lower lung function at three months was defined as a t_{PTEF}/t_E <0.25, previously associated with airway obstruction and asthma.[59,128-130] In addition, a t_{PTEF} <0.17 s (<25th percentile) was chosen as secondary exposure, as lower values have previously been found in infants and children with airway obstruction and asthma (**sub study IV**).[66-68]

High transepidermal water loss

A mean TEWL value >75th percentile denoted an impaired skin barrier function, >8.83 g/m^2/h in **sub study II** and >9.50 g/m^2/h in **sub study IV** at three months, in line with previous reports.[126,131]

Eczema

As few three-month-old infants fulfil the strict diagnostic criteria for AD by the United Kingdom Working Party (UKWP),[132] eczema was used as a proxy. Eczema at three months was defined as having clinically observed eczematous skin lesions with the exclusion of common differential diagnosis to AD, such as seborrheic and contact dermatitis, confirmed by physicians (**sub study II and IV**).[124]

Filaggrin mutations

Filaggrin mutations were defined as being carriers of any of the three mutations (R501X, 2282del4 and R2447X),[127] hypothesized to contribute to asthma **(sub study II and IV**).[36]

Higher eosinophil-derived neurotoxin levels

Higher EDN levels at one and three years equal to EDN levels ≥26.7 and ≥20.5 µg/L, respectively (**sub study III**).

3.4.2 Primary outcomes

Lower infant lung function

Lower lung function at three months was defined as a t_{PTEF}/t_E <0.25, previously associated with airway obstruction and asthma (**sub study I and II**).[59,128-130] In addition, a t_{PTEF} <0.17 s (<25th percentile) was chosen as secondary outcome, as lower values have previously been found in infants and children with airway obstruction and asthma (**sub study II**).[66-68]

Preschool asthma

Preschool asthma at three years was defined as having recurrent bronchial obstruction (≥3 episodes of recurrent bronchial obstructions) (rBO) between two and three years, and fulfilling at least one of the following criteria; 1) doctor-diagnosed asthma between zero and three years, and 2) any use of and/or prescribed asthma medications (bronchodilators, inhaled corticosteroids or leukotriene-antagonist) between nine months through three years (**sub studies III and IV**).[133]

3.4.3 Secondary outcomes

Allergic sensitization

Allergic sensitization at three years was defined as having positive SPT (≥3 mm) and/or specific IgE level (>0.35 kU$_A$/L) towards any allergen (egg, cow's milk, peanut, wheat, soy,

cod, birch, grass, mugwort, dog, cat, horse, house dust mite and/or mold) at three years (**sub study III**).

Atopic dermatitis

Atopic dermatitis at three years was defined as fulfilling the United Kingdom Working Party and/or Hanifin and Rajka criteria at three years (**sub study III**).[125]

Children with any allergic disease

Any allergic disease at three years was defined as children with either any wheeze, preschool asthma, AS and/or AD at three years (**sub study III**).

Non-atopic children

Non-atopic children at three years were defined as children with no history of any wheeze, doctor-diagnosed asthma, use of and/or prescribed asthma medications, AS or AD between birth and three years (**sub study III**).

3.5 STATISTICAL ANALYSES

Statistical analyses in the four sub studies (**I-IV**), conducted using IBM SPSS Statistics (version 26 or later) or RStudio (4.0.3), are presented in **Table 1**. Statistical significance level was set to 0.05.

Descriptive

Overall, continuous variables with normal distribution were presented with means and standard deviations (SD) and/or minimum-maximum (min-max), while numbers (n) and percentages (%) were used to describe dichotomous/categorical variables (**sub study I-IV**). In addition, median, interquartile range (IQR) and/or the 95th percentile were provided to describe continuous variables when appropriate (**sub study II**), as well as when presenting data with skewed distribution (**sub study III**).

Continuous variables were compared between independent groups using parametric and non-parametric tests; independent t-tests (parametric) for variables with normal distribution (**sub study II-IV**), while Mann Whitney-U test (non-parametric) was used for data with skewed distributions (**sub study III**). Paired samples of continuous variables with skewed distributions were compared using the non-parametric Wilcoxon's signed-rank test (**sub study I**, **III**).

The non-parametric Chi^2 tests or Fisher's exact tests (small sample sizes, expected counts <5) were used to compare distributions of dichotomous/categorical variables between independent groups (**sub study II-IV**). Paired samples of dichotomous variables with skewed distributions were compared using the non-parametric McNemar test (**sub study I**).

Associations

Univariate multivariate logistic regression was used to assess associations to dichotomous outcomes (**sub study II-IV**), while multinominal logistic regression was applied to investigate outcomes with more than two categories (**sub study IV**); all models presented with odds ratios (OR) with 95% confidence intervals (95% CI). In **sub study IV**, the relationship between dichotomized exposures and outcomes was assessed with Spearman's rank correlation, presented with Spearman's Rho coefficient (95% CI). Similarly, in **sub study III**, the relationship between exposure (repeated measurements) and outcome was explored using mixed effects logistic regression, presented with OR (95% CI). Univariate conditional logistic regression was used in **sub study I** to estimate OR (95% CI) for dichotomous outcomes of paired samples.

To study the robustness of the findings (**sub study II)**, continuous outcomes were used in univariate and multivariate linear regression models, while the univariate relationship between skewed distributions of continuous exposures at two ages were explored in robust linear regression (**sub study III)**, both presented with ß coefficients (95% CI).

Primarily based on the literature, potential covariates and confounders to exposures and outcomes were selected in prior to the analyses. However, associations between birth and background characteristics and outcomes were explored to reveal possible effect modifiers, and were included in multivariate regression models when applicable.

Interactions

Possible modification of the skin and/or food intervention on the association between exposures and outcomes were assessed in interaction analyses in **sub studies II-IV**, by including interaction terms in univariate and multivariate logistic regression models.

Predictions

To objectively define cut-off levels with highest sensitivity and specificity of the outcomes, Youden's Index was calculated,[134] whereafter continuous exposures accordingly were dichotomized (**sub study III)**. The predictive accuracy of exposures to differentiate children with the outcomes was tested using receiver operating characteristics (ROC) analyses (**sub study III-IV**), presented with area under the ROC curve (ROC-AUC) with 95% CI, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in percentages. PPV and NPV were calculated using Chi^2 tests.

Table 1. *Overview of the statistical methods used in the four sub studies (**I-IV**).*

	Sub study I-IV			
Characteristics	**I**	**II**	**III**	**IV**
Descriptive				
Continuous variables				
Independent t-test (independent, parametric)		X	X	X
Mann-Whitney U-test (independent, non-parametric)			X	
Wilcoxon's signed-rank test (paired, non-parametric)	X		X	
Dichotomous/categorical variables				
Chi^2 test or Fisher's Exact test (independent, non-parametric)		X	X	X
McNemar test (paired, non-parametric)	X			
Associations				
Dichotomous/categorical outcomes				
Logistic regression		X	X	X
Multinominal logistic regression				X
Spearman's rank correlation				X
Conditional logistic regression (paired)	X			
Mixed effects logistic regression (repeated measurements)			X	
Continuous outcomes				
Linear regression (normal distribution)		X		
Robust linear regression (skewed distribution)			X	
Interactions				
Logistic regression (interaction terms)		X	X	X
Predictions				
Youden's Index			X	
ROC analyses			X	X

3.6 METHODOLOGICAL SUMMARY

***Table 2**. Methodological summary of the four sub studies (**I-IV**).*

	Sub study I-IV			
Characteristics	**I**	**II**	**III**	**IV**
Study design	Observational study	Observational study	Observational study	Observational study
Cohort	PreventADALL	PreventADALL	PreventADALL	PreventADALL
Study population	n=91	n=899	n=1233	n=1337
Exposure(s)	Arousal state	TEWL, eczema *FLG* mutations	EDN	Lung function, TEWL, eczema, *FLG* mutations
Outcome	Lung function	Lung function	Preschool asthma	Preschool asthma
Data source(s)	3-month clinical follow-up	3-month clinical follow-up	12-, 24-, 36-month clinical follow-up	3- 12-, 24-, 36-month clinical follow-up
			Electronic questionnaires 3-36 months	Electronic questionnaires 3-36 months
			Medical records 9 months through 3 years	Medical records 9 months through 3 years
Data analyses	Descriptive	Descriptive	Descriptive	Descriptive
	Associations	Associations	Associations	Associations
		Interactions	Interactions	Interactions
			Predictions	Predictions

3.7 ETHICAL CONSIDERATIONS

All four sub studies in this thesis were conducted in line with the ethical principles of the Declaration of Helsinki,[135] and was approved by the Regional Committee for Medical and Health Research Ethics in Norway (2014/518), and the Swedish Ethical Review Authority (2014/2242-31/4) with multiple amendments, including the approval of the clinical follow-up at three years (2018/1437-32). To respect the participants autonomy and right to make informed decisions, written consent was collected from all mothers at enrolment, and from parent(s) at infant inclusion, which for the children at present acts as a surrogate consent. Acknowledging children as a vulnerable group, weighing risks and benefits, the trained investigators always discussed painful or discomfortable procedures with parents and tried to prepare the children in advance when possible. For instance, to minimize pain and discomfort a local aesthetic cream was always applied prior to venipuncture, numbing the skin. Aiming to make children feel calm and safe at the time for blood sampling, they were placed in the laps of the parents, often eating a fruit or a biscuit, watching children's television programs. The procedure is relatively quick, and only one attempt was allowed if the children were uneasy or the possibilities to sample blood were considered low. Children unsettled or unwilling to perform certain parts included in the clinical follow-up were never forced to complete the entire investigation. Ongoingly, parents are informed about the progress and novel research results published within the PreventADALL study.

To ensure the security of the study participants data, in compliance with the General Data Protection Regulation (GDPR) legislation, the retrieved information is pseudonymized and located on a secure remote desktop, only accessible for students and supervisors within the PreventADALL research group.

4 RESULTS

Main findings of the four included sub studies (**I-IV**):

4.1 INFANT LUNG FUNCTION

4.1.1 Infant lung function and arousal state

To determine if lung function differed by arousal state in early infancy, we compared TFV loops while awake to the sleeping state in 91 healthy infants at three months (**sub study I**).

In the 91 infants with TFV loops in both arousal states, t_{PTEF}/t_E was higher, t_E was shorter, while t_{PTEF} was similar while awake compared to the sleeping state (**Table 3, Figure 11**). Six (6.6%) of the 91 infants had a t_{PTEF}/t_E <0.25 in the awake state, compared to 33 (36.3%) in the sleeping state (*P*-value < .001). The OR of having a ratio of t_{PTEF}/t_E <0.25 was higher in the sleeping compared to the awake state; crude OR (95% CI) 10.0 (3.05, 32.8).

***Table 3**. Tidal-flow volume loop parameters in the awake and sleeping state at three months (n=91).*

	Awake state	**Sleeping state**	
Characteristics (n=91)	Mean (95% CI)	Mean (95% CI)	***P*-value***
Lung function parameters			
Number of loops included	21 (18, 24)	33 (30, 37)	**< .001**
Respiratory rate (*RR*) per minute	53 (51, 56)	38 (37, 40)	**< .001**
Peak tidal expiratory flow (PTEF) in ml	110 (105, 116)	84.1 (80.0, 88.2)	**< .001**
Time parameters			
Ratio t_{PTEF}/t_E	0.39 (0.37, 0.41)	0.28 (0.27, 0.29)	**< .001**
Time to PTEF (t_{PTEF}) in s	0.24 (0.23, 0.26)	0.25 (0.24, 0.26)	0.402
Expiratory time (t_E) in s	0.65 (0.61, 0.68)	0.93 (0.88, 0.98)	**< .001**
Volume parameters			
Volume at PTEF (V_{PTEF}) in ml	18.4 (17.2, 19.6)	14.2 (13.5, 15.0)	**< .001**
Expiratory volume (V_E) in ml	49.1 (46.4, 51.7)	49.7 (47.4, 51.9)	0.834
Tidal volume (V_T) in ml	49.4 (46.7, 52.1)	50.5 (48.2, 52.8)	0.598

* Wilcoxon signed-rank test of paired samples

Ratio t_{PTEF}/t_E: ratio of time to peak tidal expiratory flow to expiratory time.

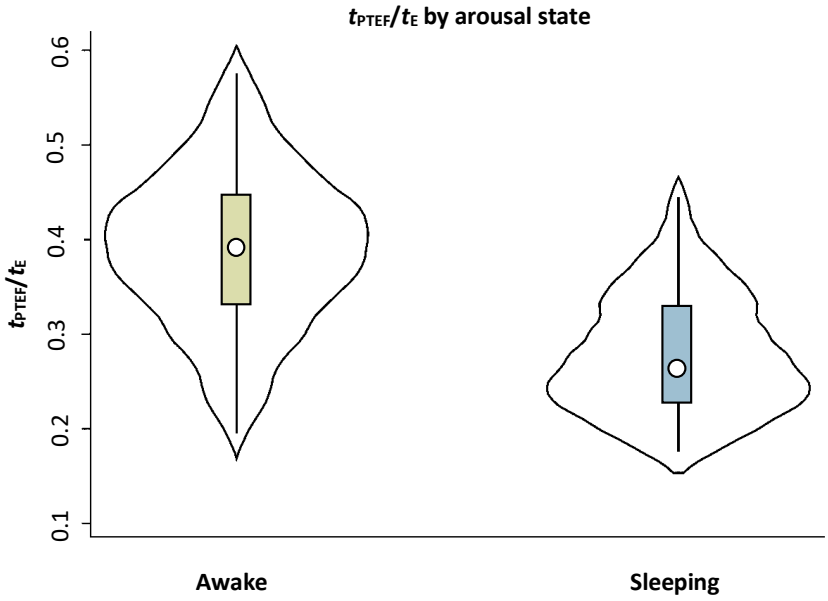

***Figure 11**. Distributions of the t_{PTEF}/t_E ratio in awake and sleeping tidal-flow volume loop measurements at three months (n=91).[136] The bottom and top of the boxes represent the second quartile and third quartile, and the circles are the means. The vertical lines below and above the boxes are the first and fourth quartiles.*

t_{PTEF}/t_E: ratio of time to peak tidal expiratory flow to expiratory time

4.1.2 Infant lung function and preschool asthma

In 1337 children with information related to the presence or absence of preschool asthma at three years as well as either TFV loops in the awake state, TEWL, eczema at three months and/or *FLG* mutations, the association between infant lung function and preschool asthma was explored (**sub study IV**). Information related to the presence or absence of preschool asthma and infant lung function was available in 563 children, of whom 74 (13.1%) had preschool asthma. The mean ± SD t_{PTEF}/t_E and t_{PTEF} in infancy were 0.39 ± 0.08 and 0.20 ± 0.05 s, respectively.

Lower lung function by lower t_{PTEF}/t_E and shorter t_{PTEF} were associated with preschool asthma; adjusted OR (95% CI) 5.44 (2.15, 13.8) and 2.71 (1.48, 4.94), respectively (**Figure 12**).

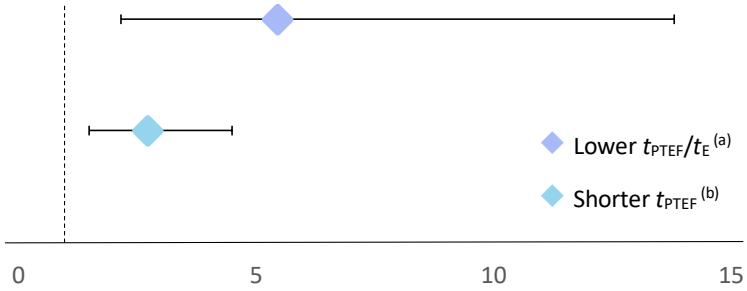

***Figure 12**. Adjusted OR (95% CI) for preschool asthma at three years, according to the presence of lower t_{PTEF}/t_E, and shorter t_{PTEF} at three months (n=563). Logistic regression models adjusted for sex, GA at birth, tobacco smoke exposure in pregnancy, urban living environment in pregnancy, breastfeeding, parental allergic disease (any), parental education level and the PreventADALL interventions.*

(a) Lower t_{PTEF}/t_E equal to a t_{PTEF}/t_E <0.25

(b) Shorter t_{PTEF} equal to a t_{PTEF} <0.17 s (<25th percentile)

t_{PTEF}/t_E: time to peak tidal expiratory flow to total expiratory time; t_{PTEF}: time to peak tidal expiratory flow, OR: odds ratio, GA: gestational age, PreventADALL: Preventing Atopic Dermatitis and ALLergies in children

4.2 SKIN BARRIER FUNCTION

4.2.1 Skin barrier function and infant lung function

In a study population of 899 infants, we explored whether reduced skin barrier function by high TEWL, manifestations of eczema at three months or *FLG* mutations, were associated with lower lung function at three months (**sub study II**). At three months, the mean ± SD TEWL was 7.94 ± 5.66 g/m^2/h (n=827), while eczema was present in 135/898 (15.0%) and *FLG* mutations identified in 73/730 (10.0%) infants. Lung function by TFV loops in the awake state was available in 899 infants, with a mean t_{PTEF}/t_E and t_{PTEF} of 0.39 ± 0.08 and 0.21 ± 0.05 s, respectively.

Overall, no associations between high TEWL, eczema or *FLG* mutations and lower lung function (t_{PTEF}/t_E <0.25) were found. However, an inverse association between high TEWL and t_{PTEF} was observed, adjusted OR (95% CI) 1.61 (1.08, 2.42) (**Figure 13**). Similar results were seen using the continuous t_{PTEF}, with a high TEWL negatively associated with t_{PTEF}; β coefficient (95% CI) -0.01 (-0.20, -0.00), while the presence of eczema or *FLG* mutations were not.

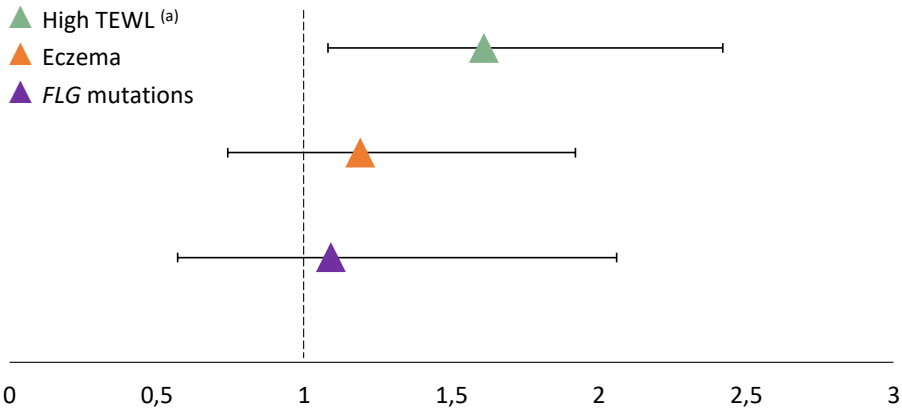

***Figure 13**. Adjusted OR (95% CI) for lower t_{PTEF} [b] at three months, according to the presence of high TEWL, eczema or FLG mutations at three months (n=899).[137] Logistic regression models adjusted for sex, GA at birth, nicotine exposure in pregnancy, parental asthma, parental education level, weight at three months and the PreventADALL skin intervention.*

[a] High TEWL equal to mean TEWL >8.83 g/m^2/h (>75th percentile) [b] Lower t_{PTEF} equal to a t_{PTEF} <0.17 s (<25th percentile)
TEWL: transepidermal water loss, FLG: filaggrin, OR: odds ratio, GA: gestational age, PreventADALL: Preventing Atopic Dermatitis and ALLergies in children

4.2.2 Infant skin barrier function and preschool asthma

To assess the associations between infant skin barrier function and preschool asthma, 1337 children with information related to the presence or absence of preschool asthma at three years as well as either TEWL, eczema at three months and/or *FLG* mutations were studied (**sub study IV**). Preschool asthma was present in 180/1337 (13.5%) children. The mean ± SD TEWL was 8.41 ± 6.12 g/m^2/h (n=1173), while 168/1318 (12.7%) had eczema at three months and 96/1045 (9.2%) carried a *FLG* mutation. In **Figure 14** and **Table 4,** the distribution of high TEWL, eczema and *FLG* mutations among the children with (n=180) and without (n=1157) preschool asthma is shown, respectively.

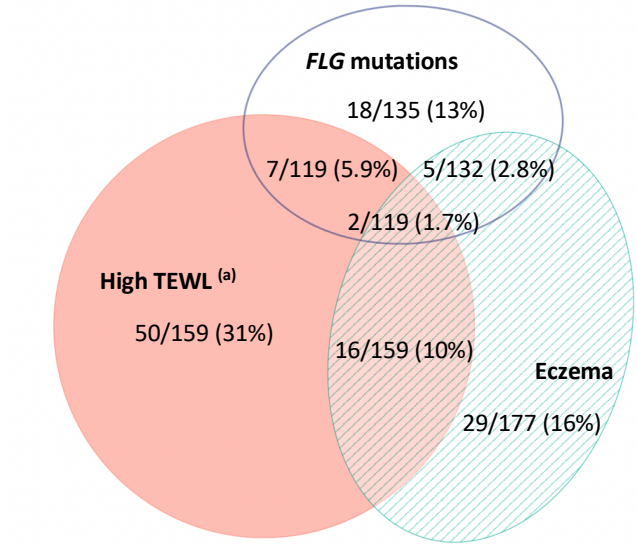

***Figure 14**. Distribution of high TEWL, eczema at three months and FLG mutations among the children with preschool asthma at three years (n=180).*[138]

(a) High TEWL equals to a mean TEWL >9.50 g/m^2/h (>75th percentile)
TEWL: transepidermal water loss, FLG: filaggrin

***Table 4**. Distribution of high TEWL, eczema at three months and FLG mutations among the children without preschool asthma at three years (n=1157).*

	Children *without* preschool asthma
Characteristics	n (%)
High TEWL (a)	244/1014 (24.1)*
Eczema	139/1141 (12.2)
FLG mutations	78/910 (8.57)

* Significant difference in distribution compared to children *with* preschool asthma at 3 years (*P*-value < .05)
(a) High TEWL equals to a mean TEWL >9.50 g/m^2/h (>75th percentile)
TEWL: transepidermal water loss, FLG: filaggrin

While a high TEWL was associated with preschool asthma; adjusted OR (95% CI) 1.61 (1.06, 2.44), eczema or *FLG* mutations were not (**Figure 15**).

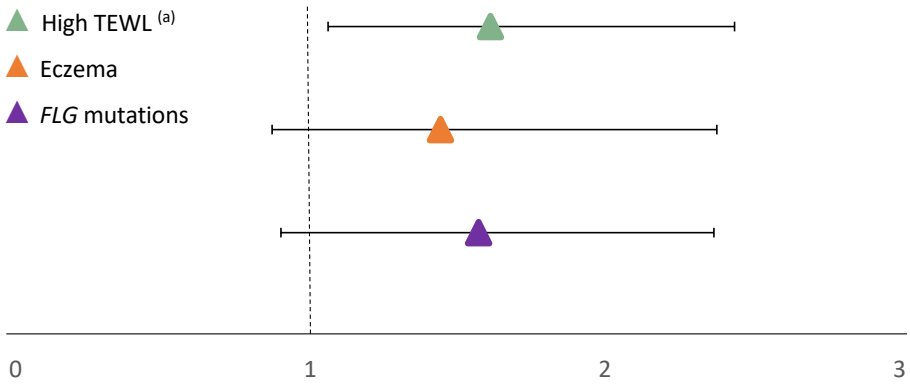

Figure 15*. Adjusted OR (95% CI) for preschool asthma at three years, according to the presence of high TEWL, eczema at three months and FLG mutations. Logistic regression models adjusted for sex, GA at birth, tobacco smoke exposure in pregnancy, urban living environment in pregnancy, breastfeeding, parental allergic disease (any), parental education level and the PreventADALL interventions. Models including FLG mutations were only adjusted for sex.*

(a) High TEWL equals to a mean TEWL >9.50 g/m^2/h (>75th percentile)
TEWL: transepidermal water loss, FLG: filaggrin, OR: odds ratio, GA: gestational age, PreventADALL: Preventing Atopic Dermatitis and ALLergies in children

4.3 EARLY-LIFE EOSINOPHIL INFLAMMATION

4.3.1 Early-life eosinophil inflammation and preschool asthma

Based on 1233 children with information on EDN levels at one and/or three years (**sub study III**), the associations between EDN levels at one and three years and preschool asthma at three years were investigated. Among the children with available EDN levels at one year and three years, 68/787 (6.8%) and 76/857 (8.9%) had preschool asthma, respectively. The median EDN levels were higher at one year compared to at three years; 27.8 µg/L versus 19.9 µg/L (*P*-value < .001) (**Figure 16**).

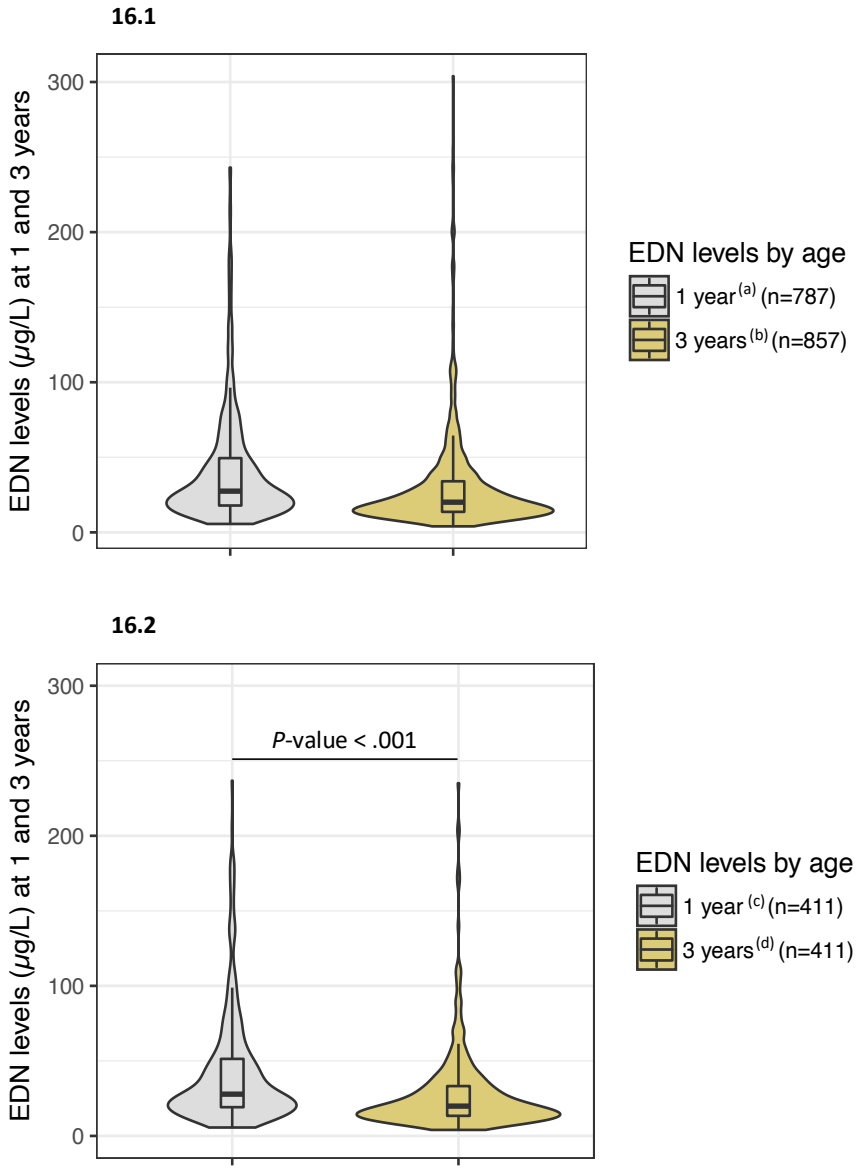

Figure 16. *EDN levels at one (n=787) and three (n=857) years (**16.1**), and in children with information on EDN at both ages (n=411) (**16.2**). From the bottom of the boxes, horizontal lines represent the second quartile, median and third quartile. Vertical lines below and above are the first and fourth quartile, respectively. The narrow tips represent EDN levels above the fourth quartile.*

(a) EDN levels at 1 year among all children with information on EDN at 1 year (b) EDN levels at 3 years among all children with information on EDN at 3 years (c) EDN levels at 1 year among children with information at both 1 and 3 years (d) EDN levels at 3 years among children with information at both 1 and 3 years
EDN: eosinophil-derived neurotoxin, µg/L: micrograms per liter

Compared to non-atopic children (no history of asthma, wheeze, doctor-diagnosed asthma, use of and/or prescribed asthma medications, AS or AD) at three years, median EDN levels at both one and three years were significantly higher in children with preschool asthma, as well as AS, AD, and any allergic disease at three years (*P*-values < .05), see **Table 5**.

Table 5*. EDN levels in children with information at one (n=787) and three (n=857) years, at both ages (n=411), as well as EDN levels at one and three years by atopic status at three years.*

	1 year		**3 years**	
Characteristics	Median (IQR; min-max)	n	Median (IQR; min-max)	n
EDN levels (µg/L) by age				
EDN at 1 year	27.4 (31.7; 5.61-243)	787		
EDN at 3 years			20.1 (20.4; 4.00-304)	857
EDN at both 1 and 3 years	27.8 (32.3; 5.61-237)	411	19.9 (20.1; 4.00-235)	411
EDN levels (µg/L) by atopic status at 3 years				
Preschool asthma [(a)]	32.6 (44.1; 6.14-237)	68	25.1 (20.5; 7.10-304)	76
Allergic sensitization [(b)]	42.0 (46.9; 9.10-205)	95	28.3 (30.5; 7.15-304)	176
Atopic dermatitis [(c)]	35.5 (43.2; 5.85-242)	154	22.5 (21.9; 5.72-254)	201
Any allergic disease [(d)]	33.5 (38.3; 5.85-242)	311	22.6 (23.0; 4.00-304)	404
Non-atopic children [(e)]	24.0 (31.1; 5.86-216)	147	17.3 (17.0; 5.00-245)	173

[(a)] Preschool asthma at 3 years was defined as having ≥3 episodes of recurrent bronchial obstruction between 2 and 3 years, and fulfilling at least one of the following criteria; 1) doctor-diagnosed asthma between 0 and 3 years, and 2) any use of and/or prescribed asthma medications (bronchodilators, inhaled corticosteroids or leukotriene-antagonist) between 9 months and 3 years [(b)] Allergic sensitization at 3 years was defined as having positive SPT (≥3 mm) and/or specific IgE level (>0.35 kU_A/L) towards any allergen (egg, cow's milk, peanut, wheat, soy, cod, birch, grass, mugwort, dog, cat, horse, house dust mite and/or mold) at 3 years [(c)] Atopic dermatitis at 3 years was defined as fulfilling the United Kingdom Working Party and/or Hanifin and Rajka criteria at 3 years [(d)] Any allergic disease at 3 years was defined as children with either any wheeze, preschool asthma, allergic sensitization and/or atopic dermatitis at 3 years [(e)] Non-atopic children were defined as children with no history of any wheeze, doctor-diagnosed asthma, use of and/or prescribed asthma medications, allergic sensitization or atopic dermatitis between birth and 3 years

IQR: interquartile range, EDN: eosinophil-derived neurotoxin, µg/L: micrograms per liter, SPT: skin prick test, kU_A/L: kilounits of allergen specific IgE per liter

Compared to non-atopic children by three years, higher EDN levels at one and three years increased the OR of preschool asthma; adjusted OR (95% CI) 2.20 (1.09, 4.41) and 4.68 (2.29, 9.55), respectively (**Figure 17**).

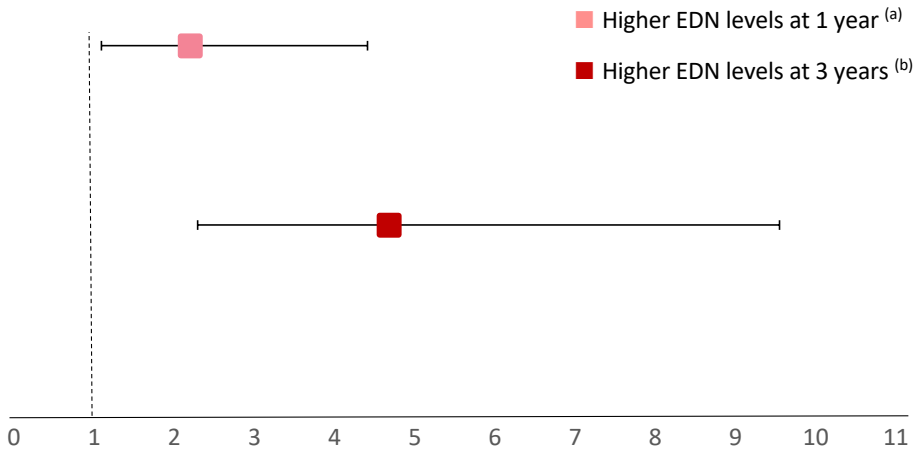

Figure 17*. Adjusted OR (95% CI) for preschool asthma at 3 years according to higher EDN levels at one and three years. Logistic regression models adjusted for sex, GA at birth, tobacco smoke in pregnancy, parental allergic disease (any), cesarean section and the PreventADALL interventions.*

(a) Higher EDN levels at 1 year by Youden's Index for preschool asthma at 3 years equal to EDN levels ≥26.7 µg/L at 1 year
(b) Higher EDN levels at 3 years by Youden's Index for preschool asthma at 3 years equal to EDN levels ≥20.5 µg/L at 3 years
EDN: eosinophil-derived neurotoxin, OR: odds ratio, GA: gestational age, PreventADALL: Preventing Atopic Dermatitis and ALLergies in children

4.4 SEX DIFFERENCES

4.4.1 Infant lung- and skin barrier function, early-life eosinophil inflammation and preschool asthma by sex

Of the 1337 children included in **sub study IV**, boys had higher rates of preschool asthma at three years (n=180) compared to girls (*P*-value < .001); 122/702 (17.4%) versus 58/635 (9.1%). Similarly, in **sub study III** (n=1233), rates of preschool asthma at three years (n=109) were higher in boys compared to girls (*P*-value =0.013); 71/482 (14.7%) versus 38/410 (9.3%). Compared to girls, the t_{PTEF}/t_E and t_{PTEF} in infancy were lower in boys (**sub study IV)**, while early-life EDN levels were higher (**sub study III**). The mean TEWL and rates of eczema in infancy and *FLG* mutations (**sub study IV**) were similar across the sexes (**Table 6**).

***Table 6**. Tidal-flow volume loop parameters (n=563), TEWL (n=1173), eczema (n=1318) at three months, FLG mutations (n=1045), as well as EDN levels at one (n=787) and three (n=857) years by sex.*

	Boys	**Girls**	
Characteristics	Descriptive statistics	Descriptive statistics	***P*-value***
TFV loop parameters at 3 months			
Ratio t_{PTEF}/t_E (n=563), mean (SD)	0.38 (0.08)	0.40 (0.08)	**0.019**
t_{PTEF} in s (n=563), mean (SD)	0.20 (0.05)	0.21 (0.05)	**< .001**
Skin conditions at 3 months			
TEWL (n=1173), mean (SD)	8.68 (5.93)	8.09 (6.32)	0.071
Eczema (n=1318), n (%)	95 (13.7)	73 (11.6)	0.244
FLG mutations (n=1045), n (%)	57 (10.2)	39 (8.0)	0.211
EDN levels (µg/L) at 1 and 3 years			
1 year (n=787), median (IQR; min-max)	32.0 (34.9; 5.95-243)	24.5 (28.1; 5.61-242)	**< .001**
3 years (n=857), median (IQR; min-max)	20.9 (21.9; 5.05-304)	19.0 (18.4; 4.00-235)	**0.018**

* Independent t-test, Chi^2 test or Mann-Whitney U-test
IQR: interquartile range, TFV: tidal flow-volume, t_{PTEF}/t_E: time to peak tidal expiratory flow to total expiratory time; t_{PTEF}: time to peak tidal expiratory flow, TEWL: transepidermal water loss, *FLG*: filaggrin, EDN: eosinophil-derived neurotoxin, µg/L: micrograms per liter

While lower t_{PTEF}/t_E and shorter t_{PTEF} were associated with preschool asthma in boys; adjusted OR (95% CI) 7.76 (2.53, 23.8) and 3.21 (1.54, 6.69), a high TEWL, eczema or *FLG* mutations were not. Carrying a *FLG* mutation was associated with the development of preschool asthma in girls; adjusted OR (95% CI) 3.48 (1.48, 8.21), while lower t_{PTEF}/t_E and t_{PTEF}, high TEWL and eczema were not (**sub study IV**), see **Figure 18**.

Higher EDN levels at one year were associated with the development of preschool asthma in boys, adjusted OR (95% CI) 4.48 (1.52, 13.2), but not in girls. At three years, higher EDN levels were associated with preschool asthma in both boys and girls; adjusted OR (95% CI) 4.22 (1.87, 9.54) and 7.58 (1.82, 31.6), respectively (**sub study III**), see **Figure 18**.

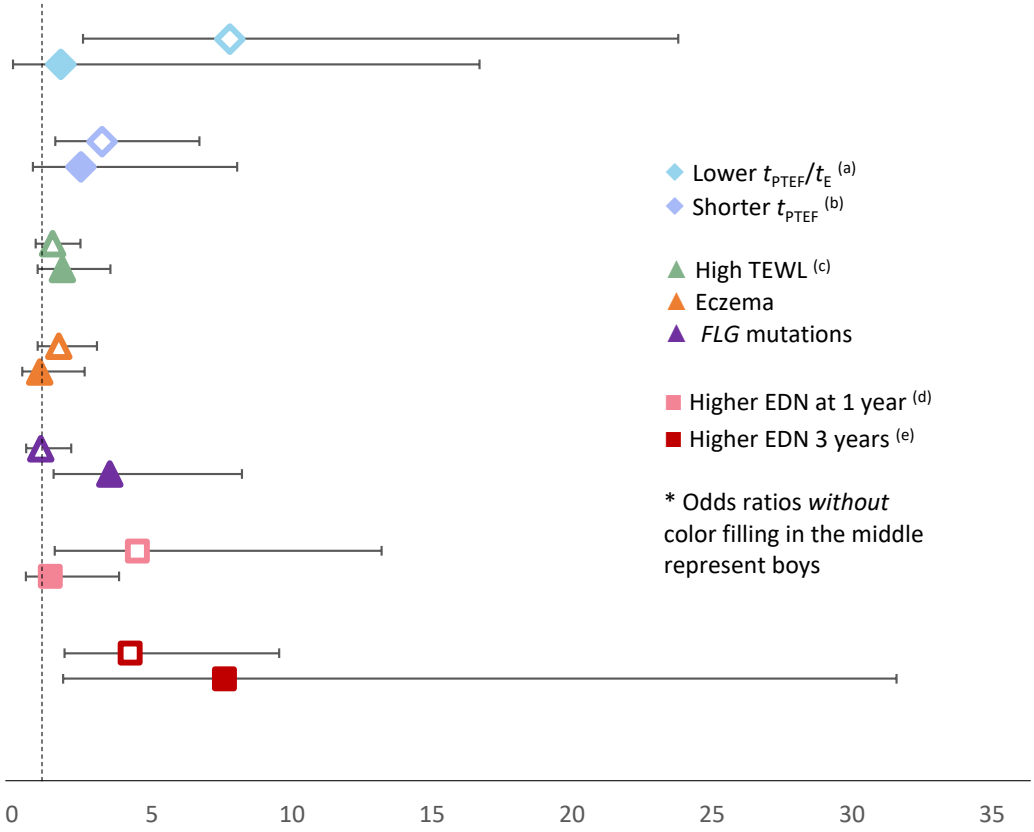

***Figure 18**. Adjusted OR (95% CI) for preschool asthma at three years by sex, according to the presence of lower t_{PTEF}/t_E and shorter t_{PTEF} (n=563), high TEWL (n=1173), eczema (n=1318) at three months, FLG mutations (n=1045) as well as higher EDN levels at one (n=787) and three (n=857) years. Logistic regression models were adjusted for GA at birth, tobacco smoke exposure in pregnancy, urban living environment in pregnancy, breastfeeding, parental allergic disease (any), parental education level, and the PreventADALL interventions. Models including FLG mutations remained unadjusted, while models including EDN were adjusted for GA at birth, tobacco smoke exposure in pregnancy, parental allergic disease (any), cesarean section and the PreventADALL interventions.*

(a) Lower t_{PTEF}/t_E equal to a t_{PTEF}/t_E <0.25 (b) Shorter t_{PTEF} equal to a t_{PTEF} <0.17 s (<25th percentile) (c) High TEWL equals to a mean TEWL >9.50 g/m^2/h (>75th percentile) (d) Higher EDN levels at 1 year by Youden's Index for asthma at 3 years equal to EDN levels ≥26.7 µg/L at 1 year (e) Higher EDN levels at 3 years by Youden's Index for asthma at 3 years equal to EDN levels ≥20.5 µg/L at 3 years
t_{PTEF}/t_E: time to peak tidal expiratory flow to total expiratory time; t_{PTEF}: time to peak tidal expiratory flow, TEWL: transepidermal water loss, *FLG*: filaggrin, EDN: eosinophil-derived neurotoxin, OR: odds ratio, GA: gestational age, PreventADALL: Preventing Atopic Dermatitis and ALLergies in children

5 DISCUSSION

5.1 MAIN FINDINGS

The overall aim of this doctoral thesis was to explore early risk factors for the development of preschool asthma, related to infant lung function, inflammation and skin barrier function in early life. The main findings of sub studies I-IV are illustrated in **Figure 19**.

First, infant lung function differed by arousal state, with overall higher TFV loop values while awake compared to the sleeping state. Both lower t_{PTEF}/t_E and shorter t_{PTEF} while awake in early infancy were positively associated with preschool asthma. Second, overall, neither eczema nor *FLG* mutations were associated with infant lung function or preschool asthma. However, positive associations between high TEWL and shorter t_{PTEF} as well as preschool asthma were seen. Third, overall, higher EDN levels in infancy and at preschool age were associated with preschool asthma.

Last, infant lung function differed by sex, with lower t_{PTEF}/t_E and t_{PTEF} observed in boys compared to girls, whereas in relation to skin barrier function, similar rates of TEWL, eczema and *FLG* mutations were seen. Regarding early-life inflammation, EDN levels were higher in boys compared to girls in both infancy and at preschool age. Preschool asthma was more frequent in boys. The associations to asthma differed according to sex, with both lower t_{PTEF}/t_E and shorter t_{PTEF} in infancy observed in boys and *FLG* mutations seen in girls only. Neither high TEWL nor eczema in early life was associated with preschool asthma when stratifying for sex. Higher EDN levels in infancy were only associated with preschool asthma in boys, whereas at preschool age, positive associations were seen among both sexes. Interestingly, at preschool age, the highest OR of preschool asthma were observed in in girls.

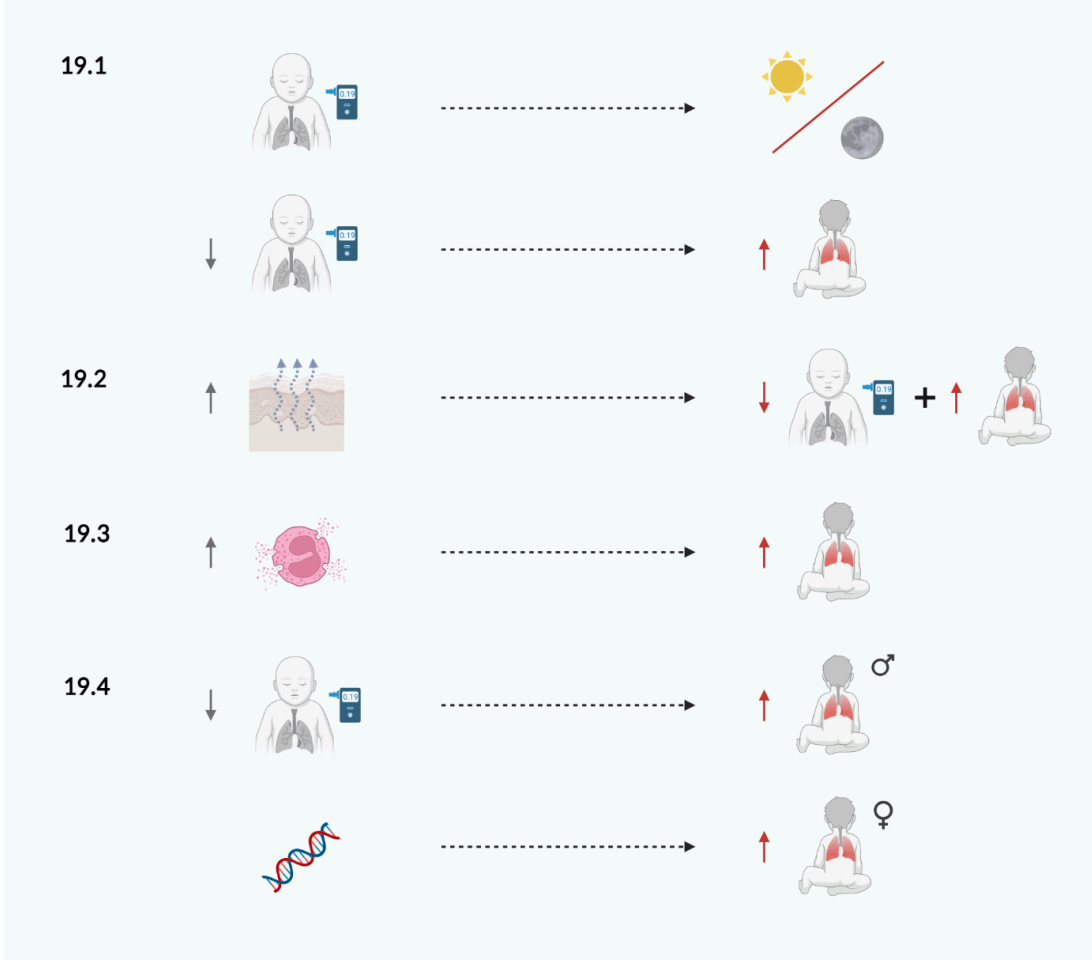

Figure 19. *Figure of the main findings of the four sub studies (**I-IV**) included in this thesis, with **19.1** illustrating that infant lung function at three months differed between awake and sleeping arousal state, as well as infant lung function in the awake state at three months being associated with preschool asthma at three years. A high TEWL at three months was associated with lower infant lung function at three months and preschool asthma at three years (**19.2**), while higher EDN levels at one and three years overall were associated with future as well as concurrent preschool asthma at three years (**19.3**). The associations to preschool asthma at three years differed by sex, with infant lung function at three months being associated with preschool asthma in boys, while FLG mutations were linked to preschool asthma in girls only (**19.4**).*

TEWL: transepidermal water loss, EDN: eosinophil-derived neurotoxin, *FLG*: filaggrin
* The illustration was created with Biorender.com

5.2 MAIN FINDINGS IN RELATION TO PREVIOUS RESEARCH

5.2.1 Infant lung function

5.2.1.1 Infant lung function and arousal state

Our findings of higher mean t_{PTEF}/t_E while awake compared to the sleeping state is in line with a study from the 1990's including 19 healthy Norwegian newborns.[139] Apart from the latter study, we are unaware of other studies assessing whether TFV loops differ according to arousal state in infants, older children and adults. Comprising the t_{PTEF}/t_E ratio, t_E was longer in the sleeping state, while t_{PTEF} was similar. During sleep, skeletal muscle tone is lower and may necessitate increased diaphragmic work of breathing,[140] which possibly can explain the longer expiratory time required (t_E) while sleeping. In support of this, one study of 70 sedated infants and children observed t_{PTEF}, t_E and t_{PTEF}/t_E varying during sleep stages, likely linked to lower tone of respiratory muscles.[141] In addition, breathing during sleep is disposed to airway obstruction,[142] with asthma symptoms commonly deteriorating,[143] possibly reflected in the lower t_{PTEF}/t_E observed while sleeping. Though all TFV loops were recorded with infants in a supine position with tidal volumes (V_T) remaining similar across the arousal states, the generally lower respiratory rates (RR) may have contributed to the lower t_{PTEF}/t_E while sleeping, supported by a previous paper of 33 sedated infants emphasizing the positive correlation between RR and t_{PTEF}/t_E.[144]

5.2.1.2 Infant lung function and preschool asthma

Lower t_{PTEF}/t_E in infancy was associated with the development of preschool asthma, in line with similar studies.[59,145] However, Guerra *et al*. used a different technique to measure lung function (forced expiratory flow-volume maneuver) among more than 100 sleeping and/or sedated American infants, and asthma was defined by doctors diagnose received at any time point between six and 36 years, reported in questionnaires.[145] In relation to asthma-like symptoms, a previous Norwegian study found an association between lower t_{PTEF}/t_E and wheezing at preschool age,[128] supporting our findings. The shorter t_{PTEF} in infancy positively associated with preschool asthma is novel. Previous studies have mainly found lower t_{PTEF} values in infants and children with airway obstruction, but support our observations.[66-68,146]

5.2.2 Infant skin barrier function

5.2.2.1 Skin barrier function and infant lung function

From a large population-based pediatric population, we are first to report an observation between high TEWL and infant lung function, indicating a potential link between the two in early life. However, as the associations to shorter and continuous t_{PTEF} only were significant in adjusted models, our observations may possibly be chance findings. In respect to asthma, a German study of 95 adolescents and adults found no difference in TEWL values between individuals with allergic asthma and controls.[147] Though no association between TEWL and t_{PTEF}/t_E was observed, a high TEWL was associated with a shorter time before reaching peak

expiratory flow (t_{PTEF}), a characteristic frequently observed in infants and children with airway obstruction.[66-68]

Though both lower lung function and AD in early life are linked to the development of preschool asthma,[36,59] we found no association between eczema and t_{PTEF}/t_E or t_{PTEF}, corresponding with earlier findings.[148,149] Hu *et al*. reported no difference in lung function by spirometry across eczema phenotypes in 4227 school-aged Dutch children.[148] Similarly, in a study of 135 Norwegian children, Håland *et al*. concluded that lung function by TFV loops appeared independent of AD.[149] Yet, evidence suggests the onset and severity of AD may be connected to asthma development,[150] factors beyond the scope of my research. In the Norwegian paper by Lødrup Carlsen *et al*., lung function from birth (TFV loops) to late adolescence (spirometry) was tracked in 329 children who since infancy had developed allergic asthma and AD.[62]

Though no associations between *FLG* mutations and t_{PTEF}/t_E or t_{PTEF} in early infancy were seen, our findings are an important contribution to the research field. In regards to airway obstruction, a large British study including 2312 infants observed an association between *FLG* mutations and wheeze in early infancy.[151] Though *FLG* mutations are hypothesized to contribute to asthma,[36] the risk is likely highest in children with a previous AD diagnosis.[71,77]

5.2.2.2 Infant skin barrier function and preschool asthma

The significant association between a high TEWL in infancy and preschool asthma, hitherto not reported, provide support to the hypothesized role of the skin barrier in asthma development.[74] To our knowledge, the only paper with a similar objective was performed by Löffler *et al*. (n=95), reporting no differences in TEWL values using a patch test across 25 subjects with allergic asthma and 26 healthy controls aged 14 and older, recruited from out- and in-patient clinics in Germany.[147] However, with several differences in methodology and study population, the results reported in the latter study[147] may not be comparable to our findings.

Overall, no associations between eczema in infancy or *FLG* mutations and preschool asthma were seen in the absence of AD or AS, in line with several previous studies.[152-154] Thus, our results suggest asthma with allergic comorbidities is more likely to represent an asthma phenotype persisting beyond preschool age.[155] With an itchy rash present at enrollment, a large Swedish report including 3124 children observed a two-fold risk of developing asthma,[156] contradictory to our observations. However, children were older (1-2 years) at baseline and follow-up was performed later in childhood (5 years after recruitment). In addition, a different definition of eczema was used and was reported by parents.[156] To the best of our knowledge, evidence of a direct association between *FLG* mutations and asthma independent of allergic comorbidities is lacking, as confirmed by our study.

5.2.3 Early-life eosinophil inflammation

5.2.3.1 Early-life eosinophil inflammation and preschool asthma

To our knowledge, we are first to investigate the relationship between EDN levels at one year and future preschool asthma, as well as EDN levels at three years and concurrent preschool asthma, demonstrating a positive correlation between higher EDN levels at both ages and preschool asthma. In line with our findings, Remes *et al*. reported significant associations between EDN as well as ECP levels and concurrent asthma in school-aged Finnish children, compared to healthy controls.[101] In contrast to our study, the latter study population was smaller (n=235), heterogeneous in terms of age (7-12 years) and asthma was parental-reported,[101] and may thereby not be comparable to our population. Nonetheless, several studies have reported higher EDN levels in children with concurrent asthma,[101,105-111] associated with bronchial hyper-responsiveness,[107,108] higher respiratory symptom burden[106,107,112] and disuse of anti-inflammatory medications,[101] supporting our findings. In relation to wheeze among 200 South Korean infants and toddlers recovering from bronchiolitis, Kim CK *et al*. reported a positive association between EDN levels and future wheezing episodes, further supporting our findings.[115]

5.2.4 Sex differences

5.2.4.1 Infant lung- and skin barrier function, early-life eosinophil inflammation and preschool asthma by sex

The associations between TFV loops in healthy, awake infants and preschool asthma differed by sex, with lower t_{PTEF}/t_E and shorter t_{PTEF} in infancy observed in boys compared to girls, are novel findings. However, our results are in line with the tendency of lower lung function in young boys observed in previous cohort studies.[157] Further supporting our findings, in 56 British infants born preterm, Stocks *et al.* reported lower t_{PTEF}/t_E and t_{PTEF} in boys compared to girls in the natural, unsedated sleeping state using the multiple occlusion technique.[158] In contrast, a larger Norwegian study (n=803) reported higher t_{PTEF}/t_E in the awake state in newborn boys compared to girls.[57] Compared to girls, the narrower airways relative to lung size in early life in boys[117] may contribute to the lower TFV loop parameters observed in boys. In addition, both male sex and lower infant lung function are known risk factors for asthma.[59,65] In line with the global trend in young children,[4] boys had higher rates of preschool asthma compared to girls (17.4% versus 9.1%) in our study. All things considered, both differences in lung structure and asthma prevalence at preschool age between the sexes may help explain our findings.

In regard to early skin barrier function, *FLG* mutations were associated with the development of preschool asthma in girls only, whereas neither high TEWL nor eczema remained significant risk factors when stratifying for sex. To our knowledge, we are first to report a sex-dependent association between *FLG* mutations and preschool asthma. In relation to AD, a previous Russian study (n=1312) found a positive link between single nucleotide polymorphisms in the *FLG* gene in adult women, but not men.[159] We are unaware of

previous publications assessing the association between TEWL and preschool asthma by sex, wherefore we provide new knowledge. The non-significant association between eczema and preschool asthma observed in both girls and boys differs from the findings in a previous Australian publication investigating 620 infants with a family history of allergic diseases, in which eczema during the first two years of life increased the risk of preschool asthma in boys.[160] Apart from that the latter study included infants at higher risk of developing allergic diseases,[160] one possible explanation is that we only focused on eczema at three months, a single time point in early infancy where an association might be harder to detect. Further, of the children identified with eczema in early life, many may not progress to develop AD in later infancy.

Concerning eosinophil-inflammation in early life, in both infancy and at preschool age, serum EDN levels were higher in boys compared to girls and is in line with a previous study of adults.[161] In the Finnish study (n=235) by *Remes et al.,* no significant differences in EDN or ECP levels were observed between the sexes, though school-aged boys tended to have higher serum EDN levels.[101] Similarly, in two population-based studies of 216 and 968 school-aged children and adolescents in Norway and Australia, respectively, no differences in serum ECP levels were found.[96,97] Helping to understand the observed sex differences in our study, EDN correlates to eosinophil count,[109,161-163] and male sex is linked to higher eosinophil counts.[164] Further, we are first to report that higher EDN levels in infancy were positively associated with preschool asthma in boys but not girls, whereas higher EDN levels at preschool age were linked to concurrent preschool asthma among both sexes, with the highest OR observed in girls although rates of preschool asthma were higher in boys (14.7%) compared to girls (9.3%). The latter finding differs to the Swedish report of Mogensen *et al*. (n=403) with similar objective, demonstrating the highest risk of having fixed airflow obstruction by spirometry in males with elevated serum ECP levels, compared to girls.[165] However, with only adult participants (17-75 years), all with self-reported doctor-diagnosed asthma and/or chronic rhinosinusitis,[165] their results may not be comparable to our findings.

5.3 STRENGTHS AND LIMITATIONS

The four sub studies included in this thesis have several mutual strengths. First, all sub studies are based on information prospectively gathered from children and mothers participating in the PreventADALL birth cohort, antenatally enrolled from the general population. Further are the children clinically well-characterized, with detailed information on birth and background data as well as exposures and outcomes, increasing the generalizability of our findings. However, with slightly higher education level among the parents in the cohort compared to the general population, the generalizability of our observations may be limited to similar populations. To increase the inter-observer reliability, health care professionals were trained together, and the study centers in Norway and Sweden used the same standard operating procedures to assess lung function, TEWL and eczema.

Based on a power analysis performed prior to data collection, the population size was sufficient to compare TFV loop parameters between paired awake and sleeping lung function

measurements in 91 infants the different arousal states. Exploring the association between skin barrier function and infant lung function (n=899), the high numbers of infants with information on TFV loops (100%) as well as TEWL (91.9%), eczema (99.9%) and/or *FLG* mutations (81.2%) are a strength, as a larger study size may reduce the risk of type II errors; when researchers fail to reject the null hypothesis when it is actually false.[166] Similarly, when assessing the correlations between infant lung function, skin barrier function and preschool asthma (n=1337), the high number of children with information on preschool asthma (100%) as well as TEWL (87.7%), eczema (98.6%) and/or *FLG* mutations (78.2%) are a strength, whilst fewer children had information on TFV loops (42.1%).

Although TFV loops, as a non-invasive technique, is an asset in epidemiological research, questions regarding the possible influence of conditions such as arousal state, positioning, respiratory rate and age have earlier been raised.[167] The process of recording and analyzing has also been discussed, in which the difficulties of obtaining reproducible TFV loops has been a concern.[67] Aiming to control for some of these issues, standard operating procedures to measure as well as manually assess the quality of infant lung function measurements were used,[123] attaining a large material on TFV loops from healthy infants in the awake (n=899) and sleeping (n=375) state, collected at a specific time point under similar conditions by trained investigators. Further, TFV loops were obtained in early infancy, limiting the risk of postnatal adverse events influencing lung function development. While several studies have demonstrated that a t_{PTEF}/t_E <0.25 in early life is associated with the development of asthma,[59,128-130] no clear cut-off for shorter t_{PTEF} has previously been determined and is a limitation. However, as lower values have been reported in infants and children with airway obstruction,[66-68] a t_{PTEF} in the lower range (<25th percentile) was chosen. Lung function was not measured in the less densely populated county of Østfold (Norway), where socioeconomical status in general was lower compared to the capital cities Oslo (Norway) and Stockholm (Sweden),[168] and limits the generalizability of our observations to similar populations.

By including TEWL measurements to objectively assess skin barrier function and using established cut-offs (>75th percentile) we strengthen our findings.[126,131] Choosing physician-confirmed eczema instead of AD fulfilling the UKWP criteria in early infancy might be considered as a limitation. However, the major UKWP AD criteria of an itchy rash has been questioned in a previous PreventADALL study, showing less than one fifth of the children with eczema at three months later diagnosed with AD by one year fulfilled this requirement in early infancy.[132] In support, advanced motor skills are needed to locate an itchy target on the body, an ability which may not yet fully developed in early infancy.[169] The rate of *FLG* mutation carriers in the sub studies (9.0-10.0%) corresponds with the prevalence in the PreventADALL study as well as in European populations.[127]

To the best of our knowledge, we are first to provide population-based serum EDN levels retrieved from a large pediatric population at two specific time points in childhood. Though previous studies foremost analyzed serum EDN with manual enzyme-linked immunosorbent

assays,[170] the automated ImmunoCAP EDN research assay used appears to be a reliable complement with high precision.[109] As infections in addition to allergens can trigger the secretion of EDN from eosinophils,[90] the exclusion of data on infections is a limitation, but was beyond the scope of my research. With eosinophil counts not available, we are also unable to elaborate on previous reports of correlations between eosinophil counts and EDN[109,161-163] or sex,[164] and is a limitation to our findings.

While wheeze is the most commonly occurring symptom of preschool asthma, all wheezing is not related to asthma[5] wherefore incidence, severity and symptom patterns must therefore be taken into consideration in diagnosis at this age.[10] To capture the incidence and symptom pattern, rBO ($\geq$3 episodes of wheeze) in the recent year was a mandatory criterion, together with a history of doctor-diagnosed asthma and/or asthma medication use,[133] attempting to create a strict definition for preschool asthma. However, in many children preschool asthma-like symptoms resolve with age,[171] while other children experience the first manifestations of chronic asthma at preschool age, pointing to the uncertainty of diagnosing asthma at this age. In addition to respiratory symptoms such as rBO, most guidelines therefore advise basing possible asthma diagnosis on response to asthma medications and the exclusion of differential diagnoses,[11] information which was not available to evaluate and is a limitation. Nevertheless, a recent birth cohort study reported a 20-150-fold higher risk of asthma at five years among children experiencing wheeze reported between three months and five years.[12] It would however be interesting to assess the associations between infant lung function, skin barrier function, EDN and asthma in children with persisting symptoms at an older age.

In none of the included sub studies were significant interaction effects between the PreventADALL skin or food intervention groups and the exposures on the outcomes observed, wherefore all children were included in our analyses. To reduce the possibility that effect-modifying factors, such as the PreventADALL interventions, heredity for allergic diseases or sex, had influence on our findings, based on the literature we adjusted for possible confounders. The majority of the potential risk factors used to adjust multivariate regression analyses were recorded prior to the clinical investigation from which all results are presented, and is a strength. Further, when applicable, continuous variables were used in addition to dichotomized variables, such as TFV loop parameters and TEWL.

6 CONCLUSIONS

Based on the results from the four sub studies (**I-IV**) included in this thesis, we conclude that both infant lung- and skin barrier function as well as early-life eosinophil inflammation may be linked to the development of preschool asthma, in which sex appears to influence (**Figure 20**).

Infant lung function

- The t_{PTEF}/t_E differed between the arousal states, with overall higher TFV loop values while awake compared to sleeping state. TFV loops should be analyzed and interpreted according to arousal state, using separate reference values.
- Lower t_{PTEF}/t_E and shorter t_{PTEF} in the awake state in early infancy were associated with prechool asthma.

Infant skin barrier function

- High TEWL was inversely associated with t_{PTEF} in early infancy as well as the development of preschool asthma, indicating a possible link between the skin barrier- and lung function in early infancy as well as later preschool asthma.

Early-life eosinophil inflammation

- We propose population-based serum EDN reference values, displaying higher EDN levels in infancy compared to at preschool age. Higher EDN levels at both ages were associated with preschool asthma, indicating early-life eosinophil inflammation may play a role in preschool asthma development.

Sex differences

- Associations to preschool asthma differed by sex, with lower t_{PTEF}/t_E and shorter t_{PTEF} observed in boys only.
- *FLG* mutations were associated with preschool asthma in girls only, while neither eczema or high TEWL remained significant risk factors in either sex.
- Higher EDN levels in infancy were associated with preschool asthma in boys only. At preschool age, higher EDN leveles were associated with concurrent preschool asthma among both sexes, with the highest OR observed in girls.

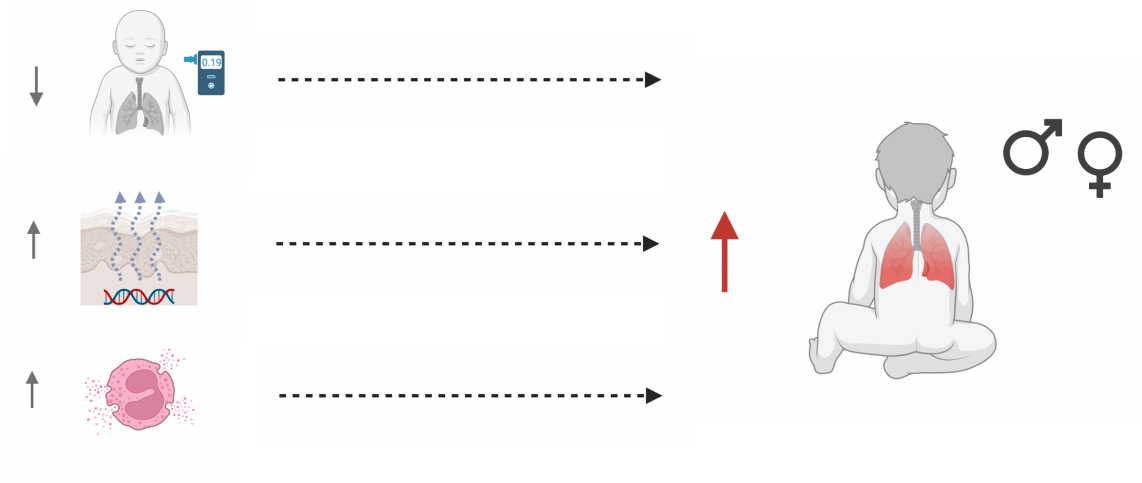

***Figure 20**. In summary, lower infant lung function, skin barrier impairment as well as eosinophil inflammation in early life may be linked to the development of preschool asthma, in which sex appears to influence.*

* The illustration was created with Biorender.com

7 POINTS OF PERSPECTIVE

7.1 CLINICAL IMPLICATIONS

While it is possible to obtain objective lung function tests from awake children actively participating, it has historically been challenging assessing lung function in non-sedated infants.[50] To our knowledge, we provide the largest material on TFV loops in the awake and/or natural sleeping state (n=1183), demonstrating that lung function measurements in both arousal states in early infancy are feasible, but ought to be assessed individually using separate reference values. However, lung function techniques allowing for repeated measurements in awake young children are warranted,[49] favoring measuring TFV loops in the awake state. We further suggest that lower t_{PTEF}/t_E and shorter t_{PTEF} while awake may be useful in the assessment of lung function and preschool asthma. Reflected in the overall lower t_{PTEF}/t_E observed while sleeping compared to the awake state, one may speculate that different cut-off levels of t_{PTEF}/t_E according to arousal state are needed. With t_{PTEF} remaining stable across awake and sleeping TFV measurements, t_{PTEF} may prove useful in future studies in which lung function with mixed arousal states are evaluated, though this is beyond the scope of the current research project.

The inverse associations between high TEWL and t_{PTEF} in infancy suggest a potential link between the skin barrier- and lung function in early life. From this significant finding, we additionally bring evidence of a possible association between high TEWL and preschool asthma. In summary, our significant findings suggest a possible link between early-life skin barrier impairment and respiratory health. In research and possibly clinical practice, TEWL may not only prove useful to identify infants at risk of AD[78] and AS,[131] but possibly also preschool asthma. Overall, no associations between eczema in infancy or *FLG* mutations and infant TFV loops or preschool asthma were observed.

Retrieved from a large pediatric general population, we suggest the documented EDN levels may be considered as reference values in similar low-risk populations, showing higher levels in infancy compared to at preschool age, in line with the ECP levels reported in a study of 245 Norwegian children.[172] Although higher EDN levels at both ages were observed in children with preschool asthma as well as AS, AD and any allergic disease compared to non-atopic children, levels largely overlapped, in line with the Finnish study of Remes et al. of school-aged children (n=235).[101] Still, we observed that higher EDN levels at both ages positively correlated to preschool asthma. It is therefore more likely that EDN, in combination with clinical characteristics and medical history, may help assist clinicians to identify children at higher risk of preschool asthma,[173] rather than single-handedly be of diagnostic or prognostic value in pediatric clinical practice. In relation to existing biomarkers reflecting a Th2-driven inflammation, all face difficulties.[174,175] Being easily-obtained in various body tissues such as in saliva, urine and blood, requiring small sample volumes and with stable concentrations after collection, EDN may have a potential in pediatric clinical practice.[93]

In infancy, t_{PTEF}/t_E and t_{PTEF} were lower in boys compared to girls, while TEWL, eczema and *FLG* mutations were similar between the sexes. Levels of EDN in both infancy and at preschool age were higher in boys than girls. Stratifying for sex, lower t_{PTEF}/t_E and shorter t_{PTEF} were associated with preschool asthma in boys, while *FLG* mutations positively correlated to preschool asthma in girls, only. The several differences in rates and the sex-dependent associations observed suggest sex ought to be taken into consideration.

7.2 FUTURE RESEARCH

With some exceptions, developmental factors for asthma from early infancy to later childhood are not well understood.[44] Observing possible links between infant lung- and skin barrier function as well as early-life eosinophil inflammation and preschool asthma, in a time period rarely studied, future research in older children and other study populations is warranted to confirm or contradict our findings. Further, no attempts to investigate the possible underlying mechanisms of our observations were made, which overall require more evidence.

Infant lung function

- As possible effect-modifying factors of TFV loops may balance out with age,[176] such as respiratory rate,[177] future research is needed to determine whether TFV loops differ between the awake and sleeping arousal state in older children.
- While we reported an association between shorter t_{PTEF} in infancy and preschool asthma, a TFV loop parameter seldomly used, previous research has mainly described lower t_{PTEF} values in infants and children with airway obstruction.[66-68,146] For this reason, future longitudinal studies are required to determine if t_{PTEF} while awake, sleeping, or possible mixed arousal states, may be relevant for the identification and prediction of infants and older children at risk of developing asthma.

Infant skin barrier function

- Though an inverse association between high TEWL and t_{PTEF} was observed in infancy, and high TEWL as well as shorter t_{PTEF} correlated to preschool asthma, additional research in older children is needed to detangle the potential relationship between the skin barrier- and lung function in the development of asthma. Similarly, the role of TEWL in asthma development also remains to be determined.

Early-life eosinophil inflammation

- With overlapping ranges in serum EDN levels in infancy and at preschool age between children with preschool asthma and non-atopic children, but with a two-fold higher OR of developing preschool asthma among children with higher levels in infancy, the role of EDN as a biomarker for asthma in pediatric clinical practice remains undetermined. To document reference values and assess associations to asthma, future prospective longitudinal birth cohort studies are warranted including samples from other body tissues, collected at various ages over time in childhood.

- It would also be valuable to longitudinally evaluate the relationship between EDN levels and eosinophil counts as well as other markers of eosinophil inflammation. Further, the associations between lung function in early infancy and EDN levels in late infancy, as well as EDN and concurrent and future lung function, would be interesting to assess.

Sex differences

- As associations to preschool asthma differed by sex, with lower t_{PTEF}/t_E and shorter t_{PTEF} as well as higher EDN levels in infancy seen in boys and *FLG* mutations in girls, only, it would be interesting to investigate if the same sex-dependent associations remained significant at an older age and in other pediatric populations retrieved from the general population.

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