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THE RISK OF SCHOOL-AGE ASTHMA AFTER THE FIRST SEVERE RHINOVIRUS-INDUCED WHEEZING

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To my family

ABSTRACT

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The risk of school-age asthma after the first severe rhinovirus-induced wheezing

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Background: The rhinovirus etiology of wheezing is an important risk factor for developing recurrent wheezing and asthma, especially in children with atopic predisposition. However, rhinovirus infection has not yet been included in the risk assessment of different asthma phenotypes at school-age.

Aims: To study 1) the impact of known risk factors and rhinovirus etiology of the first severe virus-induced wheezing episode for developing persistent asthma; 2) risk factors for developing atopic and non-atopic asthma at school-age; and 3) whether prednisolone treatment of the first wheezing episode may prevent development of asthma symptoms.

Methods: Risk factors for asthma symptoms were studied in a 7-year follow-up of Vinku study (n=111, median age 12 months at the first wheezing). Risk factors for atopic and non-atopic school-age asthma were studied in steroid-naive children jointly in Vinku and Vinku2 studies (n=127; 11 months, respectively). The preventive effect of prednisolone was assessed in two randomized trials; *post hoc* in Vinku study and prospectively in Vinku2 study.

Results: Early-onset food sensitization and rhinovirus etiology of the first wheezing episode predicted persistent asthma symptoms, and development of atopic asthma at school-age. Parental smoking and age <12 months predicted non-atopic asthma at school-age. The children with rhinovirus-induced first wheezing in the Vinku study, and those with high rhinoviral load in the Vinku2 study benefitted from prednisolone in terms of less persistent asthma symptoms.

Conclusions: Virus etiology and atopic status are worth assessing in wheezing children to recognize those with increased asthma risk. The separate risk factors of asthma phenotypes suggest different mechanisms underlying atopic and non-atopic asthma in children. This knowledge could provide a mean to identify children who would benefit from early anti-inflammatory treatment to prevent asthma.

Keywords: asthma, atopy, bronchiolitis, child, oral corticosteroids, phenotype, respiratory syncytial virus, rhinovirus, sensitization, virus, wheezing

TIIVISTELMÄ

LL Minna Lukkarinen

Kouluiän astmariski ensimmäisen rinoviruksen aiheuttaman uloshengitysvaikeuskohtauksen jälkeen

Turun yliopisto, Lääketieteellinen tiedekunta, Kliininen laitos, Lastentautioppi, Turun yliopiston kliininen tohtoriohjelma**,** Turun yliopistollinen keskussairaala, Lasten ja nuorten klinikka**,** Turku, Suomi

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Tausta: Varhaisen uloshengitysvaikeuden rinovirusetiologia on toistuvien uloshengitysvaikeuskohtausten ja astman kehittymisen tärkeä riskitekijä etenkin varhain herkistyneillä lapsilla. Tietoa uloshengitysvaikeuden rinovirusetiologiasta ei ole vielä kuitenkaan hyödynnetty kouluiän astman eri fenotyyppien riskiarvioinnissa.

Tavoite: Tutkia, 1) tunnettujen riskitekijöiden ja ensimmäisen uloshengitysvaikeuskohtauksen rinovirusetiologian merkitystä pysyvien astmaoireiden kehittymisessä; 2) kouluiän allergisen ja ei-allergisen astman riskitekijöitä; sekä 3) vähentääkö ensimmäisen uloshengitysvaikeuskohtaukseen hoidoksi annettu prednisoloni astmaoireita.

Menetelmät: Astman riskitekijöitä tutkittiin Vinku-tutkimuksen seitsemän vuoden seurannassa (n=111, mediaani-ikä 12 kk tutkimuksen alussa). Kouluiän allergisen ja ei-allergisen astman riskitekijöitä tutkittiin steroidia saamattomilla lapsilla yhdistetysti Vinku- ja Vinku2-tutkimuksissa (n=127, mediaani-ikä 11 kk). Prednisolonin suojaavaa vaikutusta arvioitiin kahdessa randomoidussa tutkimuksessa; Vinku-tutkimuksessa *post hoc* ja Vinku2-tutkimuksessa prospektiivisesti.

Tulokset: Varhainen ruoka-aineherkistyminen ja ensimmäisen uloshengitysvaikeuden rinovirusetiologia ennustivat pysyviä astmaoireita ja kouluiän allergisen astman kehittymistä. Vanhempien tupakointi ja alkuvaiheessa <12 kuukauden ikä ennustivat kouluiän ei-allergista astmaa. Prednisoloni vähensi astmaoireita niillä lapsilla, joilla oli ensimmäisen uloshengitysvaikeuden yhteydessä rinovirus Vinku-tutkimuksessa ja korkea rinovirusmäärä Vinku2-tutkimuksessa.

Päätelmät: Herkistymisen ja virusetiologian tutkiminen on kannattavaa uloshengitysvaikeuskohtauksen yhteydessä, jotta tunnistetaan astmariskilapset. Lapsuusiän astmafenotyypeillä on todennäköisesti eri mekanismit, koska niillä on eri riskitekijät. Tämä tieto voisi edesauttaa myös niiden lasten tunnistamista, jotka hyötyisivät astman ehkäisystä varhaisella anti-inflammatorisella lääkkeellä.

Avainsanat: astma, atopia, bronkioliitti, fenotyyppi, herkistyminen, kortikosteroidi, lapsi, respiratory syncytial virus, rinovirus, uloshengitysvaikeus, virus

TABLE OF CONTENTS

ABBREVIATIONS

ANOVA	Analysis of variance
API	Asthma Predictive Index
B-eos	Blood eosinophil count
CAS	Childhood Asthma Study
CDHR3	Cadherin-related family member 3
CI	Confidence interval
COAST	Childhood Origins of ASThma
CRS	Children's Respiratory Study
FEV1	Forced expiratory volume in one second
GRS	Genetic risk score
HR	Hazard ratio
ICAM-1	Intercellular adhesion molecule-1
ICS	Inhaled corticosteroid
IFN	Interferon
IFWIN	Inhaled Fluticasone in Wheezy INnfants
Ig	Immunoglobulin
IL	Interleukin
ISAAC	International Study of Asthma and Allergies in Childhood
IQR	Interquartile range
LDLR	Low-density lipoprotein receptor
mAPI	Modified API
MAS	Multicenter Allergy Study
NAEPP	The National Asthma Education and Prevention Program
NPA	Nasopharyngeal aspirate
OCS	Oral corticosteroid
OR	Odds ratio
PAC	Prevention of Asthma in Childhood
PCR	Polymerase chain reaction
PEAK	Prevention of Early Asthma in Kids
RCT	Randomized clinical trial
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
RT	Reverse transcriptase
RV	Rhinovirus
SABA	Short-acting beta$_2$-agonist
SD	Standard deviation
Th	T helper cell

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications which are referred to in the text by the Roman numbers I-IV.

I Lukkarinen Minna, Lukkarinen Heikki, Lehtinen Pasi, Vuorinen Tytti, Ruuskanen Olli, Jartti Tuomas. Prednisolone reduces recurrent wheezing after first rhinovirus wheeze: a 7-year follow-up. Pediatr Allergy Immunol. 2013; 24: 237-43.

II Lukkarinen Minna, Vuorinen Tytti, Lehtinen Pasi, Ruuskanen Olli, Jartti Tuomas. Sensitization at the first wheezing episode increases risk for long-term asthma therapy. Pediatr Allergy Immunol. 2015; 26: 687-91.

III Koistinen Annamari, Lukkarinen Minna, Turunen Riitta, Vuorinen Tytti, Vahlberg Tero, Camargo Carlos Arturo Jr, Gern James, Ruuskanen Olli, Jartti Tuomas. Prednisolone for the first rhinovirus-induced wheezing and 4-year asthma risk: a randomized trial. Submitted.

IV Lukkarinen Minna, Koistinen Annamari, Turunen Riitta, Lehtinen Pasi,Vuorinen Tytti, Jartti Tuomas. Rhinovirus-induced first wheezing episodepredicts atopic but not non-atopic asthma at school-age. J Allergy Clin Immunol in press.

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1 INTRODUCTION

Approximately 30% of all children suffer from wheezing during a respiratory infection by the age of three years (Taussig *et al.* 2003, Matricardi *et al.* 2008). Of these children, 40% continue with recurrent wheezing and 20% become sensitized to aeroallergens before school-age (Taussig *et al.* 2003, Illi *et al.* 2006, Piippo-Savolainen and Korppi 2008). In 2-3% of the infants, the wheezing/bronchiolitis is severe enough to need hospitalization (Smyth and Openshaw 2006). Thereafter, 15-40% of the hospitalized children suffer from asthma at early school-age (Sigurs *et al.* 2000, Kotaniemi-Syrjänen *et al.* 2003, Henderson *et al.* 2005). The majority of wheezing children outgrow their asthma symptoms. Based on the results of wheezing/bronchiolitis studies, the later asthma development has been associated with atopic characteristics, such as parental asthma, maternal smoking, early allergic sensitization, and wheezing induced by rhinovirus (Kotaniemi-Syrjänen *et al.* 2003, Piippo-Savolainen and Korppi 2008, Göksor *et al.* 2013). Though, the risk factors of non-atopic are still unrecognized.

There is current data on short- and long-term efficacy of inhaled and systemic corticosteroids concerning the risk reduction of wheezing disorders. The periodic or regular therapy with inhaled corticosteroids has not been preventive from asthma progression. In bronchiolitis with respiratory syncytial virus (RSV) systemic corticosteroids have not been shown effective, and are therefore not recommended (Ralston *et al.* 2014, Meissner 2016). However, oral corticosteroids for the first wheezing episode reduced the risk of physician-confirmed wheezing recurrence up to 12 months compared to placebo in rhinovirus-affected children in Vinku study (Lehtinen *et al.* 2007), and in children with high rhinovirus load in Vinku2 study (Jartti *et al.* 2015). It is of note that all other studies on the efficacy systemic corticosteroids have not included the rhinovirus etiology of the wheezing.

The childhood asthma predictive indices have mainly been based on wheezing recurrence and atopic risk factors, but still, do not separate between atopic or non-atopic asthma phenotypes (Castro-Rodriguez *et al.* 2000, Guilbert *et al.* 2004a). It has been hypothesized, whether these phenotypes have different underlying mechanisms and risk factors. Also, waiting for the symptom recurrence may delay the recognition and/or treatment of the asthmatic children. The aims of this thesis were to study the risk factors at the first severe virus-induced wheezing episode for asthma symptoms in the 7-year follow-up, and for atopic and non-atopic asthma phenotypes at school-age. The rhinovirus etiology was added to the risk assessment as a central part. The effect of the prednisolone intervention at study entry was studied on the persistency of asthma symptoms.

2 REVIEW OF LITERATURE

2.1 Definitions and diagnosis

2.1.1 Acute wheezing

Acute wheezing is defined as a continuous high-pitched sound with musical quality emitting from the chest during expiration (Elphick *et al.* 2001, NAEPP 2007). Wheezing is expiratory and the end result of narrowing of intrathoracic airways and expiratory flow limitation. The underlying process includes bronchospasm, inflammation of the airways, intraluminal mucus production, or reversible tightening of the smooth muscles in the airway walls (de Benedictis and Bush 2017). The narrowing of the intrathoracic airways leads to increased expiratory breathing work, which is clinically seen as nasal flaring, chest retractions, prolonged duration of expiration, and the use of accessory respiratory muscles (Brand *et al.* 2008).

2.1.2 Recurrent wheezing

The early-life virus-induced wheezing episodes in toddlers are usually called ”wheezy bronchitis” or ”wheezing associated with respiratory infections”. Recurrent wheezing is defined as wheezing occurring recurrently *ie.* more than once. There are phenotypic differences in children with recurrent wheezing, for in others the wheezing episodes may only be induced by viral infection, while in others they are a sign of childhood asthma attacks promoted by additional causes such as exercise and/or allergy (NAEPP 2007). Also, recurrent wheezing episodes are treated the same way as acute asthma attacks; the symptoms can be reduced by bronchodilators and continuous medication with inhaled corticosteroids (ICS) (Guilbert *et al.* 2006, Ducharme *et al.* 2014). Thereby, the clinical definition of recurrent wheezing is overlapping with the definition of childhood asthma. Between acute wheezing episode and asthma lay children who seemingly fall, at least for a period, between these two diagnoses. Asthma should be considered in any child with recurrent wheezing (Reddel *et al.* 2015).

2.1.3 Bronchiolitis

Bronchiolitis is an acute virus-induced infection of the lower respiratory tract (Smyth and Openshaw 2006, Nair *et al.* 2010). The virus infection causes extensive inflammation and oedema in the distal airways, bronchioles, and also in

the surrounding pulmonary tissue. The oedema, increased mucus production, and necrosis of airway epithelial cells lead to airway obstruction and air trapping in these distal airways (AAP 2006). Bronchiolitis is a clinical syndrome. The clinical disease initiates with upper respiratory symptoms including coryza and fever, and after 3-5 days develops to respiratory distress associated with cough, dyspnea, tachypnea (≥50/min), poor feeding, and hypoxemia (oxygen saturation <92%) (Smyth and Openshaw 2006). The characteristic clinical finding is the fine crepitation with diffuse crackles with or without expiratory wheezing heard by auscultation (Jartti *et al.* 2009). Bronchiolitis responds poorly to bronchodilators (Gadomski and Brower 2010).

The definition of bronchiolitis varies between countries. In Europe bronchiolitis is defined as the first viral infection of the lower respiratory tract in children aged <12 months with the presence of crackles with or without expiratory wheezing in the pulmonary auscultation (Scottish Intercollegiate Guidelines Network (SIGN). Bronchiolitis in children. A national clinical guideline. 2006, Smyth and Openshaw 2006, Ralston *et al.* 2014). In the United States and Canada it is particularly the first wheezing episode in children aged <24 months (AAP 2006, Smyth and Openshaw 2006, Ralston *et al.* 2014). The difference in definitions lead to discrepancy in terminology; the UK definition describes the clinical condition as virus-induced wheezing or asthma in children aged >12 months, while the US definition terms it still as bronchiolitis.

2.1.4 Asthma

Asthma is a chronic inflammatory disorder of airways associated with variable airflow obstruction and bronchial hyper-responsiveness presenting with recurrent episodes of wheezing, cough, shortness of breath, and chest tightness (GINA 2006, NAEPP 2007, Papadopoulos *et al.* 2012, GINA 2016). Airflow limitation is caused by inflammatory changes in the airway (Busse and Lemanske 2001). It is accompanied by alterations in patterns of vascularization, innervation and airway smooth muscle growth, and disturbances of the epithelial-mesenchymal trophic unit throughout the conducting airways. Airway edema develops as the disease becomes more persistent and inflammation becomes more progressive. Edema, mucus hypersecretion, and formation of mucus plugs further limit airflow. Airway hyper-responsiveness is an exaggerated bronchoconstrictor response to stimuli. Bronchoconstriction is caused by bronchial smooth muscle contraction that narrows the airways in response to exposure to a variety of stimuli, including allergens, viruses or other irritants (NAEPP 2007).

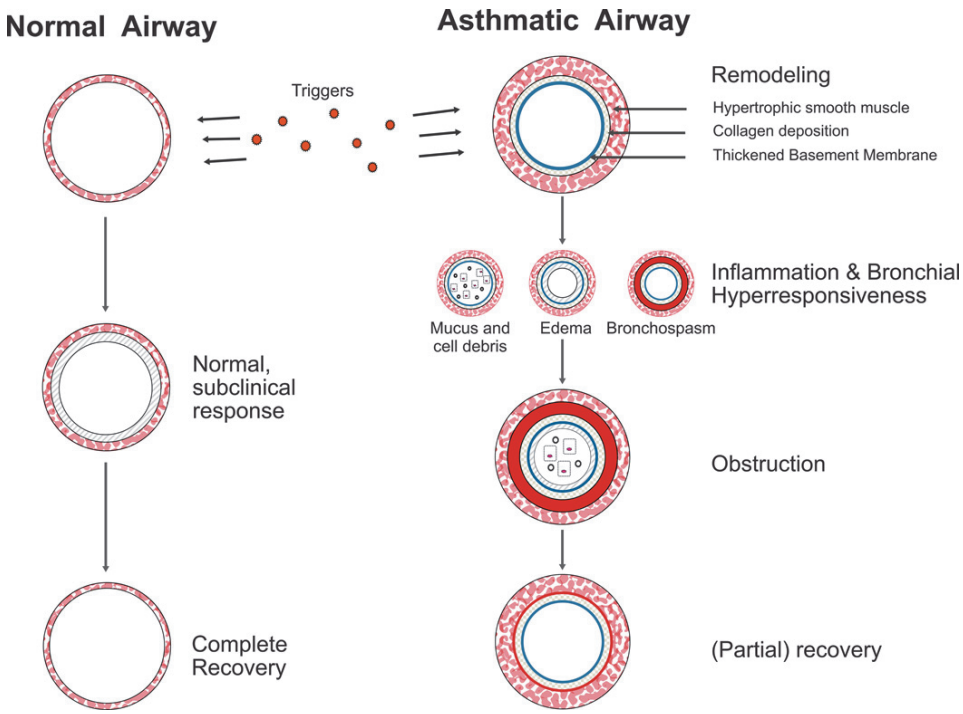


Figure 1. In children, pathological changes are present in the peripheral airways. Inflammation and hyper-reactivity are triggered by a variety of factors leading to airway obstruction. From the article of Papadopoulos *et al.* 2012.

Diagnosis

In pre-school children, the diagnosis of asthma is based on clinical criteria and the absence of an alternative diagnosis (NAEPP 2007, Brand *et al.* 2008, GINA 2016). In pre-school and school-aged children and in adults the diagnosis is made clinically and whenever possible, using non-invasive lung-function tests (Beydon *et al.* 2007). The finding of reversible airway obstruction can also be confirmed by a therapeutic trial with inhaled bronchodilators or corticosteroids. The physical examination may reveal symptoms of asthma, but the absence of these symptoms does not exclude asthma, since the disease is variable and signs may be absent between episodes. Indicative for asthma are the auscultatory sounds of expiratory wheezing during normal or forced breathing and/or prolonged expiration, use of accessory muscles, appearance of hunched shoulders, or chest deformity. The patient history indicates asthma if the child has continuous cough, recurrent wheezing, chest tightness, and if these symptoms worsen with exercise, viral respiratory infections, inhalant allergens, cold (dry) air, strong emotional expression, tobacco smoke, or stress (Busse and Lemanske 2001, NAEPP 2007, Papadopoulos *et al.* 2012).

The Asthma Predictive Index (API) has become the diagnostic criteria of asthma in pre-school children, although it was first offered as a method for asthma

prediction (Guilbert *et al.* 2004b, NAEPP 2007). Therefore, its use should be questioned to dissociate current diagnosis from the prediction of remission. However, in small children, the regular asthma control therapy with ICS is used to prevent more episodes (Guilbert *et al.* 2006). It is started after ≥4 wheezing episodes within the past 12 months lasting >1 day and affected sleep. Additionally, 1 major risk factor (physician-diagnosed eczema, aeroallergen sensitization, or parental asthma) or 2 minor risk factors (wheezing apart from colds, blood eosinophil count ≥4% or food sensitization), and/or prolonged symptoms lasting >4 weeks and requiring symptomatic treatment >2 days a week, and/or two exacerbations requiring systemic corticosteroids within 6 months are recommended (Guilbert *et al.* 2004b, Guilbert *et al.* 2006, NAEPP 2007).

In children ≥5 years of age, along the clinical symptoms above, the diagnosis of asthma requires objective documentation of at least partly reversible airway obstruction and hyper-responsiveness, usually by spirometry (Beydon *et al.* 2007, NAEPP 2007, Papadopoulos *et al.* 2012). In spirometry, the reversibility of the airflow obstruction is defined by an increase of >200 mL and ≥12% in forced expiratory volume in one second (FEV1) in the bronchodilatation test with inhalation of short-acting beta$_2$-agonist (SABA). The airway hyper-responsiveness can be defined as a decrease of ≥15% in FEV1 in exercise-challenge test.

2.1.5 Asthma phenotypes

Asthma is a syndrome of overlapping phenotypes with defined clinical and physiological characteristics (Beasley *et al.* 2015). In children, the most common classification is to devide childhood asthma immunologically into atopic and non-atopic phenotypes (Beasley *et al.* 2015, GINA 2016). This classification may slightly be simplifying due to heterogeneity and complex nature of the disease, but the children can be defined clinically. While the risk factor profile and pathogenesis of atopic asthma is quite clear, these factors are less clear in non-atopic asthma (Strina *et al.* 2014, James and Hedlin 2016). Most studies have ignored the distinction between atopic and non-atopic phenotypes even though these phenotypes are likely to have different causal mechanisms.

Atopic asthma is defined as having abnormal, reversible lung function test pointing towards asthma with positive IgE or skin prick-test result against food or aeroallegens (GINA 2016). The clinical course is often described as an atopic march where early-life eczema appears first, then food allergen sensitization slowly turns into aeroallergen sensitization, and recurrent wheezing finally develop into asthma triggered by viral infection, aeroallergen exposure or exercise (NAEPP 2007). Atopic asthmatics usually respond well to ICS and beta$_2$-agonist treatment (GINA 2016).

Non-atopic asthma is defined as having abnormal lung function tests described above, but no sensitization for allergens. The development of non-atopic asthma has not been well studied. While atopic asthma often responds to inhaled corticosteroids, non-atopic asthmatics typically have long exacerbations with limited bronchodilator effect and these patients may need larger doses of ICS compared to atopic asthmatics (GINA 2016).

2.2 Epidemiology

2.2.1 Incidence of wheezing in early childhood

Population-based studies have shown that approximately one third of all children have at least one wheezing episode during the first three years of life (Martinez *et al.* 1995, Illi *et al.* 2004, Bisgaard and Szefler 2007). The German Multicenter Allergy Study (MAS) specified how the first virus-induced wheezing occurs in 18% during the first 12 months while the incidence of first wheezing declines being 9% during the second and 4% during the third year of life (Matricardi *et al.* 2008). In infants with atopic predisposition in family (asthma or sensitization in at least one first-degree relative) the incidence of early-life wheezing is over 50 % (Kuiper *et al.* 2007).

Of the first-time wheezing children, approximately 30% continue with the tendency of wheezing after the first three years (Martinez *et al.* 1995, Matricardi *et al.* 2008). The increasing age at wheezing was associated with the increased incidence of recurrent wheezing. In Tucson study the incidence of wheezing at age 3 years was 20% in children with wheezing during the first year of life, and likewise 40% and 60% with second and third year wheezing (Taussig *et al.* 2003). However, almost 60% of the children with wheezing before the age of 3 years had stopped wheezing by the age of 6 years (Martinez *et al.* 1995, Just *et al.* 2008). About 2% of wheezing infants need hospitalization due to the wheezing severity (Koehoorn *et al.* 2008). After hospitalization, the incidence of recurrent wheezing is high. During the following year after the hospitalization, 70-75 % of the infants had at least one physician-diagnosed wheezing episode (Korppi *et al.* 1993, Reijonen and Korppi 1998), and about 40-50 % had wheezing at least twice (Reijonen and Korppi 1998). A major clinical cause of early-life wheezing is the bronchiolitis caused by RSV (Marguet *et al.* 2009, Mansbach *et al.* 2012, Meissner 2016). Bronchiolitis affects 10% of children during the first year of life, and 90% have been affected by the age of 2 years (Koehoorn *et al.* 2008). Although bronchiolitis is generally benign, 2-3% of the infants need hospitalization during the first year of life (Smyth and Openshaw 2006).

2.2.2 Incidence of asthma development after wheezing

In a subset of children, transient wheezing turns into a persistent form which is suggestive of early-onset asthma. This persistent wheezing phenotype has strongly been associated with atopic predisposition, such as early sensitization especially to aeroallergens (Martinez *et al.* 1995, Illi *et al.* 2006, Just *et al.* 2008, Holt and Sly 2012, Jackson *et al.* 2012, Kusel *et al.* 2012). Likewise, the prevalence of recurrent asthma symptoms or asthma at age 6 years was 30-60 % depending on the viral etiology of the early wheezing in high-risk children with atopic predisposition (Jackson *et al.* 2008). The increasing age at wheezing and wheezing severity predict also asthma development. Prospective birth-cohort-studies, usually based on self-reported symptom history (Martinez *et al.* 1995, Just *et al.* 2008, Matricardi *et al.* 2008), have demonstrated that 25% of infants who developed asthma had started wheezing by the age of 6 months, while 75% by the age 3 years (Martinez *et al.* 1995, Lau *et al.* 2003). The prevalence of asthma at pre-school age was 16 % after a health-care specialist-confirmed bronchiolitis diagnosis on outpatient visit (Carroll *et al.* 2009). However, after hospitalization for wheezing at infancy, 18-53 % experienced frequent asthma symptoms at pre-school age (Wennergren *et al.* 1992, Valkonen *et al.* 2009) and 15-40% had asthma at school-age (Sigurs *et al.* 2000, Kotaniemi-Syrjänen *et al.* 2003, Henderson *et al.* 2005). In hospitalized infants, the RSV-wheezing at age <12 months predicted recurrent wheezing symptoms up to teenage years suggesting that severe RSV disease in a young infant may have a different impact on the development of asthmatic symptoms than a milder disease (Sigurs *et al.* 2005).

2.2.3 Prevalence of childhood asthma

According to the International Study of Asthma and Allergies in Childhood (ISAAC), the world-wide prevalence of current wheeze is 12 %, frequent or severe asthma symptoms 5 %, and 9% for asthma ever in 6-7 year-old children (Lai *et al.* 2009). The respective rates were 14%, 7%, and 13% in 13-14 year-old children (Lai *et al.* 2009). The prevalence of asthma symptoms differs regionally; the rates of current wheeze are highest in Oceania (22-29%) and in Australia, Canada, Isle of Man, New Zealand and the UK (23-28%), and lowest in Africa (3-11%) and Northern and Eastern Europe (4-5%) (Lai *et al.* 2009, Beasley *et al.* 2015) (Figure 2). The prevalence of asthma symptoms in Europe is 3.2-6.2% including Finland (5.1%) and Sweden (3.4%) (Lai *et al.* 2009). A Finnish epidemiological study, based on the ISAAC methodology, with over 10 000 school-aged children (13-14 years) showed the prevalence of doctor-diagnosed asthma 4-7%, whereas 10-12% had had asthma-like symptoms (Pekkanen *et al.* 1997).

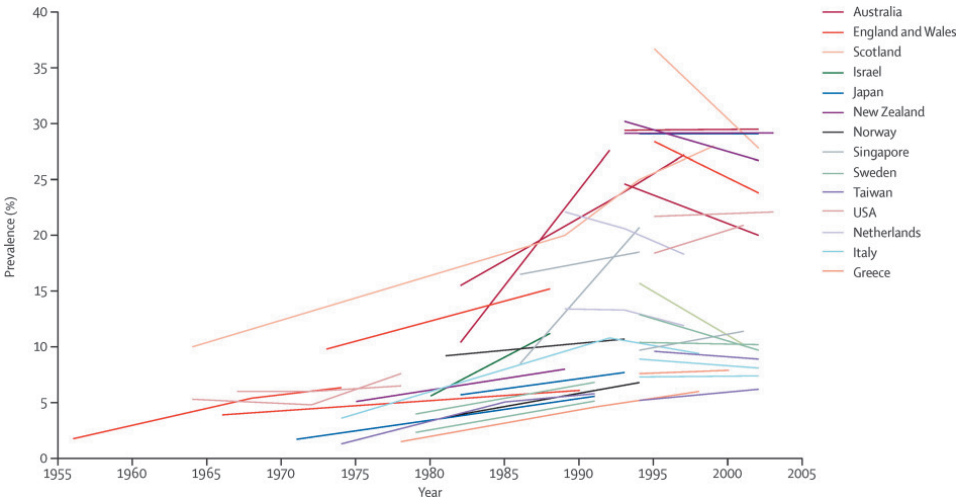


Figure 2. Global trends in asthma symptom prevalence in children by country. Studies were selected in which at least two prevalence datapoints were obtained with the same asthma symptom criteria in the same age group, population and geographical area. Countries were included if initial data available was from before 1985, to provide long-term international trends in asthma symptom prevalence. Prevalence datapoints were a minimum of four years apart. The same diagnostic criteria were used in each study, although these were not standardised between studies. The populations studied included children, ranging from 5 to 18 years. From the review of Beasley *et al.* 2015.

2.3 Virus etiology of wheezing and asthma

2.3.1 Rhinoviruses

Rhinoviruses belong to the *Enterovirus* genus in the *Picornaviridae* family. The genus *Enterovirus* consists of 13 species of which *Enterovirus* A-D and *Rhinovirus* A, B and C (RV-A, RV-B and RV-C, respectively) are human pathogens. Picornaviruses are small (~30 nm), non-enveloped ribonucleic acid (RNA) viruses containing a single-stranded RNA (Lee W 2017). Rhinoviruses were identified in the 1950s when studies finding cure to the common cold started (Andrewes *et al.* 1953). Currently, over 160 RV types have been identified; the genetically based classification assigns 80 RV-A and 32 RV-B serotypes, and 65 RV-C genotypes (Jacobs *et al.* 2013, Bochkov and Gern 2016). The first RV-C viruses were identified in 2006 when the reverse transcriptase polymerase chain reation (RT-PCR) method became the main method for diagnosis of picornaviruses (Lamson *et al.* 2006). RV-Cs do not grow in a standard cell culture (Bochkov *et al.* 2011). Previously, the diagnostics of rhinovirus infections was mainly based on virus culture. The method was slow and relatively insensitive, and the epidemiology of rhinovirus infections remained uncertain (Lee W 2017).

Rhinoviruses are widely spread circulating year-round, with peaks in late spring and early autumn (Rollinger and Schmidtke 2011, Hodinka 2016). They cause respiratory tract infections (mostly common cold-associated symptoms) and predispose to otitis media, but also lower respiratory tract infections, such as pneumonia, bronchiolitis, wheezing, and asthma (Khetsuriani *et al.*, 2008). Though, up to 60% of small children infected with rhinoviruses may be asymptomatic (van Gageldonk-Lafeber *et al.* 2005, Lee W 2017). In infants aged <12 months rhinovirus is the second most common etiological agent of wheezing after RSV, causing up to 30% of the first wheezing episodes in hospitalized infants (Marguet *et al.* 2009, Midulla *et al.* 2010, Mansbach *et al.* 2012). However, after the age >12 months, rhinovirus predominates causing up to 50% of wheezing episodes (Rakes *et al.* 1999, Kotaniemi-Syrjänen *et al.* 2003, Jartti *et al.* 2004, Kusel *et al.* 2006, Jackson *et al.* 2008, Jartti *et al.* 2009). Rhinovirus is also the leading cause of bronchiolitis leading to hospitalization outside the winter RSV bronchiolitis season (Miller *et al.* 2007, Rossi and Colin 2015, Meissner 2016).

Pathophysiology

Rhinoviruses are spread by contact (typically by hands) or via large or small aerosol particles. Rhinoviruses can live on surfaces for several hours to days, and on healthy skin for a couple of hours (Winther *et al.* 2011). The incubation of rhinovirus infection lasts 2-3 days (Lessler *et al.* 2009). They are inoculated by intranasal route, and replication occurs in the nasal epithelium, pharyngeal mucosa, or lower respiratory tract (Gern *et al.* 1997, Malmstrom *et al.* 2006, Renwick *et al.* 2007).

Most rhinovirus A types and all B types utilize intercellular adhesion molecule 1 (ICAM-1) (major types) as their cell entry receptor. Some of the rhinovirus A types (minor types) utilize low-density lipoprotein receptor (LDLR). ICAM-1 and LDLR expression has been confirmed in ciliated and non-ciliated cells of airway epithelium, and rhinovirus infection further induces ICAM-1 expression in lower airway epithelium (Papi and Johnston 1999, Blaas and Fuchs 2016, Lee W 2017). Recent data suggest that at least some rhinovirus C strains could utilize the cadherin-related family member 3 (CDHR3) with yet unknown biological function (Bochkov and Gern 2016).

Once rhinovirus infection has been established, respiratory symptoms are the result of few processes; slight destruction of normal airway tissue due to direct effects of the virus, pro-inflammatory immune responses to the infection, and up-regulation of cellular receptors (Jacobs *et al.* 2013, Blaas and Fuchs 2016). Rhinoviruses, unlike other viruses, cause minimal cytotoxicity (Nakagome et al. 2014). Also, the amount of epithelial damage does not correlate with the severity of the symptoms,

suggesting that symptoms are not produced by direct virus-induced damage to the epithelium. The first line of defense against rhinovirus infection is the airway epithelium which serves as a relatively resistant barrier against infection when undamaged. Rhinoviruses themselves can disrupt the barrier function (Blaas and Fuchs 2016). The early innate responses initiate with the attachment of the virus to its cellular receptor followed by uncoating of the virus in the cellular endosomes. After uncoating, infected cells recognize rhinovirus RNA with the interaction of toll-like receptors (especially toll-like receptor -2, -3, -7 and -8), melanoma differentiation-associated gene 5, and retinoic acid-inducible gene 1 (Jacobs *et al.* 2013, Royston and Tapparel 2016). This results in early innate immune responses with the expression of type I interferon (IFN)-ß and type III IFN-lambda enhancing the antiviral activity. Further, the epithelial cells start the expression of pro-inflammatory cytokines e.g. interleukin (IL)-6 and IL-8, which then attract inflammatory cells (neutrophils, lymphocytes, and eosinophils) at the site of infection (Jacobs *et al.* 2013). Inflammation of the airways causes epithelial edema, increased mucus production and results in airway obstruction and wheezing *in vivo* (Gern 2010, Hershenson 2013, Royston and Tapparel 2016, Lee W 2017). As a sign of adaptive immune responses, serotype-specific neutralizing serum IgG antibodies and IgA secretory antibodies in the airways are detectable usually after one or two weeks after inoculation and maintained for at least one year (Jacobs *et al.* 2013).

Association of rhinoviruses with wheezing illnesses

Certain features of rhinovirus infection point to the possibility that rhinovirus infection induces asthma symptoms. Rhinovirus infection intensifies the allergic airway inflammation in murine models by inducing the expression of eotaxin and IL-4 and IL-13, as well as increased infiltration with inflammatory cells, such as eosinophils, macrophages, and neutrophils in the respiratory tract (Nagarkar *et al.* 2010, Hammond *et al.* 2015). Rhinoviruses also stimulate the synthesis of factors that may influence airway remodeling, such as vascular endothelial growth factor, nitric oxide, and transforming growth factor beta during in vitro experiments with cultured human epithelial cells (Stone and Miller 2015). Airway inflammation itself may increase the expression of ICAM-1 and suppress toll-like receptor 7 signaling in the airways, which could be the link between increased severity of rhinovirus-induced illnesses using ICAM-1 and diseases of chronic airway inflammation (Bochkov and Gern 2016). The probable explanation for the association of asthma in children with rhinovirus-induced wheezing is that susceptibility to rhinovirus-induced early wheezing is a consequence of genetic variation at the 17q21 locus in rhinovirus-affected children (i.e., may markedly increase the risk of asthma) (Caliskan *et al.* 2013).

Early-onset sensitization and rhinovirus-triggered wheezing

Several studies support the so-called multiple-hit hypothesis, whereby infants with immune dysregulation (favoring the atopic phenotype) develop lower respiratory viral illnesses during a critical period of lung development and progress to asthma (Lemanske 2002, Oddy *et al.* 2002, Wu *et al.* 2008, Carroll *et al.* 2012, Holt and Sly 2012, Jackson *et al.* 2016). The Childhood Origins of ASThma (COAST) study group showed earlier that rhinovirus-induced wheezing predicted subsequent asthma development (Jackson *et al.* 2008), and more recently in a statistical model the chronological order of causality that early-life aeroallergen sensitization precedes rhinovirus illnesses and asthma (Jackson *et al.* 2012). They hypothesized that pre-existing sensitization leads to the vulnerability for rhinovirus-related wheezing. Also, the Australian high-risk cohort documented an increased risk for asthma at age 5 years in infants with pre-existing sensitization, when exposed to early wheezing with rhinovirus or RSV (Kusel *et al.* 2007), or to repeated severe lower respiratory tract infections in the first two years of life (Holt *et al.* 2010, Kusel *et al.* 2012). Concluded, increased risk for asthma development is mainly observed when sensitization and wheezing occur concomitantly, suggesting the possibility of direct interactions between the underlying inflammatory pathways involved.

The susceptibility for rhinovirus-induced wheezing in sensitized, asthma-prone children has been explained by alterations in innate immune responses, elevated levels of T helper cell (Th) type 2 cytokines and early airway inflammation (i.e. damaged airway epithelium) (Jakiela *et al.* 2008, Hammond *et al.* 2015). The impaired innate immune responses with impaired Th1 type responses and decreased production of IFN α/β/γ/λ and IL-10 may lead to decreased virus clearance and thereby to increased virus replication (Wark *et al.* 2005, Contoli *et al.* 2006, Durrani *et al.* 2012, Sykes *et al.* 2012). The pronounced Th2 responses resulting from increased production of Th2 type cytokines IL-4, IL-5 and IL-13 in airway secretions impaire Th1 responses, but also lead to increased expression of ICAM-1 on epithelial cells, and thereby contributing to susceptibility for rhinovirus infections (Contoli *et al.* 2015, Jackson *et al.* 2016). This cascade promotes the airway inflammation, more severe wheezing and asthma exacerbations (Wark *et al.* 2005, Contoli *et al.* 2006, Durrani *et al.* 2012, Jackson *et al.* 2016).

2.3.2 Respiratory syncytial virus

RSV belongs to the family *Paramyxoviridae* and is a member of the genus *Pneumovirus*. The family also includes parainfluenza types 1-4 viruses and metapneumovirus. RSV is a single-stranded enveloped RNA-virus with two major

antigenic groups, A and B. The genetic diversity of proteins among groups RSV A and B form several subgroups with 10 A genotypes and 13 B genotypes (Williams *et al.* 2017). RSV has a clear seasonality in Northern Europe with the peak prevalence yearly between late fall and early spring (Rossi and Colin 2015). Moreover, in Finland RSV epidemics follow a regular long-term biannial double-humped pattern (Waris 1991).

RSV is the main causative virus for bronchiolitis causing as much as 80% of the cases, the peak incidence being in infants between 3 and 6 months of age (Mansbach *et al.* 2012, Meissner 2016). Of the annual birth cohort during the first year of life, approximately 20% require outpatient medical care, whereas 2-3% with more severe illness need hospitalization due to RSV bronchiolitis/pneumonia (Smyth and Openshaw 2006). Currently, RSV is diagnosed with PCR even though time-saving rapid detection of RSV antigens is still used in clinical decision-making (Hodinka 2016, Griffiths *et al.* 2017). Risk factors for severe RSV bronchiolitis are the age <3 months, prematurity with the presence of chronic lung disease, congenital heart disease, immunodeficiency and neuromuscular disorders (Scottish Intercollegiate Guidelines Network (SIGN). Bronchiolitis in children. A national clinical guideline. 2006, AAP 2006, Ralston *et al.* 2014, Meissner 2016, Williams *et al.* 2017). In young infants, especially in preterm infants, the apnea without wheezing or other clinical findings may be the early manifestation of viral bronchiolitis (Schroeder *et al.* 2013).

Mechanisms of RSV infections

The pathogenesis of RSV bronchiolitis is unique and thereby cannot directly be compared with wheezing caused by other viruses (Rossi and Colin 2015, Griffiths *et al.* 2017). RSV causes direct damage to the airway epithelium so that it exposes the airway structures to extensive damage through apoptosis and necrosis by upregulating type I IFNs. Besides the direct cytopathic effect of the virus, the inflammatory response has an important role in the development of the signs and symptoms charasteristic to RSV. The inflammatory response in the airway epithelial cells occurs through massive release of pro-inflammatory mediators, with emphasis on tumour necrosis factor α and chemokines *e.g.* CCL-3 and -5 and CXCL10 and -11, which then contribute to activation of leukocytes (monocytes and polymorphonuclear cells) at the infection site. This combined effect of the virus and the inflammatory response to it leads to epithelial damage, sloughing off of epithelium, mucus production and ultimately airway obstruction leading to wheezing (Tregoning and Schwarze 2010, Guo-Parke *et al.* 2013, Rossi and Colin 2015, Russell *et al.* 2017, Williams *et al.* 2017).

2.3.3 Clinical differences between rhinovirus and respiratory syncytial virus

Rhinovirus and RSV are the major pathogens of virus-induced lower respiratory tract infections and wheezing representing distinct characteristics and pathogenetic mechanisms (Rossi and Colin 2015, Vandini *et al.* 2017). Children hospitalized for rhinovirus-induced wheezing tend to be older, are more likely to have wheezed previously (Rakes *et al.* 1999, Korppi *et al.* 2004, Jartti *et al.* 2009, Turunen *et al.* 2016b), have more allergic sensitization compared to RSV (Jartti *et al.* 2010, Turunen *et al.* 2014), and they also show a more favourable response to oral corticosteroid (OCS) treatment than children with RSV (Korppi *et al.* 2004, Mansbach *et al.* 2016). Rhinovirus usually causes wheezing in children older than 12 months, while RSV causes wheezing during the first year of life (Jartti *et al.* 2009, Turunen *et al.* 2014). In the COAST study with high-risk children, RSV caused more severe infections than rhinovirus (Gern *et al.* 2002), which was also reported in hospitalized small infants (Mansbach *et al.* 2008, Marguet *et al.* 2009, Turunen *et al.* 2014). In children with rhinovirus the start of the wheezing illness was more rapid and the duration was shorter compared to RSV infection (Mansbach *et al.* 2008, Mansbach *et al.* 2012, Dumas *et al.* 2016). However, Korppi et al found no differences in the clinical severity between rhinovirus and RSV bronchiolitis in hospitalized infants (Korppi *et al.* 2004).

2.3.4 Other viruses

The association between wheezing illnesses and virus infections is evitable (Meissner 2016). The wheezing episodes are triggered by viral infections in up to 95% of the cases during the first three years of life (Jartti *et al.* 2004, Jackson *et al.* 2008, Jartti *et al.* 2009, Marguet *et al.* 2009). Human bocavirus has been found an important viral pathogen causing up to 20% of the wheezing episodes in children (Jartti *et al.* 2004, Söderlund-Venermo *et al.* 2009). Most bocavirus findings have been co-infections with other viruses. Other noteworthy respiratory viruses include metapneumovirus with detection rate up to 12% in the first wheezing episode (Jartti *et al.* 2009, Marguet *et al.* 2009, Midulla *et al.* 2010, Nascimento *et al.* 2010). Parainfluenza types 1-4 viruses are responsible for nearly 14% of the wheezing episodes in infants (Kotaniemi-Syrjänen *et al.* 2003, Jackson *et al.* 2008). From the influenza A, B and C viruses only influenza A and B cause significant diseases and about 5-8% of wheezing episodes in infants aged less than two years (Kotaniemi-Syrjänen *et al.* 2003, Kusel *et al.* 2007). Adenoviruses are grouped into 7 species (A-G) comprising more than 68 types being responsible for up to 5% of wheezing episodes in hospitalized wheezing children (Jartti *et al.* 2004). Human coronaviruses 229E and OC43 (identified in the mid 1960s), and more novel NL63 and HKU1 (found 2003 and 2004) have been associated with early-life wheezing episodes in 3-13% of the cases (Kusel *et al.* 2007, Bisgaard *et*

al. 2010, Berry *et al.* 2015). Enteroviruses cause bronchiolitis (~10%), first wheezing episode (1.2-21%) and asthma exacerbations (16%) (Andreoletti *et al.* 2000, Thumerelle *et al.* 2003, Jartti *et al.* 2009, Marguet *et al.* 2009, Nascimento *et al.* 2010).

2.4 Risk factors for school-age asthma

Multiple studies during the past 3 decades have demonstrated that the most important risk factors for childhood asthma are: atopy defined as sensitization against food or perennial allergens, eczema at early childhood, parental asthma, and parental smoking (NAEPP 2007, Rubner *et al.* 2017). Except for parental smoking, other risk factors are associated with the development of atopic asthma (Rönmark *et al.* 1999, Civelek *et al.* 2011, Göksor *et al.* 2013). Children who develop atopic asthma have most likely atopic diseases in family, eczema at early life and wheezing due to rhinoviral infections (NAEPP 2007). Risk factors such as parental smoking (Rönmark *et al.* 1999, Civelek *et al.* 2011, Göksor *et al.* 2013) and short or no breast-feeding (Rönmark *et al.* 1999) are associated with later development of non-atopic asthma in childhood. This fact underlines the need to better define the phenotypes of childhood asthma as their pathogenesis may be of different origin. Good characterization of the infants suffering from their first wheezing episode may provide a window to understand the natural course of atopic and non-atopic asthma as well as to develop novel preventive treatment strategies. However, the march from early life wheezing to persistent asthma is complex and evidently multi-factorial with many host- and environmental factors involved in the process.

2.4.1 Atopic characteristics

Aeroallergen sensitization

Allergic sensitization, especially to aeroallergens during early childhood is a major risk factor for recurrent wheezing and childhood asthma (Illi *et al.* 2006, Kusel *et al.* 2007, NAEPP 2007, Jackson *et al.* 2008, Matricardi *et al.* 2008, Baris *et al.* 2011, Kusel *et al.* 2012, Wisniewski *et al.* 2013, Chiu *et al.* 2014). The German MAS cohort is a birth cohort with infants at high risk for sensitization. It presented data that positive family history of atopy, and wheezing together with sensitization, especially to perennial aeroallergens defined by specific immunoglobulin E (IgE) before age of 3 years, predicted persistent wheezing at age of 11-13 years (Illi *et al.* 2006, Matricardi *et al.* 2008). Interestingly, in a study including only children with eczema, early cat sensitization, and more specifically IgE ab towards Fel d 4 and Fel d 1, was strongly associated with wheezing (Wisniewski *et al.* 2013).

Likewise, in a Finnish population-based study on hospitalized <2 year-old wheezing children, early aeroallergen sensitization predicted asthma at adolescence (Piippo-Savolainen *et al.* 2007).

Food allergen sensitization

As defined by old "atopic march" definition of atopic diseases, food sensitization precedes the aeroallergen sensitization, and is thereby an early marker of the atopic immune responses in the host. The early appearance of food sensitization is high in asthma-prone children predicting well childhood asthma (Kusel *et al.* 2007, 2007, Jackson *et al.* 2008, Baris *et al.* 2011, Kusel *et al.* 2012). The German MAS cohort demonstrated that the risk of asthma at age 5 years was 5-fold higher if a child still had food sensitization at age 2 compared to infants whose food sensitization disappeared by the age 2 years (Kulig *et al.* 1998). In the Autralian birth cohort study the sensitization by age 2 years was an independent risk factor for asthma at 5 years (odds ratio [OR] 3.1) compared to never sensitized (OR 0.4) (Kusel *et al.* 2007).

It has been shown in birth cohort studies how food sensitization is common during the first two years of life persisting throughout childhood. The aeroallergen sensitization starts to manifest after that (Nissen *et al.* 2013, Chiu *et al.* 2014). The early appearance of food sensitization is likewise supported by studies on high-risk populations including only children with eczema (Wisniewski *et al.* 2013) or allergic patients (Melioli *et al.* 2012). The prevalence of any sensitization (including both food and aeroallergens) in general at ages 1.5 and 5 years is 6-12% and 23%, whereas in children with early wheezing/asthma as high as 20% and 50% (Nissen *et al.* 2013). This suggests that early food sensitization could serve as a risk marker for childhood asthma, since it is usually detectable with laboratory testing by the time of the first wheezing episode. The role of aeroallergen sensitization becomes more pronounced later.

Eczema

Eczema has been considered a risk factor for childhood asthma since it usually initiates the so called atopic march and is associated with asthma (Wahn *et al.* 1997, Spergel and Paller 2003). The MAS cohort demonstrated that 22% of children had eczema by the age of 2 years, but it resolved in over 40% by age 3 years (Illi *et al.* 2004). They showed that early-onset eczema alone constituted no increased risk for school-age asthma but only if there was a previous wheezing or wheezing at the onset of eczema. Likewise, a systematic review of thirteen prospective cohort studies with 4 birth cohorts and 9 eczema cohorts showed a risk of asthma 2.1 after eczema, but only a third of the children with eczema developed

asthma during later childhood (van der Hulst *et al.* 2007). On the contrary, early wheezing and sensitization predicted school-age asthma, irrespective of eczema, also suggesting different phenotype rather than a direct continuum from eczema to asthma (Illi *et al.* 2004). To support this, the ORCA Cohort applied cluster analysis to study the role of early-onset eczema for the risk of asthma at age 6 years (Amat *et al.* 2015). The analysis consisted of 214 children with atopic characteristics and early-onset eczema revealing that eczema phenotypes with multiple sensitizations or with family history of asthma had higher prevalences of asthma (33-36%) at age 6 years compared to the phenotype with low or no sensitization (15%), emphasizing the significance of concomitant atopic characteristics when estimating future asthma risk. Eczema and asthma may involve mutual genetic mechanisms since mutations in filaggrin gene combined with eczema in the first year of life were associated with a later development of asthma and hay fever (Schuttelaar *et al.* 2009). Concluded, the question regarding the early-onset eczema is whether it expresses different phenotypes with different mechanisms and risks for subsequent asthma development.

2.4.2 Viruses which induce early-life wheezing

The role of virus infections of lower respiratory tract as predicting factors for childhood asthma has been recognized for years (Busse 1989). However, a more profound understanding of these virus infections has become evident relatively recently after the results from long-term birth cohort studies that have followed-up community-based populations through the school years, and in which the collection of infection history data was a central part of the study design. The studies are the COAST (Lemanske 2002) and the Tuscon Children's Respiratory Study (CRS) (Stein *et al.* 1999) in the United States, the Childhood Asthma Study (CAS) in Australia (Kusel *et al.* 2007), and The German MAS in Europe (Illi *et al.* 2001).

The significant point here is the associations between different respiratory viruses and asthma development. Previous studies emphasized the role of RSV (Martinez 2005, Sigurs *et al.* 2010), whereas other have highlighted the role of rhinovirus (Kotaniemi-Syrjanen *et al.* 2003, Kusel *et al.* 2007, Jackson *et al.* 2008), and especially the RV-C rhinovirus as an agent of severe asthma symptoms (Bizzintino *et al.* 2011, Turunen *et al.* 2016a). However, even though RSV is a major pathogen in infant wheezing (Rakes *et al.* 1999), the relative roles of these two viruses in resulting childhood asthma are still being debated (Stein and Martinez 2010). Still, there are major gaps in current knowledge considering the roles of rhinovirus and RSV infections in the asthma pathogenesis.

Rhinovirus

Recently, the improvement of molecular diagnostics has allowed several groups to demonstrate that early-life wheezing caused by rhinoviruses is potentially a more robust marker of asthma development than wheezing episodes caused by RSV (Kotaniemi-Syrjänen *et al.* 2003, Jackson *et al.* 2008, Kotaniemi-Syrjanen *et al.* 2008, Turunen *et al.* 2014, Rubner *et al.* 2017). In high-risk birth cohorts, the early-life rhinovirus-induced wheezing has been linked to school-age asthma (Kusel *et al.* 2007, Jackson *et al.* 2008). The American COAST and the Australian CAS birth cohort studies included only wheezing children with a familial predisposition with at least one atopic parent. The COAST study demonstrated that the risk for recurrent wheezing and asthma by age 6 years was increased if the children had wheezing with rhinoviruses (OR 9.8) *vs.* RSV (OR 2.6) during the first 3 years, and furthermore, 90% of the children with rhinovirus-induced wheezing in the third year of life had asthma by age 6 years (OR 26) (Lemanske *et al.* 2005, Jackson *et al.* 2008). Although rhinovirus wheezing during infancy was an independent asthma risk factor, children who had aeroallergen sensitization and rhinovirus-induced wheezing by age 1 year had the greatest risk of asthma at school age (Jackson *et al.* 2008). The Australian birth cohort study showed that the risk for wheezing at age five years was increased if the wheezing at age <1 year was associated with rhinovirus either alone (OR 3.2) or with concomitant RSV (OR 4.1) but only in children with sensitization at age <2 years (Kusel *et al.* 2007). Therefore, the data of these high-risk birth cohorts may reflect a different susceptibility of atopic airways to rhinovirus infections.

In addition, the subsequent asthma risk has also been demonstrated in population-based long-term follow-up-studies in children hospitalized for the wheezing episode (Kotaniemi-Syrjänen *et al.* 2003, Midulla *et al.* 2012). The school-age asthma was more common after early rhinovirus-induced wheezing (52%, OR 4.1) *vs.* RSV or other viruses (15%) (Kotaniemi-Syrjänen *et al.* 2003). Of the long-term follow-up studies, only one study has focused on the first episode of lower airway infection, and it showed an association between rhinovirus etiology and recurrent wheezing (OR 3.3) in a 12-month follow-up in children with bronchiolitis at age <1 year (Midulla *et al.* 2012).

Respiratory syncytial virus

There are a number of hospital-based long-term follow-up studies examining the association between RSV-induced wheezing and asthma. Thus, early RSV-induced wheezing/lower respiratory tract infection may lead to a phenotype of recurrent wheezing, but it is less commonly associated with asthma or sensitization. In a prospective Swedish study, hospitalization for RSV bronchiolitis

at age <12 months was found to be a risk factor for asthma and allergy at the follow-up visits at the ages 3, 7, 13 and 18 years compared to the matched controls (Sigurs *et al.* 1995, Sigurs *et al.* 2000, Sigurs *et al.* 2005, Sigurs *et al.* 2010). However, a reduction in risk ratios was seen with increasing age, suggesting a resolution of the asthma-increasing effect of early RSV infection. Interestingly, a large retrospective cohort study of unselected population reported that infants born 3 months prior to the peak of the RSV season had the greatest risk for hospitalization due to lower respiratory tract illness and asthma between ages 4 and 5.5 years aiming to support the causal role of RSV bronchiolitis in asthma inception (Wu *et al.* 2008). Similarly, in a birth cohort study, RSV-bronchiolitis requiring hospitalization by age 12 months was associated with asthma by the age 7 years but not with the development of sensitization compared to the other population in the cohort (Henderson *et al.* 2005). These studies are cohort studies showing associations, but they do not answer questions on causality.

On the contrary, studies argue against the causal role for RSV in asthma inception. Two separate Finnish cohorts of hospitalized wheezing children demonstrated that RSV-induced wheezing/lower tract infection was not associated with asthma incidence at school-age (Juntti *et al.* 2003, Kotaniemi-Syrjänen *et al.* 2003) and was associated with negative allergy tests at school-age when compared to matched controls (Juntti *et al.* 2003). Similarly, the data from a large twin registry suggested that although severe RSV illnesses necessitating hospitalization can lead to short-term recurrent wheeze, it is not causal in long-term asthma development (Stensballe *et al.* 2009, Thomsen *et al.* 2009). Likewise, the Tuscon CRS is a non-selected population based birth cohort including healthy infants. They showed that RSV-induced lower respiratory tract illnesses, particularly those severe enough to lead to hospitalization, were associated with an increased risk of frequent wheeze at school age, but the risk decreased being insignificant by 13 years, and there was no link between RSV infections and sensitization (Stein *et al.* 1999).

2.4.3 Age and wheezing severity

The RSV-induced wheezing before age 12 months predicted recurrent wheezing and symptoms up to adolescence in hospitalized infants (Sigurs *et al.* 2005), suggesting that severe RSV disease in a young infant may have different impact on the asthma development than a milder disease. The Tuscon CRS and German MAS studies have showed that virus-induced wheezing is common during infancy, but that this phenotype usually is transient and resolving spontaneously by the age of 3 years (Martinez *et al.* 1995, Matricardi *et al.* 2008). However, in a subset of children, transient wheezing turns into a persistent clinical form which is suggestive of early-onset asthma. This persistent wheezing phenotype has strongly been associated with early sensitization (Taussig *et al.* 2003, Illi *et al.* 2006, Holt

and Sly 2012, Jackson *et al.* 2012, Kusel *et al.* 2012). The increasing age at wheezing was associated with increased incidence of asthma in birth cohort studies. In the Tucson CRS the incidence of wheezing at age three years was 20% in children with wheezing only during the first year of life, while the incidences were 40% and 60% with second and third year wheezing, respectively (Taussig *et al.* 2003). The COAST study demonstrated in children with atopic family members that 90% of the children with rhinovirus-induced wheezing in the third year of life had asthma by age 6 years (OR 26) suggesting that increasing age at wheezing is strongly associated with asthma development, especially in children with atopic characteristics (Lemanske *et al.* 2005, Jackson *et al.* 2008).

2.4.4 Reduced pulmonary function and pre-existing lung inflammation

Reduced pulmonary function

Reduced pulmonary function is seen already in infancy in children with later asthma, suggesting that the chronic course of asthma, characterized by continuing atopic airway inflammation derives from infancy. Reduced pulmonary flow volumes in infancy have been associated with transient wheezing during the three first years of life but not thereafter (Morgan *et al.* 2005), but on the contrary also with wheezing persisting up to teenage independent of sensitization (Turner *et al.* 2004). In turn, infants at risk for asthma (with asthmatic mothers) and with significant airflow deficit at age 1 month, developed asthma by age 7 years (Bisgaard *et al.* 2012). Children with recurrent wheezing and eczema by age 2 years had significantly lower lung volume at birth and at 2 years compared to children without wheezing and eczema (Haland *et al.* 2007). Bronchial hyper-reactivity to histamine provocation in early infancy was related to transient wheezing (Wilson *et al.* 2004), but if present at age 1 year the wheezing remained and was associated with asthma by teenage (Turner *et al.* 2009) supporting the view that future childhood asthma could emerge through infantile airway hyper-reactivity already by the age of 1 year. The response to bronchodilator was seen in 2-year-old asymptomatic children with wheezing history, especially in those with multiple asthma risk factors, compared to never-wheezing controls (Lodrup Carlsen *et al.* 2004). Summarized from these studies, the asthmatic disorders may be related to genetic predisposition, atopic susceptibility or a factor during pregnancy, since the lung fuctions were reduced already in infancy and asthma symptoms seen in later childhood.

Pre-existing lung inflammation

Children who start wheezing early and/or develop persistent symptoms may have long-term changes in lung function. Children with wheezing only before age 3 years or wheezing persisting beyond that, had decreased a lung function up to teenage years compared with children with wheezing onset after 6 years of age and those without wheezing (Martinez *et al.* 1995, Morgan *et al.* 2005). This suggests early changes in airways in wheezing pre-school children, irrespective of age at onset. However, there is conflicting data from the MAS study that children with transient wheezing sustain normal lung function and growth (Lau *et al.* 2003). The changes are probably established by 3 years of age, suggesting early airway remodelling. Children (median age 12-15 months) with recurrent wheezing had an increased level of inflammatory cells and markers in airways compared to a group of healthy controls, but not the characteristic eosinophil predominance or reticular basement membrane thickening (Krawiec *et al.* 2001, Saglani *et al.* 2005). In further studies of older children with recurrent wheezing (median age 29 months), the presence of eosinophilic inflammation and reticular basement membrane thickening was seen (Saglani *et al.* 2007). This suggest that early wheezing in preschool-age may cause long-term deficits in lung growth patterns and lung function predisposing to asthma.

2.4.5 Genetics

The genome-wide association studies have developed over the last decade and many loci across several chromosomes have been associated with asthma. Still, efforts to fully define the disease at the genetic level have failed due to the inconsistency in replicating linkages and presumably due to the heterogeneity of asthma phenotypes (Guerra and Martinez 2008). The chromosome locus 17q21 containing the ORMDL3 and GSDMB genes has most frequently been linked to childhood asthma (Moffatt *et al.* 2007, Galanter *et al.* 2008). Further, it has been demonstrated that the genetic variation of ORMDL3 is associated with non-atopic asthma phenotype in infants with early bronchial hyper-responsiveness but without a risk for atopic characteristics (Bisgaard *et al.* 2009). It has also been shown that genetic variations at the locus 17q21 were associated with early-life rhinovirus-induced wheezing and increased risk for childhood asthma (Caliskan *et al.* 2013). Eczema and the development of asthma and sensitization may involve mutual genetic mechanisms since mutations in filaggrin gene combined with early-onset eczema were associated with the development of asthma and later hay fever (Schuttelaar *et al.* 2009, Bonnelykke *et al.* 2010).

The genetic risk score (GRS) yields a quantitative index of genetic asthma risk derived from 17 asthma-associated single-nucleotide polymorphisms located in or

near the genes *IL18R1, IL13, HLA-DQ, IL33, SMAD3, ORMDL3, GSDMB, GSDMA, IL2RB* (Belsky et al. 2013). This score demostrated that childhood asthma persisted into mid-adulthood 2-3 times more likely in subjects with higher GRS than in those with median or low GRS (Belsky *et al.* 2013). Interesting is that genetic variants in children have been shown to respond differently to therapies; for example, a beneficial effect of ICS on airway hyper-responsiveness (Tantisira *et al.* 2004) or a decreased response to corticosteroids (Tantisira *et al.* 2011).

2.4.6 Parental smoking

Smoking rates during pregnancy have only slightly decreased over the last decade, with rates varying from 5-40% in Europe (Smedberg *et al.* 2014). About 50% of pregnant smokers quit smoking within the first trimester, and 50% smoke throughout pregnancy despite well-known risks (Alshaarawy and Anthony 2015). The prenatal exposure to tobacco smoke is an independent risk factor for childhood wheezing and asthma and is more critical than postnatal exposure, leading to permanent damage to the airways and reduced lung function until adulthood (Gilliland *et al.* 2000, Gilliland *et al.* 2001, Svanes *et al.* 2004, Lannero *et al.* 2006, Pattenden *et al.* 2006). Maternal smoking during pregnancy was associated with physician-diagnosed asthma (OR 1.8) (Gilliland *et al.* 2001), while a pooled analysis of 8 birth cohorts with 21000 children showed a risk for wheezing (OR 1.4) and for asthma at 4-6 years of age (OR 1.7) (Neuman *et al.* 2012). The Swedish study demonstrated that harmful effects of prenatal or early postnatal exposure to tobacco smoke are mediated by non-reversible changes in the airways, persistence or development of bronchial hyper-responsiveness, and increased smoking in mid-adulthood (Göksor *et al.* 2007).

Mothers who continued smoking beyond the first trimester delivered lighter infants with reduced lung function and an increased need for asthma therapy at age 5 years (Prabhu *et al.* 2010). This suggests that smoking cessation during the first trimester may be sufficient to prevent the fetus from harmful effects of maternal smoking. Nicotine is assumed to be the responsible component of tobacco smoke that affects lung development in fetuses. Noteworthy is that fetus' lungs can be exposed to nicotine concentrations similar to that in the blood of smokers. Nicotine predisposes fetus' lungs to thicker alveolar walls, increased collagen deposition and airway smooth muscle, and airway hyper-responsiveness with airflow restriction (Wongtrakool *et al.* 2007). It also leads to longer and more tortuous airways, and thereby decreases forced expiratory flows (Wongtrakool *et al.* 2007).

2.5 Predictive indices of childhood asthma

Early identification of children at high asthma risk would be useful in finding children who require closer monitoring, but also for prevention strategies or interventions. Therefore, the accurate prediction of asthma development would be desirable for physicians, families, and also for researchers. Of note is, that all current predictive indices of childhood asthma used in children <4 years old require recurrent wheezing, and therefore are not suitable for use at the first wheezing episode (Castro-Rodriguez *et al.* 2000, Kurukulaaratchy *et al.* 2003, Guilbert *et al.* 2004a, Devulapalli *et al.* 2008, Chang *et al.* 2013, Hafkamp-de Groen *et al.* 2013) (Table 1). Only one congress abstract has replaced the requirement of recurrent wheezing with a single rhinovirus-induced wheezing episode (Jackson *et al.* 2009).

The API was developed by the Tuscon study group to predict on-going asthma activity at the age of 6, 8 and 11 years in children <3 years with a history of recurrent wheezing (Castro-Rodriguez *et al.* 2000, Taussig *et al.* 2003). The asthma risk was based on risk factors from questionnaire data at ages 2 and 3 years on an unselected birth cohort of 1246 infants (Table 1). A positive API requires fulfilling the stringent criteria. By the age of 3 years, positive API was associated with 76% risk of active asthma from age 6 years, compared to <5% in those with negative API. The algorithm exhibited good specificity of 85-97%, but a low sensitivity of 16-42%, only.

Later, the modified API (mAPI) was developed by the Prevention of Early Asthma in Kids (PEAK) trial, to select children with recurrent wheezing for a study of secondary prevention of asthma with ICS in high-risk children (Guilbert *et al.* 2004b). The mAPI used more objective criteria than the API. Likewise, the COAST study group tested the asthma predictive ability of the mAPI in a high-risk cohort with a 30% pretest probability to 90% posttest probability (Chang *et al.* 2013). However, the current predictive indices do not recognize the future asthma phenotype, and there is no specific risk index for a prediction of non-atopic asthma in children (Table 1).

Table 1. Predictive indices of childhood asthma.

	Criteria	Stringent API	Loose API	mAPI	m2API				
First author and year		Castro-Rodriquez 2000	Castro-Rodriquez 2000	Guilbert 2004b	Chang 2013	Jackson 2009	Devulapalli 2008	Hafkamp-de Groen 2013	Kurukulaaratchy 2003
Study		Tuscon	Tuscon	COAST	COAST	COAST	Oslo*	PIAMA	Isle of Wight
Study set-up		General	General	Risk of atopy	Risk of atopy	Risk of atopy	General	General	General
Number of children		1246	1246	259	259	259	3752	3967	1034
Age at inclusion		≤3 years	≤3 years	≤3 years	≤3 years	≤3 years	2 years	≤4 years	≤4 years
Primary criterion	Wheezing nr/ last 12 months	≥3	<3	≥4	≥2	≥1 rhinovirus wheezing	Wheezing nr/ hospitalizations		
Major criteria	Parental asthma	yes	yes	yes	yes	yes	no	Family history of asthma	Family history of asthma
	Eczema	yes	yes	yes	yes	yes	no	Eczema	Eczema
	Aeroallergen sensitization	no	no	no	yes	yes	no	Frequent wheezing	Positive SPT at 4 years
	Allergic rhinitis	yes	yes	no	no	no	no	Wheeze without cold	Recurrent RTI at 2 years
Minor criteria	Allergic rhinitis	yes	yes	yes	no	no	no	Dyspnoea	
	Wheezing apart from colds	yes	yes	yes	yes	yes	no	Birth <37 weeks	
	B-eos ≥4%	yes	yes	yes	yes	yes	no	Sex	
	Food sensitization		no	no	yes	yes		SET	
Required nr of major criteria		1	1	1	1	1	Severity scoring	Severity scoring	Risk scoring
OR									
Required nr of minor criteria		2	2	2	2	2			
Asthma outcome		Parent-reported doctor-dg	Parent-reported doctor-dg	Doctor-dg	Doctor-dg	Doctor-dg	Doctor-dg	Parent-reported doctor-dg	Parent-reported wheezing
Outcome age		8 years	8 years	8 years	8 years	6 years	10 years	6 years	10 years
Incidence of asthma		14 %	14 %	33 %	33 %	28 %	36 %	6 %	37 %
Sensitivity		17%	51 %	19 %	28 %	59 %	52 %	64 %	53 %
Specificity		97 %	81 %	100 %	98 %	87 %	88 %	74 %	85 %
Positive predictive value		44 %	29 %				54 %	12 %	68 %
Negative predictive value		88 %	91 %				87 %	97 %	74 %
Positive likelihood ratio		5.1		55	13		4.3	2.4	3.4
Negative likelihood ratio		0.86		0.83	0.73		0.55	0.49	0.56

RTI: Respiratory tract infection; *SET:* Socioeconomic status; *SPT:* skin prick test

* The Environment and Childhood Asthma study

2.6 Primary prevention strategies of wheezing and asthma development

Atopic asthma progresses through recognizable stages during childhood, but the developmental stages of non-atopic asthma are not well established. The early recognition of children at high risk for distinct asthma phenotypes would be ideal in finding those who would benefit from early targeted prevention strategies to reduce inflammatory process and episodes, and thereby prevent asthma progression (Holt and Sly 2012, Nieto *et al.* 2014, Szefler 2014, Jackson *et al.* 2016, Wawrzyniak *et al.* 2016). The development of asthmatic disorders may result from a genetic predisposition or factors during pregnancy or infancy influencing the atopic susceptibility or airway physiology (Beasley *et al.* 2015, DeVries *et al.* 2016). Therefore, for children, a reasonable time frame for primary prevention of asthma might be *in utero* or in infancy. Though, prevention studies are scarce. In a clinical setting, the advice about primary prevention of asthma development has been separated from the secondary prevention of symptoms in children with an existing asthma diagnosis. In the following, there are the current data on primary prevention of childhood asthma.

2.6.1 Prevention of asthma susceptibility in-utero

Maternal diet is no longer restricted during pregnancy, since there is no evidence for the preventive effect of allergen-free diet on allergies or asthma in the offspring. The recommendations are merely vice versa, so that mothers are encouraged to eat diversely, and to include D-vitamin substitution to reduce the child's risk for atopic diseases (Fleischer *et al.* 2013, GINA 2016, Christensen *et al.* 2017). The risk for asthma in early childhood is increased by maternal antibiotic use during the third trimester of pregnancy, whereas decreased by the use of prebiotics or probiotics decreases (Rautava *et al.* 2012, Stensballe *et al.* 2013, DeVries *et al.* 2016, Wolsk *et al.* 2017). This suggests that microbial immune and metabolic programming begins during fetal life. It is noteworthy that prenatal maternal psychosocial wellness may contribute to the child's health. It has been shown that maternal stress symptoms during pregnancy have been associated with proallergenic cytokines in mothers in mid-pregnancy, but also in atopic disorders in children (Andersson *et al.* 2016a, Andersson *et al.* 2016b, Karlsson *et al.* 2017). All mothers should be encouraged to avoid/stop smoking during pregnancy for it is known to be a risk factor for childhood wheezing and asthma, particularly non-atopic asthma (Gilliland *et al.* 2000, Gilliland *et al.* 2001, Svanes *et al.* 2004, Lannero *et al.* 2006, Pattenden *et al.* 2006, Göksor *et al.* 2013, GINA 2016).

2.6.2 Prevention of asthma susceptibility in infancy

It has been noted that diversity of natural and human microbiota decreases the risk for allergies and asthma in children. The emerging amount of allergy and asthma may result from reduced exposure to natural environments with rich microbiota and diet, leading to a conclusion that early-life microbial exposures may decrease the risk for development of allergic diseases (Haahtela *et al.* 2015, Ruokolainen *et al.* 2015, von Hertzen *et al.* 2015, Jackson *et al.* 2017). In line, a delayed introduction of solid food is no longer recommended for the prevention of early atopic susceptibility, and therefore complementary foods are to be introduced between 4 and 6 months of age (Greer *et al.* 2008, Fleischer *et al.* 2013, Lau 2013, GINA 2016). Likewise, the reduction of early-life exposure to common allergens is not recommended, since early-life avoidance of inhalant allergens (house dust mite or pets) may not reduce the risk for allergies and asthma (Abramson *et al.* 2013, Beasley *et al.* 2015, GINA 2016). The type of delivery, that is ceasarean section, and the exposure to broad-spectrum antibiotics in the first week of life are shown to increase the risk of school-age (atopic) asthma and allergic rhinitis (Göksor *et al.* 2013, Alm *et al.* 2014, Black *et al.* 2016, Wu *et al.* 2016). Therefore, vaginal delivery is encouraged (GINA 2016). It is attempting to speculate that the manipulation of infant gut microbiome within the first 4-6 weeks in high-risk infants would affect the disease development (Chu *et al.* 2017, von Mutius 2017). Using prebiotics or probiotics may be beneficial, since a new target of potential preventive intervention could be the human microbiome as a key player in the development of inflammatory diseases such as allergy and asthma (Luoto *et al.* 2014, Collado *et al.* 2015, Hendaus *et al.* 2016). Breast-feeding is being advised merely for its many health benefits than for asthma prevention (GINA 2016). It has only some protection against early non-atopic wheezing in low-income countries (Nagel *et al.* 2009), suggesting a confounding factor, such as fewer smoking mothers among breast-feeders.

2.6.3 Prevention of asthma susceptibility in sensitized infants

Reduction of sensitization

Early-life sensitization is a hallmark for underlying susceptibility to viral-induced and atopic respiratory symptoms. In transiently wheezing age group the evidence between allergic sensitization and virus-induced wheezing is strong in early asthma development. Thus, the reduction of early sensitization is likely to be in a key position in the prevention of development of atopic asthma. Hypothetically, prophylactic immunotherapy in carefully selected patients would be useful in preventing the onset of new allergen sensitizations, and the disease progression to asthma (Di Bona *et al.* 2016). Though, the adequate time frame for the

immunotherapy in high risk children might be much earlier than already researched, and thus warrants further studies.

Reduction of respiratory infections and virus-induced exacerbations

The growing evidence indicate that early lower respiratory infections may play a central role in the development of asthma in sensitized children, and thus has put the reduction of viral infections to the list for asthma primary prevention. The reduction of virus-induced infections and exacerbations in infancy would hypothetically decrease the postnatal inflammatory insults to the growing lung, which otherwise would contribute to potent long-lasting damaging effects on the lung function. The development of vaccines against the most important pathogens, rhinovirus and RSV, has remained challenging (Kelly and Busse 2008, Holt and Sly 2012, Rossi and Colin 2015). The development of vaccine against rhinoviruses has been difficult due to great number of rhinovirus types and subtypes with variable antigenic sites (Holt and Sly 2012). Also, the variation of possible asthmagenic properties of rhinovirus types should be considered in the vaccine development (Stone and Miller 2015). However, a recent study generated a vaccine capable of inducing virus-neutralizing antibodies to numerous and diverse rhinovirus types in rhesus macaques (Lee *et al.* 2016). In preterm infants, the immunoprophylaxis with monoclonal antibody treatment against RSV reduced severe infections (Simoes *et al.* 2007), and the risk for recurrent wheezing in non-atopic children (Simoes *et al.* 2007, Simoes *et al.* 2010, Blanken *et al.* 2013), but was not preventive for asthma at age of 6 years (Carroll *et al.* 2017).

Hand washing with soap and water instead of ethanol-based hand disinfectants has been found effective in removing rhinoviruses, since they are non-enveloped viruses (Savolainen-Kopra *et al.* 2012). Two randomized clinical trials (RCT) conducted in Turku, Finland, showed that pre- and/or probiotics reduced the incidence of respiratory tract infections during the first year of life, particularly rhinovirus infections reduced in preterm infants when pre- and probiotics were administered on days 3-60 (Rautava *et al.* 2009, Luoto *et al.* 2014). These findings suggest that modification of gut microbiota might offer a novel and cost-effective way to reduce respiratory infections. However, the reduction of respiratory infections *per se* may be insufficient in asthma prevention if the genetic predisposition or atopic susceptibility have the key role in promoting asthma. The causative role of respiratory viruses in asthma pathogenesis could be established only when the incidence of childhood asthma is reduced by intervention trials targeting the viruses.

2.6.4 Reduction of early airway inflammation

A proportion of the children with transient wheezing phenotype go on with more persistent symptoms. This period would potentially be an ideal therapeutic window for long-term disease modification. One prevention alternative would be to tackle the pre-existing airway inflammation in sensitized children before asthma develops. It has been demonstrated that in children with atopic asthma, the use of seasonal monoclonal antibodies against IgEs *ie.* anti-IgE reduced asthma exacerbations by blocking IgE-mediated inflammation (Busse *et al.* 2011, Teach *et al.* 2015). However, safety data is lacking for young children. The current clinical practice prefers the use of non-specific anti-inflammatory drugs, typically inhaled corticosteroids together with symptom-relieving therapy against airway narrowing (NAEPP 2007). This practice is merely empirical, with no specified target to fundamental cause or asthma phenotype. There is current data on short- and long-term efficacy of inhaled and systemic corticosteroids concerning the risk reduction of wheezing disorders.

Inhaled corticosteroids

The National Asthma Education and Prevention Program (NAEPP) Guidelines recommend to initiate long-term asthma control therapy with ICS to reduce the impairment and the risk for exacerbations in 0- to 4-year-old children, if they have at least four wheezing episodes in the past 12 months and positive mAPI, or at least two exacerbations requiring systemic corticosteroids within 6 months based on the results of the PEAK trial (Guilbert *et al.* 2006, NAEPP 2007). However, the periodic or regular daily ICS therapy in children aged <3 years with recurrent wheezing and atopic predispotion has not been preventive from asthma progression in 1- and 8-year follow-ups (Bisgaard *et al.* 2006, Guilbert *et al.* 2006, Murray *et al.* 2006, Devulapalli *et al.* 2007). The treatment strategies differed in the studies, but all birth cohorts consisted of high risk children with atopic conditions. The Inhaled Fluticasone in Wheezy INnfants (IFWIN) study used a step-up/step-down strategy (Murray *et al.* 2006). The PEAK study used 2 years with continuous ICS followed by the third, treatment-free year (Guilbert *et al.* 2006). The Prevention of Asthma in Childhood (PAC) used short courses of ICS (Bisgaard *et al.* 2006). Regular daily control therapy with ICS rather achieved symptom control and reduced the risk for exacerbations (Guilbert *et al.* 2006).

Systemic corticosteroids

Another alternative for asthma prevention might be the use of systemic corticosteroids, carefully targeted to subset of children with most benefit from them. The results evaluating short-term efficacy of systemic corticosteroids in

reducing subsequent wheezing symptoms have been conflicting in children aged 6-24 months and hospitalized or cared on the emergency department for acute wheezing. In infants with at least one previous wheezing episode, the OCS did not reduce treatment failures (Fox *et al.* 1996), or shorten the duration of hospitalization. (Panickar *et al.* 2009). However, a beneficial efficacy of systemic corticosteroids has been shown. Hospitalization rate was lower after a single dose intramuscular steroids compared to placebo (Tal *et al.* 1990) and after combined therapy with OCS and nebulized epinephrine compared to both alone or only placebo in children aged <1 year with bronchiolitis (Plint *et al.* 2009). Likewise, the duration of hospitalization was shorter after OCS with salbutamol in infants with eczema or family history of asthma (Alansari *et al.* 2013). Taken the idea of subsets even further, Jartti *et al*. demostrated in the present Vinku cohort in infants experiencing their first or second wheezing episode induced by rhinovirus or RSV (mean age 1.1 year) that OCS reduced relapses during a 2-month period in children with rhinovirus infection or blood eosinophil count (B-eos) $\geq 0.2 \times 10^9$/L (Jartti *et al.* 2006). This suggests that the heterogeneity of early childhood wheezing might contribute to the results of lacking efficacy of OCS (Collins and Beigelman 2014, Castro-Rodriguez *et al.* 2016). It is of note that all other studies on the efficacy OCS have not included the viral etiology of the wheezing. However, studies on longer-term efficacy of OCS (*ie.* >2 months) are scarce. OCS for the first wheezing episode reduced the risk of physician-confirmed recurrence up to 12 months compared to placebo in rhinovirus-affected children in Vinku study (Lehtinen *et al.* 2007), and in children with high rhinovirus load in Vinku2 study (Jartti *et al.* 2015).

3 AIMS OF THE STUDY

The main aim of this thesis was to increase the knowledge about risk factors for asthma in children, in particular the impact of early-life rhinovirus-induced wheezing, and whether the systemic steroid treatment of the first wheezing episode may prevent from later asthma development.

The specific aims of the thesis were:

1. To evaluate the risk factors for childhood asthma — defined as 1) recurrent wheezing and 2) persistent asthma symptoms after the first wheezing episode in a 7-year follow-up study (I, II).

2. To assess the impact of known risk factors and rhinovirus etiology of the first severe wheezing episode for the development of atopic and non-atopic asthma phenotypes at age 8 years (IV).

3. To assess the effect of the anti-inflammatory treatment at study entry on the development of recurrent wheezing, and on the duration of long-term asthma control therapy overall, and in subgroups according to viral etiology, rhinovirus load and eczema status (I, II, III).

4 MATERIALS AND METHODS

4.1 Study subjects, designs and protocol

The Vinku study was carried out in the Department of Paediatrics, Turku University Hospital (Turku, Finland) from September, 2000 to May, 2002 (Lehtinen *et al.* 2007). Only those experiencing their first or second episode of wheezing, hospitalized and being aged <3 years were included in the long-term follow-up (Fig. 1) (Lehtinen *et al.* 2007). The exclusion criteria were inhaled or systemic corticosteroids within 4 weeks before the study, chronic disease, and need for intensive care. No stratified randomization was done for eligible participants because the long-term follow-up protocol was implemented during the efficacy trial.

The Vinku2 study was a prospective, randomized, double blind, placebo controlled, parallel, one-center trial. The recruitment for the Vinku2 trial was carried out in Turku University Hospital from June 2007 to March 2009. The inclusion criteria were the age of 3 to 23 months, delivery at 36 gestational weeks or later, first wheezing episode (based on parental report and confirmed from medical records), and written informed consent from a parent or guardian (Figure 4). Exclusion criteria were the presence of a chronic non-atopic illness, previous systemic or inhaled corticosteroid treatment, participation in another study (excluding long-term follow-up studies in childhood), varicella contact in a patient without a previous varicella illness, need for intensive care unit treatment, or poor understanding of Finnish. A double-blind RCT design was used for 12 months.

Both cohorts used similar follow-up protocols and were carried out in the Department of Paediatrics, Turku University Hospital (Turku, Finland) (Lehtinen *et al.* 2007, Jartti *et al.* 2015). At study entry, venous blood was drawn and nasopharyngeal aspirate collected, and then the children were randomized to be given either oral prednisolone or a placebo. Study physicians recruited the patients to both studies, and prospectively followed them at scheduled visits (2 weeks, 2 months, 12 months, 4 [Vinku2 only] and 7 years after the study entry) (Jartti *et al.* 2006, Lehtinen *et al.* 2007, Jartti *et al.* 2015). The children were followed for 7 years. All patient charts were reviewed for the full 7-year follow-up period for asthma symptoms, medications, and laboratory tests.

4.2 Prednisolone intervention

In order to investigate whether systemic prednisolone treatment for the first wheezing episode would affect the short- and/or long-term outcomes, the children were randomized to receive either oral prednisolone (first dose 2 mg/kg, then 2 mg/kg/day in 3 divided doses for 3 days, maximum dose 60 mg/day, Prednisolon® 5 mg tablets, Leiras Takeda, Helsinki, Finland) or a placebo.

In Vinku study, the study drug (prednisolone or placebo) was initiated immediately after nasopharyngeal aspirate (NPA) collection and blood samples were drawn. In Vinku2 study, the study drug was initiated for children with rhinovirus-positive NPA first when the PCR results were available (rhinovirus detected in a NPA sample by using PCR, ongoing signs of lower respiratory tract symptoms [cough, noisy breathing, or wheezing] at the time when PCR results were available) if the child still fulfilled all the study criteria.

4.3 Baseline data collection

4.3.1 Clinical assessment and laboratory studies

The children were examined and parents were interviewed at the acute hospitalization using standardized questionnaires on other host and environment-related risk factors of recurrent wheezing and asthma: physician-diagnosed eczema, parental history of allergy and/or asthma, parental smoking, pets at home and day care in infancy (Appendices 1 and 2). Laboratory studies included B-eos and allergen-specific serum IgE levels which were measured by the routine diagnostics of the Central Laboratory of Turku University Hospital. Serum 25-hydroxyvitamin D measurements in the Study III were done by means of liquid chromatography-tandem mass spectrometry at Massachusetts General Hospital (Boston, MA).

4.3.2 Viral studies

The NPAs for viral diagnostics were drawn using a standardized procedure (Jartti *et al.* 2004, Allander *et al.* 2007a). The NPA was taken through a nostril with a disposable catheter connected to a mucus extractor. A nasopharyngeal swab was dipped into the NPA, transported to the laboratory during the same day, and stored at -70°C before processing the samples obtained for PCR assays. At study entry, NPAs were analyzed within 3 days for rhinovirus, enterovirus and RSV.

In Vinku study, the NPAs were analyzed with PCR, virus culture and antigen detection for adenovirus, coronaviruses (229E, OC43, NL63 and HKU1), enteroviruses, human bocavirus, metapneumovirus, influenza A and B,

parainfluenza virus types 1-4, polyomaviruses WU and KI, rhinovirus, and RSV. Virus culture and antigen detection from NPA were analyzed using fresh samples by the Department of Virology, University of Turku. Virus culture was performed for adenovirus, influenza A and B viruses, PIV types 1–3, RSV, enteroviruses, rhinovirus, and metapneumovirus (Jartti *et al.* 2004), while viral antigens were detected for adenovirus, human bocavirus, influenza A and B viruses, parainfluenza virus types 1–3, and RSV. PCR was used for the detection of rhinoviruses, enteroviruses, RSV, coronaviruses (229E, OC43, NL63 and HKU1), metapneumovirus, human bocavirus, influenza A and B viruses, adenovirus, parainfluenza virus types 1-4, and WU- and KI-polyoma viruses. Moreover, formerly non-typable picornavirus and enterovirus samples were re-analyzed by RT-qPCR with improved identification of rhinovirus C strains. Of the available samples, 0/13 non-typable picornavirus samples and 1/19 (5%) enterovirus samples were rhinovirus C positive. The rhinovirus samples were not typed. Non-typable picornaviruses (rhino–enterovirus PCR positive samples that could not earlier be discriminated by hybridization) were classified as rhinoviruses according to the sequence analysis (Jartti *et al.* 2010). The PCR of rhinovirus species A, B and C was here investigated in one group, not separately.

Levels of IgG antibodies specific for adenovirus, influenza A and B viruses, parainfluenza virus types 1-3, RSV, and human bocavirus were analyzed in paired serum samples, in addition to IgM antibodies for enteroviruses and human bocavirus (Jartti *et al.* 2004, Allander *et al.* 2007b, Söderlund-Venermo *et al.* 2009). A threefold or more increase in IgG level or a positive IgM were considered acute infection.

In the Vinku2 study, the in-house RT-PCR was used for simultaneous detection of rhinovirus A, B and C, enteroviruses and RSV A and B from NPA. In addition, a multiplex PCR test (Seeplex RV12 ACE Detection; Seegene, Seoul, Korea) was used for the detection of rhinovirus, RSV, parainfluenza virus types 1-3, metapneumovirus, adenovirus, coronavirus (229E, NL63, OC43, and HKU1), and influenza A and B (Turunen *et al.* 2014). PCR products were analyzed by Screentape machine (Lab901 ScreenTape®System). Rhinovirus load was assessed from the rhinovirus-positive samples using quanitative RT-PCR. Human bocavirus infections were analyzed with serology and PCR (Allander *et al.* 2007b, Söderlund-Venermo *et al.* 2009).

4.4 Long-term data collection and follow-up visit at age 8 years

Both cohorts, Vinku and Vinku2, were combined to maximize the number of study children. Only the steroid-naive children aged 3-23 months with the first severe wheezing episode were included (Figure 5). In the Study IV, the long-term follow-

up visit was arranged at the age of 8 years after the 7-year follow-up period. In Finland, children start school at the age of 7 years. Therefore, this study visit point is called occurring at school-age.

4.4.1 Clinical assessment, follow-up data and laboratory studies

At the follow-up visit, the study children were investigated by study physicians, lung function was tested, and parents were interviewed using standardized questionnaires (Appendices 3 and 4). Laboratory studies at age 8 years included B-eos and allergen-specific serum IgE levels. All patient charts were reviewed and parents interviewed for symptoms suggestive of asthma associated with exercise, infections and allergens as well as for the symptoms suggestive of allergic rhinitis, conjunctivitis and eczema during the entire 7-year follow-up period, and preceding 12 months (NAEPP 2007). Previous and on-going asthma therapies and laboratory tests were registered.

4.4.2 Studies on lung function

The baseline flow-volume spirometry was examined by a pneumotachographic spirometer (Jaeger MasterScreen system, Jaeger GmbH, Würzburg, Germany in Vinku, and Medikro Spirometry Software, Medikro Oy, Kuopio, Finland in Vinku 2). The spirometry was measured in Vinku and Vinku2 with bronchodilatation test; spirometry at baseline and 15 minutes after 400 µg of salbutamol (Ventoline®) administered by inhalation through a spacer (Babyhaler®, both from Glaxo Smith Kline, Brentford, UK). Families were instructed to withhold the child's regular asthma medications with ICS during the preceding 4 weeks, and to withhold salbutamol for 12 hours before the spirometry. The test was re-scheduled if the child was ill or taking salbutamol for asthma symptoms. The registered index was FEV1. Lung function parameters were expressed as percentages of the gender-specific and height-related reference values (% of predicted) for Finnish children. The bronchodilatation test was regarded as positive with a reversible airflow obstruction with an increase of ≥12% in FEV1 in the bronchodilatation test (Beydon *et al.* 2007, NAEPP 2007).

In Vinku2, the exercise challenge test was performed according to international recommendations as a free-running test designed to measure bronchial hyper-reactivity in children; spirometry at baseline and 1, 5, and 10 minutes after exercise testing (Beydon *et al.* 2007, 2007). The children were urged to run 6-8 minutes at an exercise level where the heart rate was held at 85-90% of their estimated maximum heart rate (205 - (1/2) x age), assessed with a heart rate monitor (Polar Sport Tester, Polar Elektro Ltd, Kempele, Finland). Air temperature and humidity were measured, and the exercise test was performed outside when air temperature

was ≥5°C, otherwise inside. The challenge was performed under the supervision of a pediatrician. The exercise challenge test was regarded as positive if there was a decrease of ≥15% in exercise-challenge test at 5, 10 or 15 minutes after the running (Beydon *et al.* 2007, NAEPP 2007).

4.5 Definitions

Sensitization was defined as positive IgE antibodies against common allergens (cut-off level 0.35 kU/L for codfish, cow's milk, egg, peanut, soybean, wheat, cat, dog, horse, birch, mugwort, timothy, *Cladosporium herbarum* and *Dermatophagoides pteronyssinus*; fluoro-enzyme immunoassay, CAP FEIA, Phadiatop Combi®, Phadia, Uppsala, Sweden) (Jartti *et al.* 2015). Aeroallergen sensitization was defined as IgE antibodies to any of the latter 8 allergens. B-eos was expressed as cells $x10^9$ /L, and the cut-off limit for the elevated B-eos value was 0.4 cells x 10^9/L (Jartti *et al.* 2010). Eczema was a physician-made diagnosis with typical symptoms including pruritus, typical morphology and chronicity of disease (NAEPP 2007).

In Studies I, II and IV of this thesis, viral findings were combined into 3 subgroups according to the viral etiology of the first wheezing episode at study entry: the rhinovirus group (rhinovirus alone or with other viruses, RSV included), the RSV group (RSV alone or with other viruses, rhinovirus excluded), and the RSV-/rhinovirus-negative group (other viruses or no viruses found) (Lehtinen *et al.* 2007, Bergroth *et al.* 2016). This grouping was based on the earlier hypothesis that rhinovirus-associated wheezing is a stronger risk factor for recurrences than RSV-associated wheezing (Kotaniemi-Syrjänen *et al.* 2003, Lemanske *et al.* 2005), and it agrees with the viral grouping of Lemanske et al. (Lemanske *et al.* 2005).

4.6 Outcomes

4.6.1 Recurrent wheezing (I) and initiation of asthma control therapy (III)

In Study I, recurrent wheezing was defined as the earliest date during the 7-year follow-up period when a child fulfilled one or more of the following criteria:

- 3 physician-confirmed episodes of wheezing within the past 12 months;
- continuous lower respiratory symptoms (cough, wheezing) lasting >4 weeks and relieved by recurrent use of bronchodilators; or

- moderate-to-severe wheezing episodes necessitating systemic corticosteroid use within 6 months.

This was defined slightly different from the National Asthma Education and Prevention Program (NAEPP) 2007 (NAEPP 2007).

The regular asthma control therapy is currently recommended/initiated after recurrent wheezing episodes to prevent more episodes (Guilbert *et al.* 2006, NAEPP 2007, GINA 2016). In Study III, recurrent wheezing was defined as the initiation of asthma control therapy. It was initiated after ≥4 wheezing episodes (≥1 diagnosed by a physician) within the past 12 months lasting >1 day and affected sleep. In addition,

- 1 **major** risk factor (physician-diagnosed atopic eczema, aeroallergen sensitization, or parental history of asthma), or
- 2 **minor** risk factors (wheezing apart from colds, B-eos $\geq 0.40 \times 10^9$/L or food sensitization), and/or
- prolonged symptoms lasting >4 weeks and requiring symptomatic treatment >2 days per week, and/or
- 2 exacerbations requiring systemic corticosteroids within 6 months (NAEPP 2007).

The outcome was based on the NAEPP guidelines for the initiation of asthma therapy in children aged under 5 years (NAEPP 2007).

4.6.2 Persistent asthma symptoms and asthma therapy duration (II)

In Study II, persistent asthma symptoms were defined as the need of regular long-term asthma control therapy with ICS. This would work as an indicator for asthmatic children. The asthma therapy duration was determined by assessing the first and the last date of the regular or periodic therapy. The term 'regular' refers to daily and continuous and 'long-term' to a permanent, long-lasting therapy use.

4.6.3 Current asthma (IV)

In Study IV, children were diagnosed to have current asthma at age 8 years if they met one or more of the subsequent criteria during the preceding 12 months:

- patient chart report of doctor-diagnosed asthma and need of regular doctor-prescribed asthma therapy with ICS for over a month,

- use of OCS for asthma exacerbations, acute asthma attack relieved by repeated use of bronchodilator,
- and/or hyper-reactivity in spirometry defined as reversible airflow obstruction with an increase of ≥12% in FEV1 in the bronchodilatation test, or a decrease of ≥15% in exercise-challenge test.

Current atopic asthma at age 8 years was defined as asthma with laboratory-verified sensitization or patient chart and parent-reported allergy symptoms. Non-atopic asthma was defined as asthma without these. Children were in remission if they were without asthma symptoms and therapy within 12 months prior to the study visit and/or without hyper-reactivity in spirometry at the study visit (NAEPP 2007).

4.7 Statistical analyses

Analyses were made using IBM SPSS 18.0-23.0 software (SPSS Inc, Chicago, Ill, USA). At baseline, differences between groups for dichotomous data were analyzed with Pearson's Chi square, Fisher's exact and Kruskal-Wallis tests. Fisher's exact test was used when the expected frequency for any cell was <5. Likewise, continuous data were analyzed with the independent sample T-test, one-way ANOVA or Mann-Whitney U-test.

Risk factors for childhood recurrent wheezing and persistent asthma symptoms after the first wheezing episode were studied in the 7-year follow-up (Studies I and II). For Study I, risk factors for childhood recurrent wheezing were evaluated with the Cox proportional hazard regression model (the Cox model) with survival analyses *ie*. assessing time to recurrent wheezing. In this study, the Cox hazard ratio (HR) indicated the risk for recurrent wheezing with related 95% confidence interval (CI). The studied univariable risk factors were parental asthma, any sensitization, eczema, day care, parental smoking, age at study entry, sex, and viral etiology. The analysis was repeated using multivariable backward stepwise model (exclusion criteria $p > 0.10$) including same factors. Only the significant risk factors ($p < 0.05$) were included as adjustments in the final model (Lehtinen et al. 2007).

In Study II, persistent asthma symptoms during the 7-year follow-up *ie.* the need of regular asthma therapy in different ages after the first wheezing episode was assessed with the logistic regression model. The risk of individual factor was expressed as OR or adjusted OR with related 95% CI. The univariable risk factors were studied using the baseline risk factors. The multivariable analyses were adjusted with clinically relevant risk factors such as age <1 year, B-Eos ≥ 0.40 ×

10^9/L, eczema, sex, parental asthma, prednisolone intervention, rhinovirus, and sensitization at study entry.

In Study II, risk factors for the need of regular asthma therapy throughout the 7-year follow-up period was assessed using the Cox model with HR indicating the risk. The univariable risk factors were studied using the baseline risk factors. The multivariable analyses were adjusted with clinically relevant risk factors such as age <1 year, B-Eos $\geq 0.40 \times 10^9$/L, eczema, sex, parental asthma, prednisolone intervention, rhinovirus, and sensitization at study entry.

Risk factors at the first severe wheezing episode for current asthma at age 8 years, and separately for atopic and non-atopic phenotypes were assessed using the logistic regression model in Study IV. Unadjusted analyses were done with baseline characteristics at study entry. The multivariable analyses were adjusted with eczema, any sensitization, parental smoking, rhinovirus-positivity, and age <12 months at study entry, which all showed significant effects. The logistic regression analyses were also done for atopic and non-atopic asthma outcomes separately. Because of the time difference of the two cohorts (recruited either in 2000-2002 or 2007-2010) the multivariable regression analyses were also adjusted for cohort to study whether a cohort was significant in the models, or modified the magnitude of the other factors in the models. The effect of overlapping risk factors on the incidence of asthma at age 8 years was tested with χ^2 or Fisher's exact tests.

The outcome-modifying effect of the prednisolone intervention at study entry was studied with interaction effects between treatment grouping (prednisolone *vs.* placebo) and risk factors. The prednisolone effect was evaluated on time to recurrent wheezing (Study I), on time to initiation of asthma control therapy (Study III), and on the need of long-term asthma control therapy (Study II).

In Study I, the prednisolone effect on time to recurrent wheezing was studied using the Cox model with interactions between treatment grouping and pre-specified risk factors (eczema status, rhinovirus, RSV or rhinovirus-/RSV-negative etiology) (Lehtinen *et al.* 2007). Then, the multivariable model was repeated with significant interactions and adjustments for sensitization, age <12 months, viral etiology and parental asthma (Lehtinen *et al.* 2007).

In Study III, the effect of prednisolone on time to initiation of asthma therapy was evaluated with the Cox model including the main effects of dichotomized rhinovirus genome load and treatment grouping, and the interaction effect of rhinovirus genome load by treatment grouping. Rhinovirus load was dichotomized due to positively skewed distribution. The cut-off for rhinovirus load was identified by testing different values, and selecting the approximate threshold that yielded the lowest p value for the interaction effect of rhinovirus load (Jartti *et al.*

2015). The Cox model included no adjustments, for no significant differences in patient baseline characteristics were found.

In Study II, the prednisolone effect on the regular asthma therapy need in different ages was analyzed using the logistic regression model. The modifying effect was studied *post hoc* with interactions between treatment grouping and all baseline risk factors overall, and also after adjustment for sensitization (the only risk factor with unadjusted $p < .05$).

4.8 Ethics

Vinku and Vinku2 studies were accepted by the Ethics Committee of the Turku University Hospital and were commenced only after obtaining written informed consent from the guardians.

5 RESULTS

5.1 Study populations and characteristics

5.1.1 Studies I and II

In the Vinku study, long-term follow-up criteria were fulfilled by 131 children (Figure 3). Of these, 9 children were lost to follow-up and 11 children were excluded from the analysis because ICS was used for prolonged cough. Finally, 111 (85%) children with the first episode of wheezing and aged <3 years were included. The prednisolone (n = 55) and placebo (n = 56) recipients were equally distributed according to the risk factor characteristics (Figure 3).

The median age of the included children was 12 months (interquartile range [IQR] 7, 18 months) at study entry, and 8.0 years (IQR 7.3, 8.7 years) at the end of follow-up. At study entry, 75/111 (68%) were boys, 36/111 (32%) had eczema, 16/109 (14%) were sensitized and 106/111 (95%) children were virus-positive. The rhinovirus group had a higher prevalence of allergic sensitization (29 %) than the other groups (RSV 7%; RSV-/rhinovirus-negative 9%, p = 0.010). The RSV group had lower prevalence of blood eosinophil count ≥0.4 x 10^9/L (7%) than the other groups (rhinovirus 44%; RSV-/rhinovirus-negative 36%, p = 0.001). Sensitized children were older than non-sensitized (medians 16 *vs.* 11 months, p = 0.003). The diagnosis of recurrent wheezing was set and therapy was started in 51/111 (46%) children, of whom 43 (84%) children used regular and 8 (16%) used periodic therapy. Median age at therapy start was 19 months (IQR 12, 26 months).

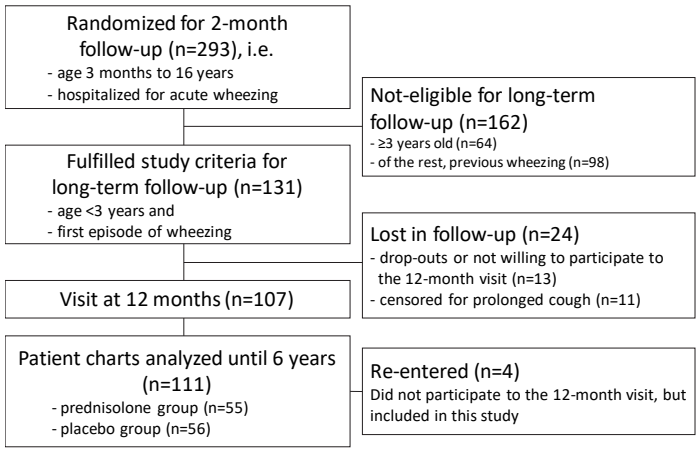


Figure 3. Study flow chart of Studies I and II.

5.1.2 Study III

The Vinku2 study consisted of 125 children, of which 79 (61 in- and 18 out-patients) were rhinovirus-positive and randomized to receive prednisolone or placebo for the first acute wheezing episode. During the follow-up, 10 children were excluded due to insufficient follow-up time (drop-outs), 9 due to insufficient data about rhinovirus genome load, and 1 due to initiation of ICS for another reason (Figure 4). Finally, 59 children were included (80% hospitalized at study entry).

At study entry, the mean age of the children was 13 months (standard deviation [SD] 6.0 months), 18 (31%) were sensitized and 23 (39%) had eczema. Twenty children (34%) had co-detection of at least 2 viruses in NPA. Twenty-three (39%) children had a rhinovirus genome load >7000 copies/mL. The treatment groups did not differ in baseline characteristics. Asthma control therapy was initiated in 40/59 (68%) children during the follow-up, 20/29 (69%) in the prednisolone and 20/30 (67%) in the placebo group.

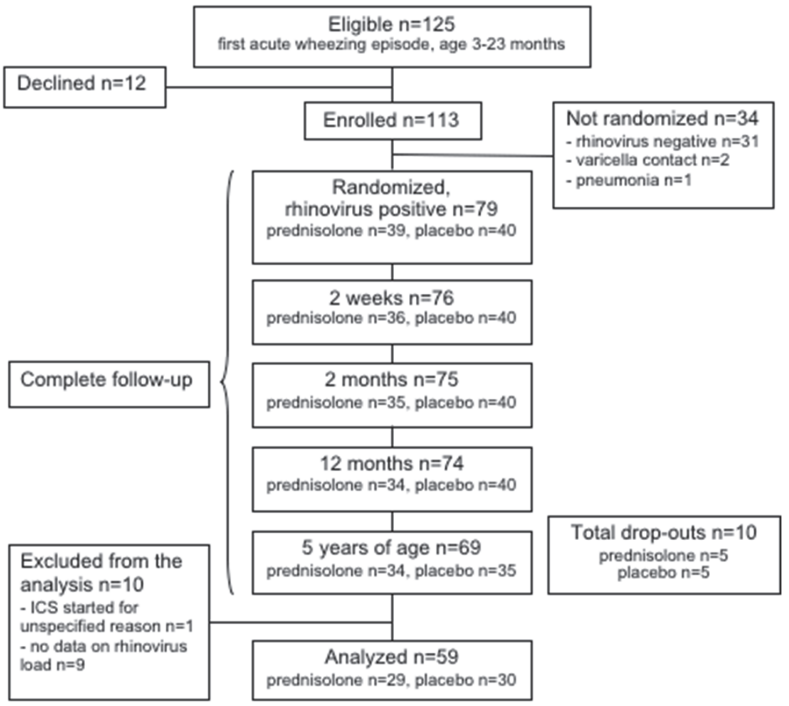


Figure 4. Study flow chart of Study III. *ICS*, inhaled corticosteroids.

5.1.3 Study IV

The study included children from Vinku and Vinku in order to increase the number of participants. The long-term follow-up criteria were fulfilled by 136 children (Figure 5). Non-eligible for the long-term follow-up were 281 children due to age ≥2 years, previous wheezing, ICS treatment, chronic disease, prednisolone intervention, or need for intensive care during the hospitalization. Nine children (mean age 15.2 months [SD 8.4 months]) declined follow-up or were lost, of whom 8 (89%) were boys, 3 (33%) were sensitized and 3 (33%) were rhinovirus-positive. Finally, 127 (93%) first-time wheezing children were enrolled, including 49 (39%) from Vinku and 78 (61%) from Vinku2. All children were followed-up using patient charts to detect asthma symptoms and medications for the full 7-year-long follow-up period. In addition, 74 (58%) children attended the follow-up visit at age 8 year (in Vinku during 2007-2008, and in Vinku2 during 2014-2015). The rest, 53 (42%) children were followed-up using patient charts (n = 38) and the information from parental interviews (n = 15) (Figure 5).

At study entry, the median age was 11 months (IQR 6;16 months), 64% of the children were boys, 17% were sensitized, 28% had eczema, and 98% were virus-positive (Table 2). At the end of the follow-up, the median age was 7.7 years (IQR 7.1; 8.2 years).

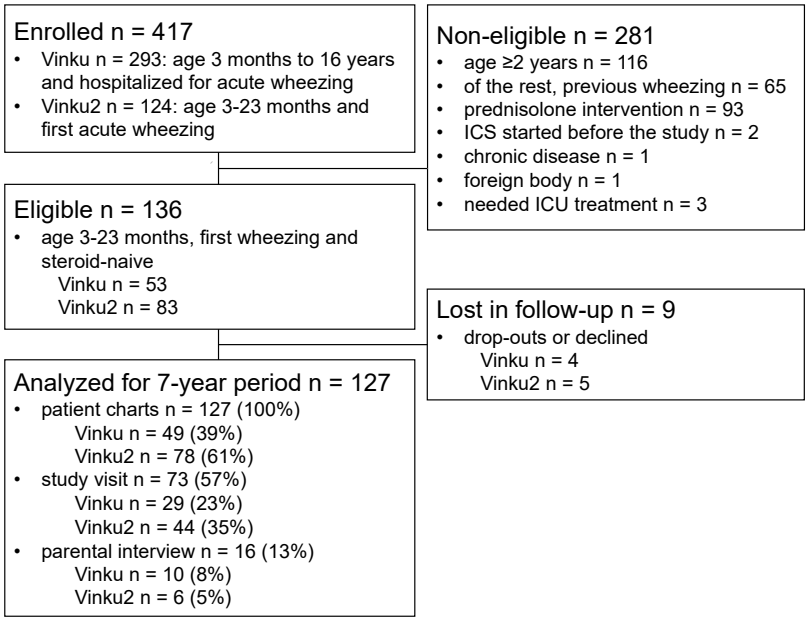


Figure 5. Study flow chart of Study IV. *ICS*, inhaled corticosteroids; *ICU*, intensive care unit.

5.2 Risk for recurrent wheezing (I) and persistent asthma (II) after the first wheezing episode

5.2.1 Risk for recurrent wheezing (I)

During the 7-year follow-up, 51/111 (46%) of the children suffered from recurrent wheezing. The incidence of recurrent wheezing was 56% (19/34) in children with rhinovirus infection, 69% (11/16) in children with sensitization, 53% (27/51) in children aged <12 months and in 56% (20/36) with eczema.

Allergic sensitization at the first wheezing episode was the only risk factor for recurrent wheezing (HR 2.25, 95% CI 1.15-4.41) in the univariable analysis of the Cox model. Sensitization was divided into aeroallergen (HR 4.40, 95% CI 1.35-14.33, respectively, n = 3) and food allergen sensitizations (HR 2.28, 95% CI 1.17-4.46, n = 16). All three children with aeroallergen sensitization had recurrent wheezing. The risk factors for recurrent wheezing in the multivariable analyses were rhinovirus (adjusted HR 3.54, 95% CI 1.51-8.30), sensitization (HR 3.47, 95% CI 1.55-8.30), age <12 months (HR 2.45, 95% CI 1.29-4.65), and eczema (HR 2.33, 95% CI 1.11-4.90).

5.2.2 Risk for persistent asthma symptoms ie. risk for need of regular and prolonged asthma therapy (II)

Long-term asthma control therapy due to recurrent wheezing was started in 51/111 (46%) children, of whom 43 (84%) used regular and 8 (16%) used periodic therapy. Median age at therapy start was 19 months (IQR 12, 26 months). The logistic regression model showed an increased risk for the use of asthma therapy during the follow-up in sensitized children (univariable $p \leq 0.039$ and multivariable $p \leq 0.029$) (Figure 6). Age <1 year increased the risk until the age 2 years (multivariable OR 5.0, 95% CI 1.7-15, p = 0.003) (Figure 6).

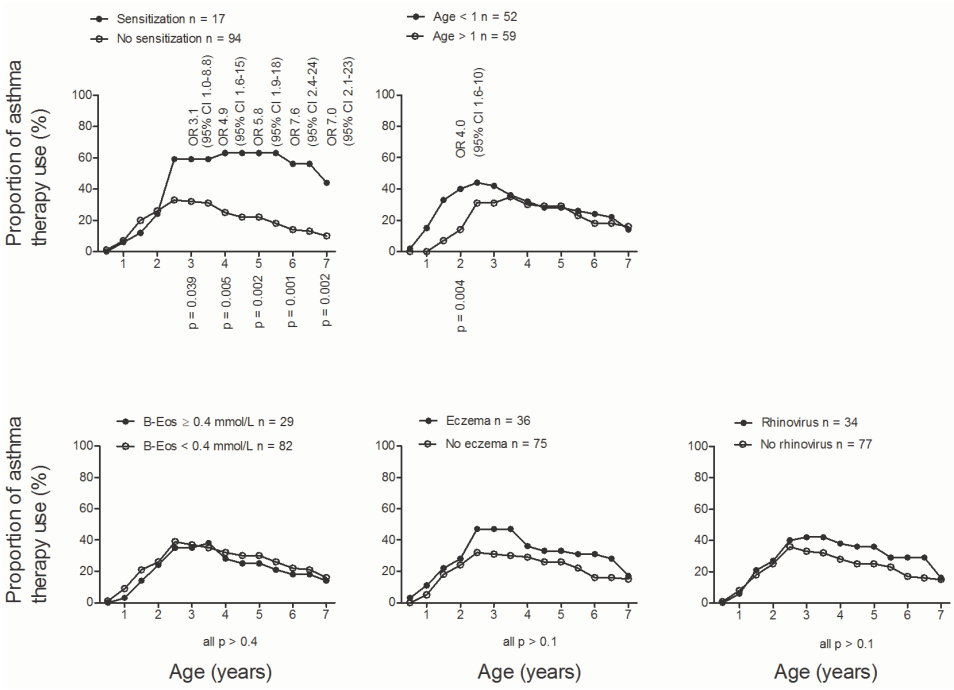


Figure 6. The yearly proportion of asthma therapy use and risk (OR) for asthma control therapy with inhaled corticosteroids in different ages during the 7-year follow-up in the univariable model. The p values indicate risk testing for asthma control therapy at 0 to 7 years of age. Risk assessed with logistic regression (n = 111). *CI*, confidence interval; *OR*, odds ratio. Modified from Study II.

Sensitization at study entry was the only risk factor for using regular asthma therapy throughout the 7-year follow-up period (unadjusted HR 2.9, 95% CI 1.3-7.4, p = 0.03 and adjusted HR 4.5, 95% CI 1.4-15, p = 0.01) in the Cox model with no significant interactions (Figure 7). The median duration of therapy was 3.1 years (IQR 1.2, 5.4 years), whereas in sensitized children it was 5.4 years (IQR 3.3, 6.5 years) *vs.* in non-sensitized 2.4 years (IQR 1.2, 5.1 years) (p = 0.02).

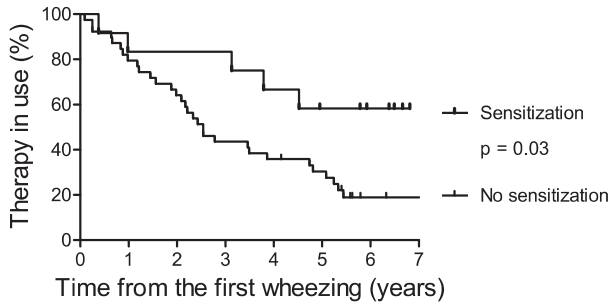


Figure 7. Time to the end of regular asthma control therapy during the 7-year follow-up. Risk assessed with the univariable Cox model (n = 111). From Study II.

5.3 Risk for asthma at age 8 years after the first severe wheezing episode (IV)

During the follow-up, 67 (53%) children were diagnosed to have recurrent wheezing or asthma ever, and regular long-term asthma control therapy with ICS was started. Thirty (24%) children who developed asthma were in remission by the end of the follow-up. Current asthma was diagnosed in 37/127 (29%) children, of whom 19/127 (15%) had atopic and 18/127 (14%) had non-atopic asthma (Table 2).

Table 2. Baseline patient characteristics at the first wheezing episode in the paper IV.

		Current asthma at age 8 years		
	All	**Any**	**Atopic**	**Non-atopic**
Risk factor	127	37 (29)	19 (15)	18 (14)
Age 3-11 months	68 (54)	25 (68)	10 (43)	15 (83)
Age 12-23 months	59 (46)	12 (32)	9 (57)	3 (17)
Male sex	81 (64)	24 (65)	15 (79)	9 (50)
Female sex	46 (36)	13 (35)	4 (21)	9 (50)
Eczema	35 (28)	16 (43)	11 (58)	5 (28)
Any sensitization*	22 (17)	11 (31)	11 (61)	0
Food	22 (17)	11 (31)	11 (61)	0
Aeroallergen	6 (5)	6 (17)	6 (33)	0
B-eos $\geq 0.4 \times 10^9$/L	41 (32)	13 (37)	9 (53)	4 (22)
Parental asthma	23 (18)	10 (27)	4 (21)	6 (33)
Parental smoking	51 (40)	20 (54)	9 (47)	11 (61)
Breast feeding ≥ 4 months	55 (43)	20 (54)	10 (53)	10 (56)
Rhinovirus alone or with other viruses, RSV included	65 (51)	22 (60)	16 (84)	6 (33)
RSV alone or with other viruses, rhinovirus excluded	35 (28)	5 (14)	2 (11)	3 (17)
RSV-/rhinovirus-negative	26 (21)	9 (24)	0	9 (50)

Values are shown as numbers (percentage within asthma subgroups) of subjects.
B-eos, Blood eosinophil count; *RSV*, Respiratory syncytial virus.
* Defined as IgE antibodies to any of the common allergens. See the Methods section for details.

5.3.1 Risk for asthma at age 8 years

At study entry, the risk factors for current asthma at age 8 years were sensitization (OR 3.0, 95% CI 1.2-7.8), eczema (OR 2.7, 95% CI 1.2-6.5) and the first wheezing episode appearing at age <12 months (OR 2.3, 95% CI 1.0-5.0) in unadjusted logistic regression (all p <0.05, Table 3). In the multivariable analyses the first wheezing episode at age <12 months (adjusted OR 3.6, 95% CI 1.4-9.5), sensitization (adjusted OR 3.5, 95% CI 1.1-11), eczema (adjusted OR 2.9, 95% CI 1.1-7.3), and parental smoking (adjusted OR 2.8, 95% CI 1.2-6.9) remained as significant risk factors (all p <0.05, Table 3).

5.3.2 Risk for atopic asthma at age 8 years

Current asthma was specified to atopic and non-atopic asthma. The unadjusted risk factors for atopic asthma at age 8 years were sensitization (OR 13, 95% CI 4.3-41), rhinovirus etiology of the first wheezing episode (OR 6.4, 95% CI 1.8-23) and eczema (OR 4.8, 95% CI 1.7-13) (all p <0.05, Table 3). In the adjusted analyses sensitization (adjusted OR 12, 95% CI 3.0-44), rhinovirus etiology (adjusted OR 5.0, 95% CI 1.1-22) and eczema (adjusted OR 4.8, 95% CI 1.4-17) remained significant (all p <0.05, Table 3).

5.3.3 Risk for non-atopic asthma at age 8 years

The unadjusted risk factors for non-atopic asthma at age 8 years were the RSV-/rhinovirus-negative etiology of the first wheezing episode (OR 5.4, 95% CI 1.9-16) and age <12 months (OR 5.3, 95% CI 1.4-19) (all p <0.05, Table 3). In the multivariable analyses RSV-/rhinovirus-negative etiology (adjusted OR 8.0, 95% CI 2.3-28), age <12 months (adjusted OR 7.3, 95% CI 1.7-31) and parental smoking (OR 3.8, 95% CI 1.2-13) remained significant (all p <0.05, Table 3).

Table 3. Risk factors at the first wheezing episode for current asthma phenotypes at age 8 years. From Study IV.

	Current asthma at age 8 years								
Unadjusted analyses	**Any**			**Atopic**			**Non-atopic**		
Risk factors	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Age 3-11 months	***2.3***	***1.0-5.0***	***0.045***	0.96	0.36-2.5	0.93	***5.3***	***1.4-19***	***0.012***
Male sex	1.1	0.48-2.4	0.87	2.4	0.74-7.7	0.15	0.51	0.19-1.4	0.19
Eczema	***2.7***	***1.2-6.5***	***0.013***	***4.8***	***1.7-13***	***0.002***	1.0	0.33-1.0	0.98
Any sensitization*	***3.0***	***1.2-7.8***	***0.023***	***13***	***4.3-41***	***<0.001***	N/A	N/A	***0.041***‡
Food	***3.0***	***1.2-7.8***	***0.023***	***13***	***4.3-41***	***<0.001***	N/A	N/A	***0.041***‡
Aeroallergen	N/A	N/A	***<0.001***†	N/A	N/A	***<0.001***†	N/A	N/A	0.59‡
B-eos ≥0.4 x 10^9/L	1.3	0.57-2.9	0.55	2.6	0.93-7.4	0.067	0.53	0.16-1.7	0.30
Parental asthma	2.1	0.83-5.4	0.12	1.2	0.36-4.0	0.76	2.6	0.86-7.9	0.089
Parental smoking	2.2	0.99-4.7	0.053	1.4	0.51-3.7	0.53	2.6	0.94-7.3	0.065
Breast feeding ≥4 months	1.8	0.85-4.0	0.12	1.6	0.59-4.1	0.38	1.8	0.65-4.9	0.26
Rhinovirus	1.6	0.74-3.5	0.23	***6.4***	***1.8-23***	***0.005***	0.42	0.15-1.2	0.11
RSV	***0.31***	***0.11-0.88***	***0.028***	0.27	0.06-1.2	0.089	0.48	0.13-1.7	0.27
RSV-/rhinovirus-negative	1.4	0.55-3.5	0.49	N/A	N/A	***0.013***§	***5.4***	***1.9-16***	***0.002***
Multivariable analyses									
Age 3-11 months	***3.6***	***1.4-9.5***	***0.009***	1.8	0.49-6.4	0.38	***7.3***	***1.7-31***	***0.007***
Eczema	***2.9***	***1.1-7.3***	***0.028***	***4.8***	***1.4-17***	***0.014***	0.66	0.18-2.4	0.53
Any sensitization	***3.5***	***1.1-11***	***0.030***	***12***	***3.0-44***	***<0.001***	¶	¶	¶
Parental smoking	***2.8***	***1.2-6.9***	***0.021***	2.3	0.63-8.5	0.21	***3.8***	***1.2-13***	***0.028***
Rhinovirus	1.5	0.61-3.7	0.38	***5.0***	***1.1-22***	***0.035***	-	-	-
RSV-/rhinovirus-negative	-	-	-	-	-	-	***8.0***	***2.3-28***	***0.001***

Risk assessed with the logistic regression model. In unadjusted analyses age 3-11 months *vs.* age 12-23 months, male sex *vs.* female, eczema *vs.* no eczema, sensitization to any allergen, food or aeroallergen *vs.* no sensitization, B-eos ≥0.4 x 10^9/L *vs.* B-eos <0.4 x 10^9/L, parental asthma and smoking *vs.* no asthma or smoking, duration of breast feeding ≥4 months *vs.* <4months. Multivariable analyses adjusted with age 3-11 months, eczema, any sensitization, parental smoking, and rhinovirus-positivity or rhinovirus-negativity (*P* near .05 in unadjusted analyses). In N/A cells *P* was assessed using Fisher's exact test due to 0 cell counts.

B-eos, Blood eosinophil count; *95% CI*, 95% Confidence interval; *N/A*: Not applicable; *OR*, Odds ratio; *RSV*, Respiratory syncytial virus.

* Defined as IgE antibodies to any of the common allergens. See the Methods section for details.

† N/A for all aeroallergen-sensitized children developed atopic asthma.

‡ N/A for none of the sensitized children developed non-atopic asthma.

§ N/A for none of the RSV/rhinovirus-negative children developed atopic asthma.

5.3.4 Overlapping conditions

The incidence of current asthma increased cumulatively if the child had concomitant risk characteristics at study entry (Figure 8 and Table 4). The incidence of asthma was higher with the presence of both eczema and sensitization (70%) *vs.* either one (37%) *vs.* neither of these factors present (21%) (p = 0.003). Respectively, the incidences of asthma were likewise higher with the presence of both sensitization and rhinovirus (59%/24%/25%) (p = 0.015), with eczema and rhinovirus (55%/27%/21%) (p = 0.018), or with age <12 months and parental smoking (56%/23%/21% with age 13-23 months and no parental smoking) (p = 0.004).

The incidence of atopic asthma increased cumulatively when the concomitant rhinovirus etiology was added on the atopic risk factors at study entry (Figure 8 and Table 4). The incidence of atopic asthma was high with eczema and sensitization (70%) *vs.* either one (23%) *vs.* neither (4%) (p <0.001), respectively with sensitization and rhinovirus (59%/12%/4%) (p <0.001), with eczema and rhinovirus (45%/15%/15%) (p <0.001), with B-eos ≥0.4 x 10^9/L and rhinovirus (27%/18%/5%) (p = 0.015), or with parental asthma and rhinovirus (29%/20%/6%) (p = 0.038) (Table 4).

The incidence of non-atopic asthma increased with age <12 months and RSV-/rhinovirus-negative etiology (50%) *vs.* either one (15%) *vs.* neither (2%) (p <0.001). Respectively, the age <12 months with parental smoking increased the asthma incidence (33%) *vs.* either one (12%) *vs.* age 13-23 months and no parental smoking (3%) (p = 0.003) (Table 4).

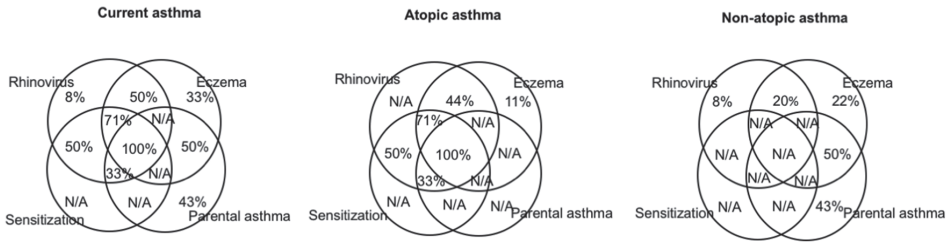


Figure 8. The incidence of current asthma phenotypes at age 8 years in children (N = 127) with sole and overlapping atopic risk factors (sensitization, eczema, rhinovirus, parental asthma) at the first wheezing episode tested with χ^2 or Fisher's exact tests. *N/A*, not applicable. From Study IV.

Table 4. The effect of concomitant characteristics at study entry for the incidence of current asthma at age 8 years. From Study IV.

	Current asthma at age 8 years					
Risk factors	**Any**	p	**Atopic**	p	**Non-atopic**	p
Age 3-11 months and no rhinovirus*	13/38 (34)		3/38 (8)		10/38 (26)	
Age 12-23 months **OR** rhinovirus	14/53 (26)	0.71	7/53 (13)	0.11	7/53 (13)	***0.014***
Age 12-23 months **AND** rhinovirus	10/36 (28)		9/36 (25)		1/36 (3)	
No eczema and no sensitization†	16/77 (21)		3/77 (4)		13/77 (17)	
Eczema **OR** any sensitization	13/35 (37)	***0.003***	8/35 (23)	***<0.001***	5/35 (14)	0.37
Eczema **AND** any sensitization	7/10 (70)		7/10 (70)		0/10 (0)	
No eczema and no rhinovirus	10/47 (21)		1/47 (2)		9/47 (19)	
Eczema **OR** rhinovirus	16/60 (27)	***0.018***	9/60 (15)	***<0.001***	7/60 (12)	0.46
Eczema **AND** rhinovirus	11/20 (55)		9/20 (45)		2/20 (10)	
No sensitization and no rhinovirus	14/56 (25)		2/56 (4)		12/56 (21)	
Any sensitization **OR** rhinovirus	12/56 (24)	***0.015***	6/50 (12)	***<0.001***	6/50 (12)	0.072
Any sensitization **AND** rhinovirus	10/17 (59)		10/17 (59)		0/17 (0)	
B-eos <0.4 x 10^9/L and no rhinovirus	14/56 (25)		3/56 (5)		11/56 (20)	
B-eos ≥0.4 x 10^9/L **OR** rhinovirus	12/38 (32)	0.65	7/38 (18)	***0.015***	5/38 (13)	0.20
B-eos ≥0.4 x 10^9/L **AND** rhinovirus	11/33 (33)		9/33 (27)		2/33 (6)	
No parental asthma and no rhinovirus	11/51 (22)		3/51 (6)		8/51 (16)	
Parental asthma **OR** rhinovirus	20/59 (34)	0.20	12/59 (20)	***0.038***	8/59 (14)	0.95
Parental asthma **AND** rhinovirus	6/14 (43)		4/14 (29)		2/14 (14)	
Age 12-23 months with RSV or rhinovirus	10/47 (21)		9/47 (19)		1/47 (2)	
Age 3-11 months **OR** RSV-/rhinovirus-negative‡	20/66 (30)	0.11	10/66 (15)	0.21	10/66 (15)	***<0.001***
Age 3-11 months **AND** RSV-/rhinovirus-negative	7/14 (50)		0/14 (0)		7/14 (50)	
Age 12-23 months and no parental smoking	7/33 (21)		6/33 (18)		1/33 (3)	
Age 3-11 months **OR** parental smoking	15/65 (23)	***0.004***	7/65 (11)	0.33	8/65 (12)	***0.003***
Age 3-11 months **AND** parental smoking	15/27 (56)		6/27 (22)		9/27 (33)	

Values are shown as numbers (percentage) of subjects. *P* was assessed using 2 or Fisher's exact tests indicating the whole group's comparisons.
B-eos, Blood eosinophil count.
* Alone or with other viruses, RSV included.
† Defined as IgE antibodies to any of the common allergens.
‡ With other viruses or no viruses.

5.4 The efficacy of prednisolone intervention (I, II and III)

5.4.1 Prednisolone reduces the risk of recurrent wheezing (I) and the initiation of asthma therapy (III)

In Study I, the Vinku cohort, prednisolone had no overall effect in reducing recurrent wheezing (Figure 9), but there were significant interactions between the treatment grouping (prednisolone *vs.* placebo), and viral etiology (p = 0.029) and eczema (p = 0.033 in the Cox model). Prednisolone prevented recurrent wheezing in rhinovirus-infected children (HR 0.37, 95% CI 0.15-0.95; adjusted HR 0.32, 95% CI 0.12-0.90) but not in the RSV-infected and in the RSV-/rhinovirus-negative groups (Figure 9). Prednisolone treatment also prevented recurrent wheezing in children with eczema (HR 0.53, 95% CI 0.21-1.33 and adjusted HR 0.27, 95% CI 0.08-0.87) (Figure 9).

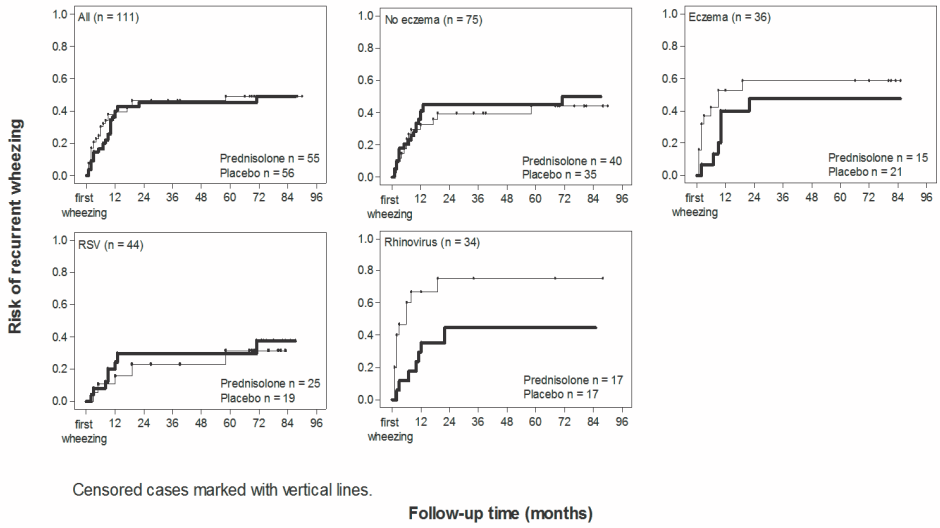


Figure 9. Risk of recurrent wheezing in prednisolone (bold line) and placebo recipients during the 7-year follow-up. The interaction analyses of the Cox model showed that prednisolone treatment provided no overall benefit, but the treatment effect showed differences related to the eczema and rhinovirus etiology of the first episode. In the eczema and rhinovirus groups, the difference between prednisolone and placebo recipients persisted for the entire 7-year-long follow-up period. In the RSV-/rhinovirus-negative group there were too few cases in the prednisolone recipients with recurrent wheezing (n = 3) for meaningful statistical analysis. *RSV*, respiratory syncytial virus. From Study I.

In Study III with the Vinku2 cohort, long-term asthma control therapy was initiated in 40/59 (68%) children for recurrent wheezing during the follow-up until age 5 years. Overall, prednisolone did not affect the time to the initiation of asthma control therapy compared to placebo (p = 0.99). However, the level of rhinovirus genome load at study entry modified the effect of prednisolone (rhinovirus load x study drug interaction, p = 0.043) (Figure 10). Hence, in children with rhinovirus genome load >7000 copies/mL, prednisolone lowered the risk for initiating asthma therapy (n = 14) compared to the placebo group (n = 9) (HR 0.38; 95% CI 0.14–1.01, p = 0.052). In the placebo group, asthma control therapy was started within subsequent 14 months in all 9 children. In children with low rhinovirus genome load <7000 copies/mL, no effect of OCS was observed.

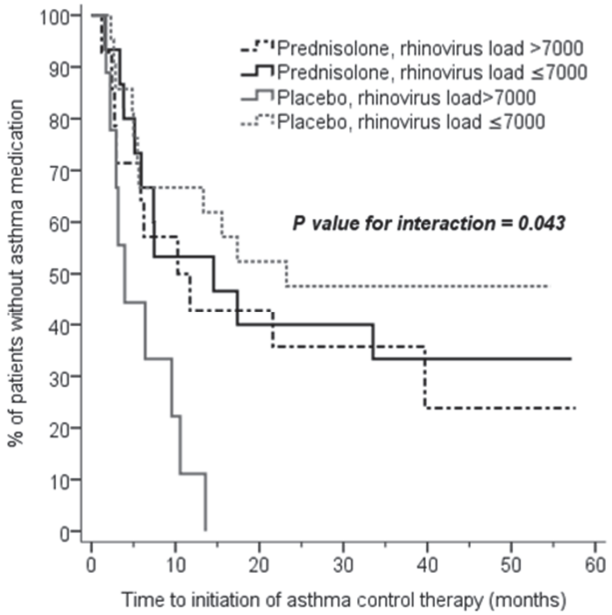
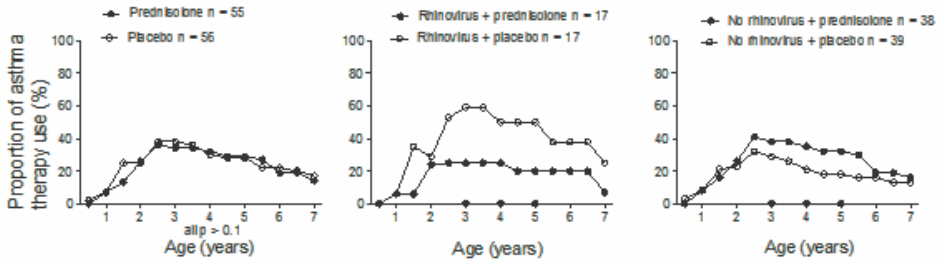


Figure 10. The time to the initiation of asthma control therapy in children randomized to receive prednisolone or placebo for the first rhinovirus-induced wheezing episode. Data are represented according to rhinovirus genome load. Children with rhinovirus genome load >7000 copies/mL had a longer time to the initiation of asthma control therapy in the prednisolone group when compared to the placebo group. In all children in the placebo group, the asthma control therapy was started within subsequent 14 months. From Study III.

5.4.2 *Prednisolone reduces the risk for persistent asthma symptoms ie. long-term asthma control therapy need (II)*

In the Vinku cohort, prednisolone intervention did not reduce the overall use of long-term asthma control therapy or shorten the therapy duration (p >0.1) (Figure 11). However, in the interaction analyses, prednisolone showed a trend for risk reduction in therapy need in the rhinovirus-group during ages 3 to 5 years, and the effect remained after adjustment for sensitization (p ≤0.051) (Figure 11).



Age (years)	Interaction between treatment and viral aetiology p	Treatment effect with prednisolone compared to placebo among rhinovirus-positive subjects OR	95% CI	Treatment effect with prednisolone compared to placebo among rhinovirus-negative subjects OR	95% CI
1	0.99	NA	NA	NA	NA
2	0.61	NA	NA	NA	NA
3	**0.040**	0.23	0.05-1.03	1.50	0.57-3.93
4	**0.051**	0.33	0.08-1.49	2.03	0.72-5.70
5	**0.029**	0.25	0.05-1.24	2.13	0.73-6.20
6	0.29	NA	NA	NA	NA
7	0.19	NA	NA	NA	NA

Figure 11. The yearly proportion of asthma therapy use and risk (OR) for asthma control therapy with inhaled corticosteroids in different ages during the 7-year follow-up by treatment effect between treatment (prednisolone *vs.* placebo) and rhinovirus status. The p values indicate risk testing for asthma control therapy at 0 to 7 years of age. Risk assessed with the logistic regression (n = 111). *CI*, confidence interval; *NA*, not applicable; *OR*, odds ratio. Modified from Study II.

6 DISCUSSION

6.1 Risk for childhood recurrent wheezing and persistent asthma symptoms after the first wheezing episode in the 7-year follow-up (I and II)

Age at the first wheezing episode

The first aim of this thesis was to evaluate risk factors for childhood asthma, defined as 1) recurrent wheezing and 2) persistent asthma symptoms, after the first wheezing episode in the 7-year follow-up. The median ages at the time of the first wheezing episodes were 11-13 months. Already at this age the risk factors for different school-age asthma phenotypes were found. The first wheezing episode at age <12 months predicted recurrent wheezing, persistent asthma symptoms and non-atopic asthma at school-age (83% *vs.* 53% in atopic asthmatics) (Studies I and II). On the contrary, in children with the first wheezing at age >12 months the incidence of atopic asthma was higher (47% *vs.* 17% in non-atopic asthmatics). Noteworthy is that in our study the risk for non-atopic asthma was inversely dependent on age, while other population-based studies have proposed that the risk for persistent asthma symptoms increases by age (Kotaniemi-Syrjänen *et al.* 2003, Jackson *et al.* 2008, Midulla *et al.* 2012). These earlier studies were conducted on children with atopic predisposition and rhinovirus-induced wheezing. This may indicate that the children with increased risk for atopic asthma start their wheezing tendency in older age, while the children with risk for non-atopic start wheezing earlier.

Even though the age range in Studies I and II was rather broad (3-35 months, median 12 months), 86% were aged <2 years. The Studies III and IV included only children aged <2 years. This makes our cohorts and results overall similar to the Kuopio bronchiolitis study which also was a population-based study with hospitalized children (Kotaniemi-Syrjänen *et al.* 2003). The polarization of ages at the first wheezing episode according to different school-age asthma phenotypes suggests heterogeneity among children with bronchiolitis. Still, many clinical trials have excluded toddlers aged >12 months or have included them as small subgroups. As shown in this thesis, bronchiolitis may overlap with rhinovirus-induced wheezing and asthma. Most importantly, the inclusion criteria age <12 months would have excluded the subgroup of children who developed atopic asthma, and who benefitted from the early OCS. Taking this into account, the

findings from trials predominantly assessing young infants might turn out to be one-sided.

Sensitization

Sensitization already at the time of the first wheezing episode predicted recurrent wheezing, need for regular long-term asthma control therapy, longer duration of therapy, and atopic asthma at school-age. It was the most significant independent risk factor predicting asthma symptoms throughout the studies in this thesis, and thereby highlights its importance, found by us and others (OR 3-16) (Illi *et al.* 2006, Jackson *et al.* 2008, Matricardi *et al.* 2008, Jartti *et al.* 2010, Kusel *et al.* , Wisniewski *et al.* 2013). The incidence of sensitization at study entry was 16-17%, but in asthmatics 31-71% (Study II). All children who were sensitized to aeroallergens at the study entry developed persistent asthma, so the aeroallergen sensitization as a diagnostic criterion had 100% sensitivity, presumably due to our severely wheezing population. In general populations, the development of aeroallergen sensitization is rather slow, and is seldom present before age one year (Illi *et al.* 2006, Jartti *et al.* 2009, Chiu *et al.* 2014). Therefore, its predictive value may not be the best when assessing the future asthma risk at the time of the first wheezing episode. In high risk groups, though, the predictive value may be better.

On the other hand, all sensitized children were sensitized to food allergens, and only a small proportion was co-sensitized to aeroallergens. We found that food sensitization effectively predicted disease persistence throughout the 7-year follow-up. Others have shown that food sensitization usually is detectable with laboratory testing by the time of the first wheezing episode before age 2 years (Kusel *et al.* 2007, 2007, Kusel *et al.* 2012, Nissen *et al.* 2013, Chiu *et al.* 2014). Therefore, testing young severely wheezing children for food sensitization would be a useful tool for clinical practice. This is supported by other studies on high-risk children (NAEPP 2007, Baris *et al.* 2011, Kusel *et al.* 2012, Melioli *et al.* 2012, Nissen *et al.* 2013, Wisniewski *et al.* 2013). The co-detection of food and aeroallergens in this subgroup is even more sensitive, but the role of aeroallergen sensitization becomes more pronounced at older age.

Eczema

Early-onset eczema is usually considered to be the first manifestation of atopic diseases followed by food allergy, wheezing/asthma and allergic rhinitis. A meta-analysis showed an OR 2.1 for asthma after early-onset eczema (van der Hulst *et al.* 2007). In this thesis, eczema-associated risk for recurrent wheezing (HR 2.3), school-age asthma (OR 2.9), and atopic asthma (adjusted OR 4.8), but not non-

atopic asthma, are in line with earlier studies showing that eczema predicts asthma if concomitant atopic characteristics are present (Göksor *et al.* 2013, Amat *et al.* 2015). In our study with severely wheezing children, only half of the children with early-onset eczema subsequently developed asthma, even though additional atopic symptoms would have been expected. The question regarding the early-onset eczema is, whether it expresses different phenotypes with different mechanisms and risks for subsequent asthma development.

Rhinovirus

The novel finding in this thesis was that rhinovirus etiology of the first wheezing episode at age 12 months predicted not only the wheezing recurrence but also atopic asthma at school-age (Studies I and IV). This is noteworthy since no other studies have been able to predict such a long-term outcome. Based on previous studies, rhinoviruses have been linked to pre-existing atopic conditions (Kusel *et al.* 2007, Jackson *et al.* 2012). We also found that rhinovirus-affected children were more often sensitized at study entry compared to RSV or RSV-/rhinovirus-negative groups (29% *vs.* 7-9%) (Study I). When coupled with clinical knowledge that children with atopic characteristics are more likely to develop asthma, it seems that rhinovirus itself would act as an early marker uncovering the underlying asthma susceptibility in atopic asthma-prone children (Lehtinen *et al.* 2007, Lukkarinen and Jartti 2016). On the other hand, Bønnelykke *et al.* and Kusel *et al.* found no particular viral or bacterial risk factor for school-age asthma (Kusel *et al.* 2012, Bønnelykke *et al.* 2015). Bønnelykke et al. suggested that underlying susceptibility to any trigger would be the risk factor instead of some specific agent (Bønnelykke *et al.* 2015). Kusel et al. hypothesized that febrile infections of the lower respiratory tract rather than wheezing would be a marker of asthma development, especially in early-onset atopics (Kusel *et al.* 2012). Asthma and persistent wheeze at age 10 years were associated with rhinovirus-induced wheezing during the first year of life only in sensitized children (Kusel *et al.* 2012). The findings of Bønnelykke and Kusel could be explained by the fact that they assessed the risk for overall asthma instead of differentiating the asthma phenotypes. It could be hypothesized that Kusel *et al.* would have had similar findings to ours if they had splitted the asthma outcome in atopic and non-atopic.

6.2 Risk for atopic and non-atopic asthma phenotypes at school-age after the first severe wheezing episode (IV)

The second aim was to assess risk factors for atopic and non-atopic asthma phenotypes at age 8 years. The novel idea was to assess risk factors for separate asthma phenotypes, and to add the presence of rhinovirus infection to this phenotype-based risk assessment. This population-based study was based on children with the first wheezing episode, among whom one third developed asthma 7 years later. The initial episode with severe wheezing was defined so that 90% of the children were hospitalized and 10% were remitted to the emergency room of the tertiary hospital. All children were steroid-naive since the prednisolone-treated children were excluded from the analyses. Therefore, our results could be valid for hospitalized first-time wheezing children, and may give new perspectives when estimating their future asthma risk.

Like in earlier studies, in this thesis the classical atopic asthma risk factors from the API and mAPI, such as sensitization (to food and/or aeroallergens), eczema at the first severe wheezing episode predicted atopic but not non-atopic asthma at school-age (Rönmark *et al.* 1999, Castro-Rodriguez *et al.* 2000, Guilbert *et al.* 2004a, Kurukulaaratchy *et al.* 2004, NAEPP 2007, Civelek *et al.* 2011, Göksor *et al.* 2013, Bousquet *et al.* 2014). However, the earlier studies differed from ours, by being conducted on birth cohorts and they included no virus etiology (Rönmark *et al.* 1999, Kurukulaaratchy *et al.* 2004, Civelek *et al.* 2011, Göksor *et al.* 2013). They included older children (Rönmark *et al.* 1999). In contrary to earlier studies, we demonstrated no clear asthma-reducing effect of breast-feeding, or asthma-increasing effect from male sex (Rönmark *et al.* 1999, Kurukulaaratchy *et al.* 2004, Civelek *et al.* 2011, Göksor *et al.* 2013).

Multiple early and overlapping atopic indicators such as eczema, food sensitization, and parental ashma, combined to the rhinovirus-etiology of the first wheezing episode, improved the recognition of children with atopic asthma at school-age (Study IV). Previously, early-life rhinovirus-induced wheezing has been linked to school-age asthma, but not specifically to atopic asthma, because the viral risk factors for separate asthma phenotypes have not been studied (Kotaniemi-Syrjänen *et al.* 2003, Kusel *et al.* 2007, Jackson *et al.* 2008). Previous studies included wheezing children with atopic predisposition, and thereby the results may have reflected susceptibility of atopic airways to rhinovirus infections (Kusel *et al.* 2007, Jackson *et al.* 2008). The underlying susceptibility, first to atopic disorders and thereafter to viral triggers, may rather be the true risk factor for atopic asthma, not the sensitization or the triggering virus itself. The interaction between sensitization and virus infections is likely to be involved (Holt and Sly 2011and 2012, Jackson *et al.* 2012, Edwards *et al.* 2013, Jackson *et al.* 2016, Rubner *et al.* 2017).

On the contrary, the factors predicting non-atopic asthma at school-age were different from atopic asthma; the first wheezing episode before age 12 months, parental smoking and the RSV-/rhinovirus-negative etiology of the first wheezing episode. This suggests a different underlying pathogenic mechanism for atopic and non-atopic asthma, and eventually a different illness. Parental smoking has strongly been linked to non-atopic asthma (Rönmark *et al.* 1999, Civelek *et al.* 2011, Göksor *et al.* 2013). It has been shown that maternal smoking during pregnancy causes even greater risk for childhood asthma than the exposure to tobacco smoke after birth (Gilliland *et al.* 2001, Lannero *et al.* 2006, Pattenden *et al.* 2006). We did not study separately maternal or parental smoking, or maternal smoking during pregnancy. Though, as much as 50% of smoking women continue with smoking during pregnancy (Alshaarawy and Anthony 2015). It could be assumed that these women continue with the habit after delivery. So, if there is questionnaire-based positive information about maternal/parental smoking at the time of the child's first wheezing episode (median age 11-12 months), the parents may presumably have smoked also during the pregnancy. On the other hand, about 50% of pregnant smokers quit smoking within the first trimester (Alshaarawy and Anthony 2015), which is crucial for the development of healthy peripheral airways in fetuses and infants (Prabhu *et al.* 2010). This suggests that the pathogenesis of smoking-induced hyper-reactivity and/or non-atopic asthma occurs during the second and third trimester of pregnancy. Nicotine is assumed to be the major causative component of tobacco smoke that affects lung development in fetuses of smokers (Wongtrakool *et al.* 2007).

Concurrently, we observed that the RSV-/rhinovirus-negative wheezing was associated with non-atopic asthma at school-age, most likely because the rhinovirus-positive wheezing children developed atopic asthma. In our study, RSV etiology was not predictive for any asthma or different phenotypes. Previously, the Tuscon Children's Respiratory Study showed in a population-based birth cohort that children with RSV-induced lower respiratory tract infections had frequent wheezing episodes at school-age, but the risk decreased by age 13 age years, and there was no link between RSV infections and sensitization (Stein *et al.* 1999). They included no rhinovirus-etiology in their analyses. Likewise, early-life RSV-induced wheezing/lower respiratory tract infection was not associated with school-age asthma (Juntti *et al.* 2003, Kotaniemi-Syrjänen *et al.* 2003) or sensitization (Juntti *et al.* 2003) in hospitalized wheezing children.

6.3 The long-term effect of the prednisolone intervention after the first wheezing episode (I, II and III)

The third aim of this thesis was to assess the long-term effect of the prednisolone intervention at study entry on asthma development. In Vinku, the early OCS halved the risk of recurrent wheezing in a 7-year follow-up in children with rhinovirus-induced first wheezing and/or eczema (Study I). Furthermore, in the same children early OCS showed a trend for less need of long-term asthma control therapy (Study II). In Vinku2, OCS reduced the risk for recurrent wheezing and asthma therapy in children with high rhinovirus genome load (>7000 copies/mL) (Study III). These results based on two separate cohorts may be two sides of the same phenomenon; the OCS inhibited the appearance of new asthma symptoms by reducing the risk of recurrence (Studies I and III), and at the same time it also reduced the persistence of asthma symptoms by decreasing the need for asthma therapy (Study II). This was exclusively after rhinovirus-induced wheezing.

The risk-reducing effect of OCS could not be confirmed as clearly in the Vinku2 cohort (Jartti *et al.* 2015)(Study III) compared to the Vinku cohort (Studies I and II). The explanation might be sensitivity differences in PCR techniques used in the two cohorts. During Vinku, conventional PCR followed by liquid hybridization was used in rhinovirus diagnostics without quantitative analyses, whereas during Vinku2, this method was replaced by quantitative RT-PCR, with higher sensitivity, detecting rhinoviruses at lower levels (Jartti *et al.* 2013). This means that the beneficial effect of OCS in our studies may be due to high rhinovirus loads, and more severe airway inflammation already at the time of the first wheezing episode (Jartti *et al.* 2015). Another possible explanation for the the smaller OCS effect in Vinku2 compared to Vinku may be the delay in initiation of OCS (45 hours in Vinku2 *vs.* 0 hours in Vinku) due to the time it took to complete rhinovirus PCR in Vinku2 (Jartti *et al.* 2015).

It is striking that these long-term disease-modifying effects could be expected after a 3-day course of OCS. The effect is presumably due to early targeting to high-risk children with pronounced atopic characteristics of sensitization and rhinovirus etiology. It has previously been shown in the studies with ICS that periodic or regular ICS therapy in children with recurrent wheezing before age 3 years did not prevent subsequent asthma symptoms (Bisgaard *et al.* 2006, Guilbert *et al.* 2006, Murray *et al.* 2006, Devulapalli *et al.* 2007). This suggests that topical therapy may not reach the disease-modifying effect but rather achieves symptom control and reduces the risk of exacerbations (Guilbert *et al.* 2006). On the other hand, in all these studies no account was taken to atopic characteristics, sensitization status or virus etiology of the wheezing episodes. Studies on systemic corticosteroids conducted on hospitalized wheezing children indicated that infants with atopic

chracteristics may benefit from OCS (Alansari *et al.* 2013). The current long-term results in this thesis support the view that a subgroup of atopic infants with airway inflammation related to early rhinovirus infection, allergic sensitization and eczema may be targeted with disease-modifying anti-inflammatory treatment in an early phase of the disease progression.

The hypothetical mechanism of OCS in rhinovirus-affected children might be that it weakens the pre-existing, likely atopy-related airway inflammation through diverse biologic mechanisms (Stellato 2007, de Benedictis and Bush 2012, Holt and Sly 2012). Glucocorticoids may have a capacity to modify/preserve epithelial barrier, and thereby protect against respiratory viral infections (Hermanns *et al.* 2004). One mechanism might also be the ability to repress the transcription of many inflammatory genes and/or transcription factors, and inducing expression of anti-inflammatory genes (Stellato 2007, de Benedictis and Bush 2012). Moreover, glucocorticoids inhibit the rhinovirus-induced up-regulation of its own major receptor ICAM-1 in pulmonary mucosa (Papi *et al.* 2000). It may be crucial to dose glucocorticosteroids systemically since it has been suggested that a local inflammation triggers a linkage to bone marrow from where cells potentially migrate to their different peripheral targets, e.g. lungs or skin (Holt and Sly 2012). Early administration of OCS may also be particularly important because rhinovirus load peaks early in rhinovirus infection (Kennedy *et al.* 2014).

Concluded for the OCS treatment, in clinical practice the challenge is the early identification of high asthma risk infants, and thereafter their selection for the effective intervention to reduce asthma morbidity. These results support the idea that early severe wheezing due to high rhinoviral load could serve as a potential marker to recognize these children with pre-existing susceptibility, and who possibly could benefit from acute treatment with OCS. This is of note, since there is supposed to be distinct bronchiolitis phenotypes that respond differently to therapies (Dumas *et al.* 2016). The natural course of asthma inception might be modifiable if high-risk children are identified early and the intervention strategy is found effective.

6.4 Prediction of asthma phenotypes

Currently, the ability to predict school-age asthma risk based on early-life characteristics is limited. Early identification of children at high asthma risk is important to find children who require closer monitoring, but also for therapy or prevention strategies. The development of asthmatic disorders may be the result from a genetic predisposition or factors during pregnancy influencing the airway physiology. This can also be derived from the findings in this thesis, that the division of distinct asthma phenotypes was already apparent at the first wheezing episode (Figure 12). Sensitization to food is likely to develop earlier than to aeroallergens being more useful in the asthma predictive indices, particularly in high risk infants (Kusel *et al.* 2007, Nissen *et al.* 2013, Chiu *et al.* 2014). A limitation with current risk indices is that they are mainly based on atopic characteristics but still used for school-age asthma risk assessment, regardless of the asthma phenotype (Castro-Rodriguez *et al.* 2000, Guilbert *et al.* 2004a, NAEPP 2007). This could explain their relatively low overall sensitivities in asthma prediction, meaning that they cannot exclude future asthma. Still, their specificities are high, meaning that a positive tes/high index confirms the possibility of asthma development. Another limitation is their requirement for several wheezing epidsodes. There is no specific risk index for the prediction of non-atopic asthma in children since the early-life risk factors for non-atopic asthma are still not well established.

Another shortage is, that virus etiology of early wheezing episodes has not been included in the asthma predictive indices even though the viral diagnostics has improved, and led to better and earlier recognition of wheezing-triggering agents (Jartti *et al.* 2013, Turunen *et al.* 2014). Only one congress abstract studied the rhinovirus etiology of wheezing in mAPI showing rather high sensitivity (59%) for asthma at age 6 years, when the ≥4 wheezing episodes in the mAPI was replaced with ≥1 rhinovirus-induced wheezing episode (Jackson *et al.* 2009). Our findings show that early rhinovirus-induced wheezing is an independent risk factor for atopic asthma. Combining the early-manifesting atopic markers and rhinovirus etiology of wheezing to one asthma predictive index in hospitalized wheezing children would offer a tool to assess asthma risk in infants more precisely and already at the first wheezing episode (Figure 12).

Figure 12. Characteristics at the first wheezing episode in hospitalized children to assess asthma risk at school-age. *RSV*, respiratory syncytial virus.

6.5 Strengths and limitations

6.5.1 Strengths

Two separate cohorts demonstrated similar results on the efficacy of OCS. This highlights the success of initial randomization. All children had the first pediatrician-confirmed wheezing episode and 90% were hospitalized for the wheezing severity. We focused on wheezing (*vs.* bronchiolitis with or without wheezing) to minimize the heterogeneity and to make results more generalizable (Marguet *et al.* 2009, Flores *et al.* 2011, Midulla *et al.* 2012). Other strengths of this thesis are comprehensive assessment of atopic characteristics and virus etiology at study entry, long-term follow-up, and the use of non-selected population. This is a population-based study meaning that all children from the area of Southwest Finland (urban or rural) who needed hospitalization for the wheezing were admitted to this hospital. Therefore, the results could be adapted to hospitalized first-time wheezing children, and may give new perspective when estimating their future asthma risk. The close verification of wheezing was important because infant wheezing may truly be an important risk factor for asthma.

The verification of wheezing episodes, asthma symptoms, medications, laboratory tests for the full 7-year follow-up period, and the duration of asthma controller therapy was confirmed in all children from health care records and from parental interviews. Children may become labelled as having wheezing even if they do not if the history of wheezing is only based on parental report. Therefore, wheezing should be physician-confirmed (Levy *et al.* 2004) since parental understanding and definition of wheezing may differ widely (Elphick *et al.* 2001, Michel *et al.* 2006).

To minimize the selection bias, in all four Studies were also included children who did not attend the long-term study visit. It is a known fact that people adhere follow-up studies that concern their interests, in our case asthmatics. To maximize the objectivity of the results regarding asthma risk at school-age, we considered children with bronchial hyper-reactivity in spirometry asthmatics, even though they were yet without a proper pediatrician-set asthma diagnose. We think this reflects well the real-life situation and completed our asthma outcome. In the Study IV concerning current asthma, all children were steroid-naive *ie.* they received no ICS/OCS before or as a treatment for the first wheezing. This is noteworthy since OCS may affect long-term asthma outcome as shown in Studies I, II and III (Lehtinen *et al.* 2007, Jartti *et al.* 2015, Lukkarinen *et al.* 2015).

The sub-studies of this thesis are presented in chronological order demonstrating the continuum through the 7-year follow-up period. The Study I presents the shortest-term outcome, the Studies II and III present the disease persistence and

the efficacy of OCS during the follow-up. The Study IV shows the asthma status in children at the end of the follow-up. We developed a new, more illustrative outcome to childhood asthma risk assessment, namely the need for long-term asthma therapy with ICS (Study II). We suggest that it illustrates well the asthma persistence because children with persistent symptoms need regular long-term asthma therapy to achieve symptom control and to reduce the risk of exacerbations (Guilbert *et al.* 2006, NAEPP 2007).

6.5.2 Limitations

We focused on children hospitalized for the first and severe wheezing episode. Therefore, the results may not be generalized for outpatient care which is different from studies on birth cohorts. Our study populations were rather small. When analyzing the risk of recurrent wheezing, the need of long-term asthma therapy and the efficacy of prednisolone in Studies I and II, the risk of bias due to multiple comparisons was avoided by using the same pre-specified subgroups as in the 1-year follow-up of same cohort (Lehtinen *et al.* 2007). Prospective intervention studies on rhinovirus-induced wheezing are challenging because the virus PCR analysis usually is an over-night diagnosis, and therefore prolongs the start of intervention, which was also the case in Vinku2 study. However, fortunately diagnostic multiplex testing for respiratory pathogens with turnaround time 1-3 hours is currently available (Rappo *et al.* 2016, Subramony *et al.* 2016). In this thesis, the PCR detection covered rhinovirus A, B and C species as one group, since they were not sequenced. However, the samples from upper airway for virus PCR analysis are considered to adequately reflect the infection status in the lower airways (Jartti *et al.* 2012).

7 SUMMARY AND CONCLUSIONS

7.1 Main findings

First, at the first wheezing episode allergic sensitization, rhinovirus etiology, the presence of eczema and age <12 months predicted recurrence of wheezing episodes. In particular, early-onset food sensitization predicted the persistence of asthma symptoms.

Second, different risk factors for atopic and non-atopic asthma phenotypes at school-age were found in first-time severely wheezing children, suggesting a different underlying pathogenic mechanism for atopic and non-atopic asthma, and eventually a different illness. Risk factors for atopic asthma were allergic sensitization, eczema and rhinovirus etiology of the first severe wheezing episode, particularly the presence of concomitant rhinovirus infection and atopic characteristics. On the other hand, risk factors for non-atopic asthma at school-age were the first wheezing episode presenting before age 12 months, parental smoking and the RSV-/rhinovirus-negative etiology of the first wheezing episode.

Third, sensitive rhinovirus diagnostics has markedly improved the early identification of children in risk of asthma. Since the rhinovirus-infected first-time wheezing children benefitted from early treatment with oral corticosteroids in terms of less wheezing recurrence and asthma persistence, this evidence may substantially influence clinical practice.

In conclusion, quick identification of the viral cause and specific risk factor patterns may be used to select first-time wheezing children at increased risk for developing atopic and non-atopic asthma. Early-life wheezing caused by rhinovirus may be a marker of later asthma development in susceptible (atopic) children. Our data from two randomized clinical trials and long-term follow-ups suggest that the natural course of asthma inception in the high-risk population may be prevented by using early and effective anti-inflammatory treatment. However, more powerful prospective randomized trials are warranted to confirm this.

7.2 Future considerations

For future wheezing studies, it is relevant to define/design similar inclusion criteria and objective follow-up protocols to maximize the homogeneity of cohorts and the objectivity of results. The accurate definition of whether the studies have been conducted on birth cohorts (with or without familial predisposition to atopic conditions) or are population based (out-patient *vs.* hospitalized) is relevant for generalizable results. Also, to use information from patient charts together with questionnaire data would serve as more objective follow-up data by reducing parental recall-bias.

It is important to have clear definitions to recognize wheezing phenotypes. The definition of wheezing (physician-confirmed *vs.* parent-reported), the certain number of wheezing episode (first *vs.* indeterminate), and whether the wheezing is the only inclusive criteria (*vs.* bronchiolitis with or without wheezing) is noteworthy since distinct clinical phenotypes of bronchiolitis/wheezing have been identified (Dumas *et al.* 2016). It is irrelevant whether the first wheezing episode is classified as bronchiolitis or not (Brand *et al.* 2008) due to the discrepancy in terminology between the UK and US definitions. Therefore, studies on bronchiolitis should clearly define the wheezing status of the subjects. Otherwise, the heterogeneity of definitions may have consequences on results on early risk factors, therapy and long-term outcomes, including future asthma risk. It may also explain the current conflicting results of the early risk factors for childhood asthma (Florin *et al.* 2017). To support this, in the Study IV risk factors for different school-age asthma phenotypes could be recognized in infancy. This finding provides an approach into the mechanisms underlying childhood wheezing and asthma, prognostics, and potentially different therapies or prevention of distinct asthma phenotypes (Szefler 2014). Early-life risk factors for non-atopic asthma should be studied.

The promising results suggest that early OCS treatment for the first severe rhinovirus-induced wheezing would reduce asthma symptoms in high risk infants. The patient group who possibly could benefit from the early OCS treatment would constitute of children aged under 2 years with severe physician-diagnosed wheezing episode associated with high rhinoviral load and atopic conditions such as early sensitization and/or eczema (de Benedictis and Bush 2017). Physicians ought to recognize this risk group. For future wheezing studies is recommended 1) a prospective cohort to study the effect of rhinovirus etiology in asthma predictive indices and in precision of separate asthma predictive indices for atopic and non-atopic asthma, as well as 2) a randomized clinical (multicenter) trial to increase the number of participants to confirm the preventive effect of early systemic anti-inflammatory treatment in early wheezing children with rhinovirus.

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REFERENCES

AAP. American Academy of Pediatrics Subcommittee on Diagnosis and management of bronchiolitis. *Pediatrics*. 2006; 118 (4): 1774-1793.

Abramson MJ, Toelle BG, James AL, Wood-Baker R, Burton D, Xuan W, Johns DP, Buist AS and Marks GB. Asthma diagnosis and treatment - 1016. Is atopy in people aged 40 and over related to fixed airflow obstruction? *World Allergy Organ J*. 2013; 6 Suppl 1 P16.

Alansari K, Sakran M, Davidson BL, Ibrahim K, Alrefai M and Zakaria I. Oral dexamethasone for bronchiolitis: a randomized trial. *Pediatrics*. 2013; 132 (4): e810-816.

Allander T, Jartti T, Gupta S, Niesters HG, Lehtinen P, Osterback R, Vuorinen T, Waris M, Bjerkner A, Tiveljung-Lindell A, van den Hoogen BG, Hyypiä T and Ruuskanen O. Human bocavirus and acute wheezing in children. *Clin Infect Dis*. 2007a; 44 (7): 904-910.

Allander T, Jartti T, Gupta S, Niesters HG, Lehtinen P, Österback R, Vuorinen T, Waris M, Bjerkner A, Tiveljung-Lindell A, van den Hoogen BG, Hyypia T and Ruuskanen O. Human bocavirus and acute wheezing in children. *Clin Infect Dis*. 2007b; 44 (7): 904-910.

Alm B, Goksor E, Pettersson R, Mollborg P, Erdes L, Loid P, Aberg N and Wennergren G. Antibiotics in the first week of life is a risk factor for allergic rhinitis at school age. *Pediatr Allergy Immunol*. 2014; 25 (5): 468-472.

Alshaarawy O and Anthony JC. Month-wise estimates of tobacco smoking during pregnancy for the United States, 2002-2009. *Matern Child Health J*. 2015; 19 (5): 1010-1015.

Amat F, Saint-Pierre P, Bourrat E, Nemni A, Couderc R, Boutmy-Deslandes E, Sahraoui F, Panse I, Bagot M, Fouere S and Just J. Early-onset atopic dermatitis in children: which are the phenotypes at risk of asthma? Results from the ORCA cohort. *PLoS One*. 2015; 10 (6): e0131369.

Andersson NW, Hansen MV, Larsen AD, Hougaard KS, Kolstad HA and Schlunssen V. Prenatal maternal stress and atopic diseases in the child: a systematic review of observational human studies. *Allergy*. 2016a; 71 (1): 15-26.

Andersson NW, Li Q, Mills CW, Ly J, Nomura Y and Chen J. Influence of prenatal maternal stress on umbilical cord blood cytokine levels. *Arch Womens Ment Health*. 2016b; 19 (5): 761-767.

Andreoletti L, Lesay M, Deschildre A, Lambert V, Dewilde A and Wattre P. Differential detection of rhinoviruses and enteroviruses RNA sequences associated with classical immunofluorescence assay detection of respiratory virus antigens in nasopharyngeal swabs from infants with bronchiolitis. *J Med Virol*. 2000; 61 (3): 341-346.

Andrewes CH, Chaproniere DM, Gompels AE, Pereira HG and Roden AT. Propagation of common-cold virus in tissue cultures. *Lancet*. 1953; 265 (6785): 546-547.

Baris S, Karakoc-Aydiner E, Ozen A, Ozdemir C, Bahceciler NN and Barlan IB. Serum immunoglobulin levels as a predictive factor for a better outcome of non-atopic childhood asthma. *Pediatr Allergy Immunol*. 2011; 22 (3): 298-304.

Beasley R, Semprini A and Mitchell EA. Risk factors for asthma: is prevention possible? *Lancet*. 2015; 386 (9998): 1075-1085.

Belsky DW, Sears MR, Hancox RJ, Harrington H, Houts R, Moffitt TE, Sugden K, Williams B, Poulton R and Caspi A. Polygenic risk and the development and course of asthma: an analysis of data from a four-decade longitudinal study. *Lancet Respir Med*. 2013; 1 (6): 453-461.

Bergroth E, Aakula M, Korppi M, Remes S, Kivisto JE, Piedra PA, Camargo CA, Jr. and Jartti T. Post-bronchiolitis Use of Asthma Medication: A Prospective 1-year Follow-up Study. *Pediatr Infect Dis J*. 2016; 35 (4): 363-368.

Berry M, Gamieldien J and Fielding BC. Identification of new respiratory viruses in the new millennium. *Viruses*. 2015; 7 (3): 996-1019.

Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, Bisgaard H, Davis GM, Ducharme FM, Eigen H, Gappa M, Gaultier C, Gustafsson PM, Hall GL, Hantos Z, Healy MJ, Jones MH, Klug B, Lodrup Carlsen KC, McKenzie SA, Marchal F, Mayer OH, Merkus PJ, Morris MG, Oostveen E, Pillow JJ, Seddon PC, Silverman M, Sly PD, Stocks J, Tepper RS, Vilozni D and Wilson NM. An official

American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. *Am J Respir Crit Care Med*. 2007; 175 (12): 1304-1345.

Bisgaard H, Bonnelykke K, Sleiman PM, Brasholt M, Chawes B, Kreiner-Moller E, Stage M, Kim C, Tavendale R, Baty F, Pipper CB, Palmer CN and Hakonarsson H. Chromosome 17q21 gene variants are associated with asthma and exacerbations but not atopy in early childhood. *Am J Respir Crit Care Med*. 2009; 179 (3): 179-185.

Bisgaard H, Hermansen MN, Bonnelykke K, Stokholm J, Baty F, Skytt NL, Aniscenko J, Kebadze T and Johnston SL. Association of bacteria and viruses with wheezy episodes in young children: prospective birth cohort study. *BMJ*. 2010; 341 c4978.

Bisgaard H, Hermansen MN, Loland L, Halkjaer LB and Buchvald F. Intermittent inhaled corticosteroids in infants with episodic wheezing. *N Engl J Med*. 2006; 354 (19): 1998-2005.

Bisgaard H, Jensen SM and Bonnelykke K. Interaction between asthma and lung function growth in early life. *Am J Respir Crit Care Med*. 2012; 185 (11): 1183-1189.

Bisgaard H and Szefler S. Prevalence of asthma-like symptoms in young children. *Pediatr Pulmonol*. 2007; 42 (8): 723-728.

Bizzintino J, Lee WM, Laing IA, Vang F, Pappas T, Zhang G, Martin AC, Khoo SK, Cox DW, Geelhoed GC, McMinn PC, Goldblatt J, Gern JE and Le Souef PN. Association between human rhinovirus C and severity of acute asthma in children. *Eur Respir J*. 2011; 37 (5): 1037-1042.

Blaas D and Fuchs R. Mechanism of human rhinovirus infections. *Mol Cell Pediatr*. 2016; 3 (1): 21.

Black M, Bhattacharya S, Philip S, Norman JE and McLernon DJ. Planned Repeat Cesarean Section at Term and Adverse Childhood Health Outcomes: A Record-Linkage Study. *PLoS Med*. 2016; 13 (3): e1001973.

Blanken MO, Rovers MM, Molenaar JM, Winkler-Seinstra PL, Meijer A, Kimpen JL, Bont L and Dutch RSVNN. Respiratory syncytial virus and recurrent wheeze in healthy preterm infants. *N Engl J Med*. 2013; 368 (19): 1791-1799.

Bochkov YA and Gern JE. Rhinoviruses and Their Receptors: Implications for Allergic Disease. *Curr Allergy Asthma Rep*. 2016; 16 (4): 30.

Bochkov YA, Palmenberg AC, Lee WM, Rathe JA, Amineva SP, Sun X, Pasic TR, Jarjour NN, Liggett SB and Gern JE. Molecular modeling, organ culture and reverse genetics for a newly identified human rhinovirus C. *Nat Med*. 2011; 17 (5): 627-632.

Bonnelykke K, Pipper CB, Tavendale R, Palmer CN and Bisgaard H. Filaggrin gene variants and atopic diseases in early childhood assessed longitudinally from birth. *Pediatr Allergy Immunol*. 2010; 21 (6): 954-961.

Bousquet J, Gern JE, Martinez FD, Anto JM, Johnson CC, Holt PG, Lemanske RF, Jr., Le Souef PN, Tepper RS, von Mutius ER, Arshad SH, Bacharier LB, Becker A, Belanger K, Bergstrom A, Bernstein DI, Cabana MD, Carroll KN, Castro M, Cooper PJ, Gillman MW, Gold DR, Henderson J, Heinrich J, Hong SJ, Jackson DJ, Keil T, Kozyrskyj AL, Lodrup Carlsen KC, Miller RL, Momas I, Morgan WJ, Noel P, Ownby DR, Pinart M, Ryan PH, Schwaninger JM, Sears MR, Simpson A, Smit HA, Stern DA, Subbarao P, Valenta R, Wang X, Weiss ST, Wood R, Wright AL, Wright RJ, Togias A and Gergen PJ. Birth cohorts in asthma and allergic diseases: report of a NIAID/NHLBI/MeDALL joint workshop. *J Allergy Clin Immunol*. 2014; 133 (6): 1535-1546.

Brand PL, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, de Blic J, de Jongste JC, Eber E, Everard ML, Frey U, Gappa M, Garcia-Marcos L, Grigg J, Lenney W, Le Souef P, McKenzie S, Merkus PJ, Midulla F, Paton JY, Piacentini G, Pohunek P, Rossi GA, Seddon P, Silverman M, Sly PD, Stick S, Valiulis A, van Aalderen WM, Wildhaber JH, Wennergren G, Wilson N, Zivkovic Z and Bush A. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. *Eur Respir J*. 2008; 32 (4): 1096-1110.

Busse WW. The relationship between viral infections and onset of allergic diseases and asthma. *Clin Exp Allergy*. 1989; 19 (1): 1-9.

Busse WW and Lemanske RF, Jr. Asthma. *N Engl J Med*. 2001; 344 (5): 350-362.

Busse WW, Morgan WJ, Gergen PJ, Mitchell HE, Gern JE, Liu AH, Gruchalla RS, Kattan M, Teach SJ, Pongracic JA, Chmiel JF, Steinbach SF, Calatroni A, Togias A, Thompson KM, Szefler SJ and Sorkness CA. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *N Engl J Med*. 2011; 364 (11): 1005-1015.

Bønnelykke K, Vissing NH, Sevelsted A, Johnston SL and Bisgaard H. Association between

respiratory infections in early life and later asthma is independent of virus type. *J Allergy Clin Immunol*. 2015; 136 (1): 81-86

Caliskan M, Bochkov YA, Kreiner-Moller E, Bonnelykke K, Stein MM, Du G, Bisgaard H, Jackson DJ, Gern JE, Lemanske RF, Jr., Nicolae DL and Ober C. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med*. 2013; 368 (15): 1398-1407.

Carroll KN, Gebretsadik T, Escobar GJ, Wu P, Li SX, Walsh EM, Mitchel E, Sloan CD, Dupont WD and Hartert TV. Respiratory syncytial virus immunoprophylaxis in high-risk infants and development of childhood asthma. *J Allergy Clin Immunol*. 2017; 139 (1): 66-71 e63.

Carroll KN, Gebretsadik T, Minton P, Woodward K, Liu Z, Miller EK, Williams JV, Dupont WD and Hartert TV. Influence of maternal asthma on the cause and severity of infant acute respiratory tract infections. *J Allergy Clin Immunol*. 2012; 129 (5): 1236-1242.

Carroll KN, Wu P, Gebretsadik T, Griffin MR, Dupont WD, Mitchel EF and Hartert TV. The severity-dependent relationship of infant bronchiolitis on the risk and morbidity of early childhood asthma. *J Allergy Clin Immunol*. 2009; 123 (5): 1055-1061, 1061 e1051.

Castro-Rodriguez JA, Beckhaus AA and Forno E. Efficacy of oral corticosteroids in the treatment of acute wheezing episodes in asthmatic preschoolers: Systematic review with meta-analysis. *Pediatr Pulmonol*. 2016; 51 (8): 868-876.

Castro-Rodriguez JA, Holberg CJ, Wright AL and Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. *Am J Respir Crit Care Med*. 2000; 162 (4 Pt 1): 1403-1406.

Chang TS, Lemanske RF, Jr., Guilbert TW, Gern JE, Coen MH, Evans MD, Gangnon RE, David Page C and Jackson DJ. Evaluation of the modified asthma predictive index in high-risk preschool children. *J Allergy Clin Immunol Pract*. 2013; 1 (2): 152-156.

Chiu CY, Huang YL, Tsai MH, Tu YL, Hua MC, Yao TC, Yeh KW and Huang JL. Sensitization to food and inhalant allergens in relation to atopic diseases in early childhood: a birth cohort study. *PLoS One*. 2014; 9 (7): e102809.

Christensen N, Sondergaard J, Fisker N and Christesen HT. Infant Respiratory Tract Infections or Wheeze and Maternal Vitamin D in Pregnancy: A Systematic Review. *Pediatr Infect Dis J*. 2017; 36 (4): 384-391.

Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD and Aagaard KM. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nat Med*. 2017; 23 (3): 314-326.

Civelek E, Cakir B, Orhan F, Yuksel H, Boz AB, Uner A and Sekerel BE. Risk factors for current wheezing and its phenotypes among elementary school children. *Pediatr Pulmonol*. 2011; 46 (2): 166-174.

Collado MC, Rautava S, Isolauri E and Salminen S. Gut microbiota: a source of novel tools to reduce the risk of human disease? *Pediatr Res*. 2015; 77 (1-2): 182-188.

Collins AD and Beigelman A. An update on the efficacy of oral corticosteroids in the treatment of wheezing episodes in preschool children. *Ther Adv Respir Dis*. 2014; 8 (6): 182-190.

Contoli M, Ito K, Padovani A, Poletti D, Marku B, Edwards MR, Stanciu LA, Gnesini G, Pastore A, Spanevello A, Morelli P, Johnston SL, Caramori G and Papi A. Th2 cytokines impair innate immune responses to rhinovirus in respiratory epithelial cells. *Allergy*. 2015; 70 (8): 910-920.

Contoli M, Message SD, Laza-Stanca V, Edwards MR, Wark PA, Bartlett NW, Kebadze T, Mallia P, Stanciu LA, Parker HL, Slater L, Lewis-Antes A, Kon OM, Holgate ST, Davies DE, Kotenko SV, Papi A and Johnston SL. Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med*. 2006; 12 (9): 1023-1026.

de Benedictis FM and Bush A. Corticosteroids in respiratory diseases in children. *Am J Respir Crit Care Med*. 2012; 185 (1): 12-23.

de Benedictis FM and Bush A. Infantile wheeze: rethinking dogma. *Arch Dis Child*. 2017; 102 (4): 371-375.

DeVries A, Wlasiuk G, Miller SJ, Bosco A, Stern DA, Lohman IC, Rothers J, Jones AC, Nicodemus-Johnson J, Vasquez MM, Curtin JA, Simpson A, Custovic A, Jackson DJ, Gern JE, Lemanske RF, Jr., Guerra S, Wright AL, Ober C, Halonen M and Vercelli D. Epigenome-wide Analysis Links SMAD3 Methylation at Birth to Asthma in Children of Asthmatic Mothers. *J Allergy Clin Immunol*. 2016, 10.1016/j.jaci.2016.10.041

Devulapalli CS, Carlsen KC, Haland G, Munthe-Kaas MC, Pettersen M, Mowinckel P and Carlsen KH. Severity of obstructive airways disease by age 2 years predicts asthma at 10 years of age. *Thorax*. 2008; 63 (1): 8-13.

Devulapalli CS, Lodrup Carlsen KC, Haland G, Munthe-Kaas MC, Pettersen M, Mowinckel P and Carlsen KH. No evidence that early use of inhaled corticosteroids reduces current asthma at 10 years of age. *Respir Med*. 2007; 101 (8): 1625-1632.

Di Bona D, Plaia A, Leto-Barone MS, La Piana S, Macchia L and Di Lorenzo G. Efficacy of allergen immunotherapy in reducing the likelihood of developing new allergen sensitizations: a systematic review. *Allergy*. 2016, 10.1111/all.13104

Ducharme FM, Tse SM and Chauhan B. Diagnosis, management, and prognosis of preschool wheeze. *Lancet*. 2014; 383 (9928): 1593-1604.

Dumas O, Mansbach JM, Jartti T, Hasegawa K, Sullivan AF, Piedra PA and Camargo CA, Jr. A clustering approach to identify severe bronchiolitis profiles in children. *Thorax*. 2016; 71 (8): 712-718.

Durrani SR, Montville DJ, Pratt AS, Sahu S, DeVries MK, Rajamanickam V, Gangnon RE, Gill MA, Gern JE, Lemanske RF, Jr. and Jackson DJ. Innate immune responses to rhinovirus are reduced by the high-affinity IgE receptor in allergic asthmatic children. *J Allergy Clin Immunol*. 2012; 130 (2): 489-495.

Edwards MR, Regamey N, Vareille M, Kieninger E, Gupta A, Shoemark A, Saglani S, Sykes A, Macintyre J, Davies J, Bossley C, Bush A and Johnston SL. Impaired innate interferon induction in severe therapy resistant atopic asthmatic children. *Mucosal Immunol*. 2013; 6 (4): 797-806.

Elphick HE, Sherlock P, Foxall G, Simpson EJ, Shiell NA, Primhak RA and Everard ML. Survey of respiratory sounds in infants. *Arch Dis Child*. 2001; 84 (1): 35-39.

Fleischer DM, Spergel JM, Assa'ad AH and Pongracic JA. Primary prevention of allergic disease through nutritional interventions. *J Allergy Clin Immunol Pract*. 2013; 1 (1): 29-36.

Flores P, Guimaraes J and Videira Amaral JM. Th1 and th2 cytokine expression in nasopharyngeal secretions during acute bronchiolitis in children younger than two years old. *Allergol Immunopathol (Madr)*. 2011; 39 (1): 3-9.

Florin TA, Plint AC and Zorc JJ. Viral bronchiolitis. *Lancet*. 2017; 389 (10065): 211-224.

Fox GF, Marsh MJ and Milner AD. Treatment of recurrent acute wheezing episodes in infancy with oral salbutamol and prednisolone. *Eur J Pediatr*. 1996; 155 (6): 512-516.

Gadomski AM and Brower M. Bronchodilators for bronchiolitis. *Cochrane Database Syst Rev*. 2010, 10.1002/14651858.CD001266.pub2(12): CD001266.

Galanter J, Choudhry S, Eng C, Nazario S, Rodriguez-Santana JR, Casal J, Torres-Palacios A, Salas J, Chapela R, Watson HG, Meade K, LeNoir M, Rodriguez-Cintron W, Avila PC and Burchard EG. ORMDL3 gene is associated with asthma in three ethnically diverse populations. *Am J Respir Crit Care Med*. 2008; 177 (11): 1194-1200.

Gern JE. The ABCs of rhinoviruses, wheezing, and asthma. *J Virol*. 2010; 84 (15): 7418-7426.

Gern JE, Galagan DM, Jarjour NN, Dick EC and Busse WW. Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. *Am J Respir Crit Care Med*. 1997; 155 (3): 1159-1161.

Gern JE, Martin MS, Anklam KA, Shen K, Roberg KA, Carlson-Dakes KT, Adler K, Gilbertson-White S, Hamilton R, Shult PA, Kirk CJ, Da Silva DF, Sund SA, Kosorok MR and Lemanske RF, Jr. Relationships among specific viral pathogens, virus-induced interleukin-8, and respiratory symptoms in infancy. *Pediatr Allergy Immunol*. 2002; 13 (6): 386-393.

Gilliland FD, Berhane K, McConnell R, Gauderman WJ, Vora H, Rappaport EB, Avol E and Peters JM. Maternal smoking during pregnancy, environmental tobacco smoke exposure and childhood lung function. *Thorax*. 2000; 55 (4): 271-276.

Gilliland FD, Li YF and Peters JM. Effects of maternal smoking during pregnancy and environmental tobacco smoke on asthma and wheezing in children. *Am J Respir Crit Care Med*. 2001; 163 (2): 429-436.

GINA. Global Initiative for Asthma (GINA), National Heart, Lung and Blood Institute (NHLBI) Global strategy for asthma management and prevention. Bethesda (MD): Global Initiative for Asthma (GINA), National Heart, Lung and Blood Institute (NHLBI); 2006. p. 339. Available from: http://www.ginasthma.com/. 2006,

GINA. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2016. Available from: http://www.ginasthma.org/. 2016,

Greer FR, Sicherer SH, Burks AW, American Academy of Pediatrics Committee on N, American Academy of Pediatrics Section on A and Immunology. Effects of early nutritional interventions on the development of atopic

disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. *Pediatrics*. 2008; 121 (1): 183-191.

Griffiths C, Drews SJ and Marchant DJ. Respiratory Syncytial Virus: Infection, Detection, and New Options for Prevention and Treatment. *Clin Microbiol Rev*. 2017; 30 (1): 277-319.

Guerra S and Martinez FD. Asthma genetics: from linear to multifactorial approaches. *Annu Rev Med*. 2008; 59 327-341.

Guilbert TW, Morgan WJ, Krawiec M, Lemanske RF, Jr., Sorkness C, Szefler SJ, Larsen G, Spahn JD, Zeiger RS, Heldt G, Strunk RC, Bacharier LB, Bloomberg GR, Chinchilli VM, Boehmer SJ, Mauger EA, Mauger DT, Taussig LM, Martinez FD, Prevention of Early Asthma in Kids Study CAR and Education N. The Prevention of Early Asthma in Kids study: design, rationale and methods for the Childhood Asthma Research and Education network. *Control Clin Trials*. 2004a; 25 (3): 286-310.

Guilbert TW, Morgan WJ, Zeiger RS, Bacharier LB, Boehmer SJ, Krawiec M, Larsen G, Lemanske RF, Liu A, Mauger DT, Sorkness C, Szefler SJ, Strunk RC, Taussig LM and Martinez FD. Atopic characteristics of children with recurrent wheezing at high risk for the development of childhood asthma. *J Allergy Clin Immunol*. 2004b; 114 (6): 1282-1287.

Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szefler SJ, Bacharier LB, Lemanske RF, Jr., Strunk RC, Allen DB, Bloomberg GR, Heldt G, Krawiec M, Larsen G, Liu AH, Chinchilli VM, Sorkness CA, Taussig LM and Martinez FD. Long-term inhaled corticosteroids in preschool children at high risk for asthma. *N Engl J Med*. 2006; 354 (19): 1985-1997.

Guo-Parke H, Canning P, Douglas I, Villenave R, Heaney LG, Coyle PV, Lyons JD, Shields MD and Power UF. Relative respiratory syncytial virus cytopathogenesis in upper and lower respiratory tract epithelium. *Am J Respir Crit Care Med*. 2013; 188 (7): 842-851.

Göksor E, Alm B, Pettersson R, Mollborg P, Erdes L, Aberg N and Wennergren G. Early fish introduction and neonatal antibiotics affect the risk of asthma into school age. *Pediatr Allergy Immunol*. 2013; 24 (4): 339-344.

Göksor E, Amark M, Alm B, Gustafsson PM and Wennergren G. The impact of pre- and post-natal smoke exposure on future asthma and bronchial hyper-responsiveness. *Acta Paediatr*. 2007; 96 (7): 1030-1035.

Haahtela T, Laatikainen T, Alenius H, Auvinen P, Fyhrquist N, Hanski I, von Hertzen L, Jousilahti P, Kosunen TU, Markelova O, Makela MJ, Pantelejev V, Uhanov M, Zilber E and Vartiainen E. Hunt for the origin of allergy - comparing the Finnish and Russian Karelia. *Clin Exp Allergy*. 2015; 45 (5): 891-901.

Hafkamp-de Groen E, Lingsma HF, Caudri D, Levie D, Wijga A, Koppelman GH, Duijts L, Jaddoe VW, Smit HA, Kerkhof M, Moll HA, Hofman A, Steyerberg EW, de Jongste JC and Raat H. Predicting asthma in preschool children with asthma-like symptoms: validating and updating the PIAMA risk score. *J Allergy Clin Immunol*. 2013; 132 (6): 1303-1310.

Haland G, Carlsen KH, Devulapalli CS, Pettersen M, Mowinckel P and Lodrup Carlsen KC. Lung function development in the first 2 yr of life is independent of allergic diseases by 2 yr. *Pediatr Allergy Immunol*. 2007; 18 (6): 528-534.

Hammond C, Kurten M and Kennedy JL. Rhinovirus and asthma: a storied history of incompatibility. *Curr Allergy Asthma Rep*. 2015; 15 (2): 502.

Hendaus MA, Jomha FA and Ehlayel M. Allergic diseases among children: nutritional prevention and intervention. *Ther Clin Risk Manag*. 2016; 12 361-372.

Henderson J, Hilliard TN, Sherriff A, Stalker D, Al Shammari N and Thomas HM. Hospitalization for RSV bronchiolitis before 12 months of age and subsequent asthma, atopy and wheeze: a longitudinal birth cohort study. *Pediatr Allergy Immunol*. 2005; 16 (5): 386-392.

Hermanns MI, Unger RE, Kehe K, Peters K and Kirkpatrick CJ. Lung epithelial cell lines in coculture with human pulmonary microvascular endothelial cells: development of an alveolo-capillary barrier in vitro. *Lab Invest*. 2004; 84 (6): 736-752.

Hershenson MB. Rhinovirus-Induced Exacerbations of Asthma and COPD. *Scientifica (Cairo)*. 2013; 2013 405876.

Hodinka RL. Respiratory RNA Viruses. *Microbiol Spectr*. 2016; 4 (4):

Holt PG, Rowe J, Kusel M, Parsons F, Hollams EM, Bosco A, McKenna K, Subrata L, de Klerk N, Serralha M, Holt BJ, Zhang G, Loh R, Ahlstedt S and Sly PD. Toward improved prediction of risk for atopy and asthma among preschoolers: a prospective cohort study. *J*

Allergy Clin Immunol. 2010; 125 (3): 653-659, 659 e651-659 e657.

Holt PG and Sly PD. Interaction between adaptive and innate immune pathways in the pathogenesis of atopic asthma: operation of a lung/bone marrow axis. *Chest*. 2011; 139 (5): 1165-1171.

Holt PG and Sly PD. Viral infections and atopy in asthma pathogenesis: new rationales for asthma prevention and treatment. *Nat Med*. 2012; 18 (5): 726-735.

Illi S, von Mutius E, Lau S, Bergmann R, Niggemann B, Sommerfeld C, Wahn U and Group MAS. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *BMJ*. 2001; 322 (7283): 390-395.

Illi S, von Mutius E, Lau S, Nickel R, Gruber C, Niggemann B, Wahn U and Multicenter Allergy Study G. The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. *J Allergy Clin Immunol*. 2004; 113 (5): 925-931.

Illi S, von Mutius E, Lau S, Niggemann B, Gruber C and Wahn U. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet*. 2006; 368 (9537): 763-770.

Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Pappas TE, Lee WM, Gern JE and Lemanske RF, Jr. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *Am J Respir Crit Care Med*. 2012; 185 (3): 281-285.

Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, Printz MC, Lee WM, Shult PA, Reisdorf E, Carlson-Dakes KT, Salazar LP, DaSilva DF, Tisler CJ, Gern JE and Lemanske RF, Jr. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med*. 2008; 178 (7): 667-672.

Jackson DJ, Gern JE and Lemanske RF, Jr. The contributions of allergic sensitization and respiratory pathogens to asthma inception. *J Allergy Clin Immunol*. 2016; 137 (3): 659-665.

Jackson DJ, Gern JE and Lemanske RF, Jr. Lessons learned from birth cohort studies conducted in diverse environments. *J Allergy Clin Immunol*. 2017; 139 (2): 379-386.

Jackson DJ, Guilbert TW, Evans MD, Gangnon RE, Roberg KA, Anderson EL, Pappas TE, Tisler CJ, DaSilva DF, Salazar LEP, Gern JE and Lemanske RF, Jr. Inclusion of Rhinovirus Wheezing History in Early Life Improves the Sensitivity of the Modified Asthma Predictive Index (mAPI). *J Allergy Clin Immunol*. 2009; 123 S82.

Jacobs SE, Lamson DM, St George K and Walsh TJ. Human rhinoviruses. *Clin Microbiol Rev*. 2013; 26 (1): 135-162.

Jakiela B, Brockman-Schneider R, Amineva S, Lee WM and Gern JE. Basal cells of differentiated bronchial epithelium are more susceptible to rhinovirus infection. *Am J Respir Cell Mol Biol*. 2008; 38 (5): 517-523.

James A and Hedlin G. Biomarkers for the Phenotyping and Monitoring of Asthma in Children. *Curr Treat Options Allergy*. 2016; 3 (4): 439-452.

Jartti T, Kuusipalo H, Vuorinen T, Söderlund-Venermo M, Allander T, Waris M, Hartiala J and Ruuskanen O. Allergic sensitization is associated with rhinovirus-, but not other virus-, induced wheezing in children. *Pediatr Allergy Immunol*. 2010; 21 (7): 1008-1014.

Jartti T, Lehtinen P, Vanto T, Hartiala J, Vuorinen T, Makela MJ and Ruuskanen O. Evaluation of the efficacy of prednisolone in early wheezing induced by rhinovirus or respiratory syncytial virus. *Pediatr Infect Dis J*. 2006; 25 (6): 482-488.

Jartti T, Lehtinen P, Vuorinen T and Ruuskanen O. Bronchiolitis: age and previous wheezing episodes are linked to viral etiology and atopic characteristics. *Pediatr Infect Dis J*. 2009; 28 (4): 311-317.

Jartti T, Lehtinen P, Vuorinen T, Österback R, van den Hoogen B, Osterhaus AD and Ruuskanen O. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. *Emerg Infect Dis*. 2004; 10 (6): 1095-1101.

Jartti T, Nieminen R, Vuorinen T, Lehtinen P, Vahlberg T, Gern J, Camargo CA, Jr. and Ruuskanen O. Short- and long-term efficacy of prednisolone for first acute rhinovirus-induced wheezing episode. *J Allergy Clin Immunol*. 2015; 135 (3): 691-698.

Jartti T, Soderlund-Venermo M, Hedman K, Ruuskanen O and Makela MJ. New molecular virus detection methods and their clinical value in lower respiratory tract infections in children. *Paediatr Respir Rev*. 2013; 14 (1): 38-45.

Jartti T, Söderlund-Venermo M, Hedman K, Ruuskanen O and Mäkelä M. New molecular virus detection methods and their clinical value in lower respiratory tract infections in children. *Paediatr. Respir. Rev.* 2012; in press.

Juntti H, Kokkonen J, Dunder T, Renko M, Niinimaki A and Uhari M. Association of an

early respiratory syncytial virus infection and atopic allergy. *Allergy*. 2003; 58 (9): 878-884.

Just J, Nicoloyanis N, Chauvin M, Pribil C, Grimfeld A and Duru G. Lack of eosinophilia can predict remission in wheezy infants? *Clin Exp Allergy*. 2008; 38 (5): 767-773.

Karlsson L, Nousiainen N, Scheinin NM, Maksimow M, Salmi M, Lehto SM, Tolvanen M, Lukkarinen H and Karlsson H. Cytokine profile and maternal depression and anxiety symptoms in mid-pregnancy-the FinnBrain Birth Cohort Study. *Arch Womens Ment Health*. 2017; 20 (1): 39-48.

Kelly JT and Busse WW. Host immune responses to rhinovirus: mechanisms in asthma. *J Allergy Clin Immunol*. 2008; 122 (4): 671-682; quiz 683-674.

Kennedy JL, Shaker M, McMeen V, Gern J, Carper H, Murphy D, Lee WM, Bochkov YA, Vrtis RF, Platts-Mills T, Patrie J, Borish L, Steinke JW, Woods WA and Heymann PW. Comparison of viral load in individuals with and without asthma during infections with rhinovirus. *Am J Respir Crit Care Med*. 2014; 189 (5): 532-539.

Koehoorn M, Karr CJ, Demers PA, Lencar C, Tamburic L and Brauer M. Descriptive epidemiological features of bronchiolitis in a population-based cohort. *Pediatrics*. 2008; 122 (6): 1196-1203.

Korppi M, Kotaniemi-Syrjänen A, Waris M, Vainionpää R and Reijonen TM. Rhinovirus-associated wheezing in infancy: comparison with respiratory syncytial virus bronchiolitis. *Pediatr Infect Dis J*. 2004; 23 (11): 995-999.

Korppi M, Reijonen T, Poysa L and Juntunen-Backman K. A 2- to 3-year outcome after bronchiolitis. *Am J Dis Child*. 1993; 147 (6): 628-631.

Kotaniemi-Syrjanen A, Reijonen TM, Korhonen K, Waris M, Vainionpaa R and Korppi M. Wheezing due to rhinovirus infection in infancy: Bronchial hyperresponsiveness at school age. *Pediatr Int*. 2008; 50 (4): 506-510.

Kotaniemi-Syrjanen A, Vainionpaa R, Reijonen TM, Waris M, Korhonen K and Korppi M. Rhinovirus-induced wheezing in infancy--the first sign of childhood asthma? *J Allergy Clin Immunol*. 2003; 111 (1): 66-71.

Kotaniemi-Syrjänen A, Vainionpää R, Reijonen TM, Waris M, Korhonen K and Korppi M. Rhinovirus-induced wheezing in infancy--the first sign of childhood asthma? *J Allergy Clin Immunol*. 2003; 111 (1): 66-71.

Krawiec ME, Westcott JY, Chu HW, Balzar S, Trudeau JB, Schwartz LB and Wenzel SE. Persistent wheezing in very young children is associated with lower respiratory inflammation. *Am J Respir Crit Care Med*. 2001; 163 (6): 1338-1343.

Kuiper S, Muris JW, Dompeling E, Kester AD, Wesseling G, Knottnerus JA and van Schayck CP. Interactive effect of family history and environmental factors on respiratory tract-related morbidity in infancy. *J Allergy Clin Immunol*. 2007; 120 (2): 388-395.

Kulig M, Bergmann R, Tacke U, Wahn U and Guggenmoos-Holzmann I. Long-lasting sensitization to food during the first two years precedes allergic airway disease. The MAS Study Group, Germany. *Pediatr Allergy Immunol*. 1998; 9 (2): 61-67.

Kurukulaaratchy RJ, Fenn M, Matthews S and Arshad SH. Characterisation of atopic and non-atopic wheeze in 10 year old children. *Thorax*. 2004; 59 (7): 563-568.

Kurukulaaratchy RJ, Matthews S, Holgate ST and Arshad SH. Predicting persistent disease among children who wheeze during early life. *Eur Respir J*. 2003; 22 (5): 767-771.

Kusel MM, de Klerk NH, Holt PG, Kebadze T, Johnston SL and Sly PD. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J*. 2006; 25 (8): 680-686.

Kusel MM, de Klerk NH, Kebadze T, Vohma V, Holt PG, Johnston SL and Sly PD. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol*. 2007; 119 (5): 1105-1110.

Kusel MM, Kebadze T, Johnston SL, Holt PG and Sly PD. Febrile respiratory illnesses in infancy and atopy are risk factors for persistent asthma and wheeze. *Eur Respir J*. 2012; 39 (4): 876-882.

Lai CK, Beasley R, Crane J, Foliaki S, Shah J and Weiland S. Global variation in the prevalence and severity of asthma symptoms: phase three of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax*. 2009; 64 (6): 476-483.

Lamson D, Renwick N, Kapoor V, Liu Z, Palacios G, Ju J, Dean A, St George K, Briese T and Lipkin WI. MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York State during 2004-2005. *J Infect Dis*. 2006; 194 (10): 1398-1402.

Lannero E, Wickman M, Pershagen G and Nordvall L. Maternal smoking during pregnancy increases the risk of recurrent wheezing during the first years of life (BAMSE). *Respir Res*. 2006; 7 3.

Lau S. What is new in the prevention of atopy and asthma? *Curr Opin Allergy Clin Immunol*. 2013; 13 (2): 181-186.

Lau S, Illi S, Sommerfeld C, Niggemann B, Volkel K, Madloch C, Gruber C, Nickel R, Forster J, Wahn U and Multicentre Allergy Study G. Transient early wheeze is not associated with impaired lung function in 7-yr-old children. *Eur Respir J*. 2003; 21 (5): 834-841.

Lee S, Nguyen MT, Currier MG, Jenkins JB, Strobert EA, Kajon AE, Madan-Lala R, Bochkov YA, Gern JE, Roy K, Lu X, Erdman DD, Spearman P and Moore ML. A polyvalent inactivated rhinovirus vaccine is broadly immunogenic in rhesus macaques. *Nat Commun*. 2016; 7 12838.

Lee W GJ. Rhinovirus. In Clinical Virology, Fourth Edition, 1143-1164; Whitley RJ Richman DD, Hayden FG. 2017. ASM Press, Washington DC. 10. 1128/9781555819439.ch27

Lehtinen P, Ruohola A, Vanto T, Vuorinen T, Ruuskanen O and Jartti T. Prednisolone reduces recurrent wheezing after a first wheezing episode associated with rhinovirus infection or eczema. *J Allergy Clin Immunol*. 2007; 119 (3): 570-575.

Lemanske RF, Jr. The childhood origins of asthma (COAST) study. *Pediatr Allergy Immunol*. 2002; 13 Suppl 15 38-43.

Lemanske RF, Jr., Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, Kirk CJ, Reisdorf E, Roberg KA, Anderson EL, Carlson-Dakes KT, Adler KJ, Gilbertson-White S, Pappas TE, Dasilva DF, Tisler CJ and Gern JE. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol*. 2005; 116 (3): 571-577.

Lessler J, Reich NG, Brookmeyer R, Perl TM, Nelson KE and Cummings DA. Incubation periods of acute respiratory viral infections: a systematic review. *Lancet Infect Dis*. 2009; 9 (5): 291-300.

Levy ML, Godfrey S, Irving CS, Sheikh A, Hanekom W, Bush A and Lachman P. Wheeze detection: recordings vs. assessment of physician and parent. *J Asthma*. 2004; 41 (8): 845-853.

Lodrup Carlsen KC, Pettersen M and Carlsen KH. Is bronchodilator response in 2-yr-old children associated with asthma risk factors? *Pediatr Allergy Immunol*. 2004; 15 (4): 323-330.

Lukkarinen M and Jartti T. The first rhinovirus-wheeze acts as a marker for later asthma in high-risk children. *J Allergy Clin Immunol*. 2016, 10.1016/j.jaci.2016.01.040

Lukkarinen MM, Koistinen AP, Turunen RM and Jartti TT. Toward Primary Prevention of Asthma: Role of Corticosteroids for the First Rhinovirus Wheeze. *Am J Respir Crit Care Med*. 2015; 192 (8): 1018-1019.

Luoto R, Ruuskanen O, Waris M, Kalliomaki M, Salminen S and Isolauri E. Prebiotic and probiotic supplementation prevents rhinovirus infections in preterm infants: a randomized, placebo-controlled trial. *J Allergy Clin Immunol*. 2014; 133 (2): 405-413.

Malmstrom K, Pitkaranta A, Carpen O, Pelkonen A, Malmberg LP, Turpeinen M, Kajosaari M, Sarna S, Lindahl H, Haahtela T and Makela MJ. Human rhinovirus in bronchial epithelium of infants with recurrent respiratory symptoms. *J Allergy Clin Immunol*. 2006; 118 (3): 591-596.

Mansbach JM, Clark S, Teach SJ, Gern JE, Piedra PA, Sullivan AF, Espinola JA and Camargo CA, Jr. Children Hospitalized with Rhinovirus Bronchiolitis Have Asthma-Like Characteristics. *J Pediatr*. 2016; 172 202-204 e201.

Mansbach JM, McAdam AJ, Clark S, Hain PD, Flood RG, Acholonu U and Camargo CA, Jr. Prospective multicenter study of the viral etiology of bronchiolitis in the emergency department. *Acad Emerg Med*. 2008; 15 (2): 111-118.

Mansbach JM, Piedra PA, Teach SJ, Sullivan AF, Forgey T, Clark S, Espinola JA, Camargo CA, Jr. and Investigators M-. Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. *Arch Pediatr Adolesc Med*. 2012; 166 (8): 700-706.

Marguet C, Lubrano M, Gueudin M, Le Roux P, Deschildre A, Forget C, Couderc L, Siret D, Donnou MD, Bubenheim M, Vabret A and Freymuth F. In very young infants severity of acute bronchiolitis depends on carried viruses. *PLoS One*. 2009; 4 (2): e4596.

Martinez FD. Heterogeneity of the association between lower respiratory illness in infancy and subsequent asthma. *Proc Am Thorac Soc*. 2005; 2 (2): 157-161.

Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M and Morgan WJ. Asthma and wheezing in the first six years of life. The

Group Health Medical Associates. *N Engl J Med*. 1995; 332 (3): 133-138.

Matricardi PM, Illi S, Gruber C, Keil T, Nickel R, Wahn U and Lau S. Wheezing in childhood: incidence, longitudinal patterns and factors predicting persistence. *Eur Respir J*. 2008; 32 (3): 585-592.

Meissner HC. Viral Bronchiolitis in Children. *N Engl J Med*. 2016; 374 (18): 1793-1794.

Melioli G, Marcomini L, Agazzi A, Bazurro G, Tosca M, Rossi GA, Minale P, Rossi R, Reggiardo G, Canonica GW and Passalacqua G. The IgE repertoire in children and adolescents resolved at component level: a cross-sectional study. *Pediatr Allergy Immunol*. 2012; 23 (5): 433-440.

Michel G, Silverman M, Strippoli MP, Zwahlen M, Brooke AM, Grigg J and Kuehni CE. Parental understanding of wheeze and its impact on asthma prevalence estimates. *Eur Respir J*. 2006; 28 (6): 1124-1130.

Midulla F, Pierangeli A, Cangiano G, Bonci E, Salvadei S, Scagnolari C, Moretti C, Antonelli G, Ferro V and Papoff P. Rhinovirus bronchiolitis and recurrent wheezing: one year follow-up. *Eur Respir J*. 2012; 39 (2): 396-402.

Midulla F, Scagnolari C, Bonci E, Pierangeli A, Antonelli G, De Angelis D, Berardi R and Moretti C. Respiratory syncytial virus, human bocavirus and rhinovirus bronchiolitis in infants. *Arch Dis Child*. 2010; 95 (1): 35-41.

Miller EK, Lu X, Erdman DD, Poehling KA, Zhu Y, Griffin MR, Hartert TV, Anderson LJ, Weinberg GA, Hall CB, Iwane MK, Edwards KM and New Vaccine Surveillance N. Rhinovirus-associated hospitalizations in young children. *J Infect Dis*. 2007; 195 (6): 773-781.

Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, Depner M, von Berg A, Bufe A, Rietschel E, Heinzmann A, Simma B, Frischer T, Willis-Owen SA, Wong KC, Illig T, Vogelberg C, Weiland SK, von Mutius E, Abecasis GR, Farrall M, Gut IG, Lathrop GM and Cookson WO. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature*. 2007; 448 (7152): 470-473.

Morgan WJ, Stern DA, Sherrill DL, Guerra S, Holberg CJ, Guilbert TW, Taussig LM, Wright AL and Martinez FD. Outcome of asthma and wheezing in the first 6 years of life: follow-up through adolescence. *Am J Respir Crit Care Med*. 2005; 172 (10): 1253-1258.

Murray CS, Woodcock A, Langley SJ, Morris J, Custovic A and team Is. Secondary prevention of asthma by the use of Inhaled Fluticasone propionate in Wheezy INfants (IFWIN): double-blind, randomised, controlled study. *Lancet*. 2006; 368 (9537): 754-762.

NAEPP. National Asthma Education and Prevention Program. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. *J Allergy Clin Immunol*. 2007; 120 (5 Suppl): S94-138.

Nagarkar DR, Bowman ER, Schneider D, Wang Q, Shim J, Zhao Y, Linn MJ, McHenry CL, Gosangi B, Bentley JK, Tsai WC, Sajjan US, Lukacs NW and Hershenson MB. Rhinovirus infection of allergen-sensitized and -challenged mice induces eotaxin release from functionally polarized macrophages. *J Immunol*. 2010; 185 (4): 2525-2535.

Nagel G, Buchele G, Weinmayr G, Bjorksten B, Chen YZ, Wang H, Nystad W, Saraclar Y, Braback L, Batlles-Garrido J, Garcia-Hernandez G, Weiland SK and Group IPIS. Effect of breastfeeding on asthma, lung function and bronchial hyperreactivity in ISAAC Phase II. *Eur Respir J*. 2009; 33 (5): 993-1002.

Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, O'Brien KL, Roca A, Wright PF, Bruce N, Chandran A, Theodoratou E, Sutanto A, Sedyaningsih ER, Ngama M, Munywoki PK, Kartasasmita C, Simoes EA, Rudan I, Weber MW and Campbell H. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet*. 2010; 375 (9725): 1545-1555.

Nakagome K, Bochkov YA, Ashraf S, Brockman-Schneider RA, Evans MD, Pasic TR and Gern JE. Effects of rhinovirus species on viral replication and cytokine production. *J Allergy Clin Immunol*. 2014; 134 (2): 332-341.

Nascimento MS, Souza AV, Ferreira AV, Rodrigues JC, Abramovici S and Silva Filho LV. High rate of viral identification and coinfections in infants with acute bronchiolitis. *Clinics (Sao Paulo)*. 2010; 65 (11): 1133-1137.

Neuman A, Hohmann C, Orsini N, Pershagen G, Eller E, Kjaer HF, Gehring U, Granell R, Henderson J, Heinrich J, Lau S, Nieuwenhuijsen M, Sunyer J, Tischer C, Torrent M, Wahn U, Wijga AH, Wickman M, Keil T, Bergstrom A and Consortium E. Maternal smoking in pregnancy and asthma in preschool children: a pooled analysis of eight birth cohorts. *Am J Respir Crit Care Med*. 2012; 186 (10): 1037-1043.

Nieto A, Wahn U, Bufe A, Eigenmann P, Halken S, Hedlin G, Host A, Hourihane J, Just J, Lack G, Lau S, Matricardi PM, Muraro A, Papadopoulos N, Roberts G, Simpson A, Valovirta E, Weidinger S, Wickman M and Mazon A. Allergy and asthma prevention 2014. *Pediatr Allergy Immunol*. 2014; 25 (6): 516-533.

Nissen SP, Kjaer HF, Host A, Nielsen J and Halken S. The natural course of sensitization and allergic diseases from childhood to adulthood. *Pediatr Allergy Immunol*. 2013; 24 (6): 549-555.

Oddy WH, de Klerk NH, Sly PD and Holt PG. The effects of respiratory infections, atopy, and breastfeeding on childhood asthma. *Eur Respir J*. 2002; 19 (5): 899-905.

Panickar J, Lakhanpaul M, Lambert PC, Kenia P, Stephenson T, Smyth A and Grigg J. Oral prednisolone for preschool children with acute virus-induced wheezing. *N Engl J Med*. 2009; 360 (4): 329-338.

Papadopoulos NG, Arakawa H, Carlsen KH, Custovic A, Gern J, Lemanske R, Le Souef P, Makela M, Roberts G, Wong G, Zar H, Akdis CA, Bacharier LB, Baraldi E, van Bever HP, de Blic J, Boner A, Burks W, Casale TB, Castro-Rodriguez JA, Chen YZ, El-Gamal YM, Everard ML, Frischer T, Geller M, Gereda J, Goh DY, Guilbert TW, Hedlin G, Heymann PW, Hong SJ, Hossny EM, Huang JL, Jackson DJ, de Jongste JC, Kalayci O, Ait-Khaled N, Kling S, Kuna P, Lau S, Ledford DK, Lee SI, Liu AH, Lockey RF, Lodrup-Carlsen K, Lotvall J, Morikawa A, Nieto A, Paramesh H, Pawankar R, Pohunek P, Pongracic J, Price D, Robertson C, Rosario N, Rossenwasser LJ, Sly PD, Stein R, Stick S, Szefler S, Taussig LM, Valovirta E, Vichyanond P, Wallace D, Weinberg E, Wennergren G, Wildhaber J and Zeiger RS. International consensus on (ICON) pediatric asthma. *Allergy*. 2012; 67 (8): 976-997.

Papi A and Johnston SL. Rhinovirus infection induces expression of its own receptor intercellular adhesion molecule 1 (ICAM-1) via increased NF-kappaB-mediated transcription. *J Biol Chem*. 1999; 274 (14): 9707-9720.

Papi A, Papadopoulos NG, Degitz K, Holgate ST and Johnston SL. Corticosteroids inhibit rhinovirus-induced intercellular adhesion molecule-1 up-regulation and promoter activation on respiratory epithelial cells. *J Allergy Clin Immunol*. 2000; 105 (2 Pt 1): 318-326.

Pattenden S, Antova T, Neuberger M, Nikiforov B, De Sario M, Grize L, Heinrich J, Hruba F, Janssen N, Luttmann-Gibson H, Privalova L, Rudnai P, Splichalova A, Zlotkowska R and Fletcher T. Parental smoking and children's respiratory health: independent effects of prenatal and postnatal exposure. *Tob Control*. 2006; 15 (4): 294-301.

Pekkanen J, Remes ST, Husman T, Lindberg M, Kajosaari M, Koivikko A and Soininen L. Prevalence of asthma symptoms in video and written questionnaires among children in four regions of Finland. *Eur Respir J*. 1997; 10 (8): 1787-1794.

Piippo-Savolainen E and Korppi M. Wheezy babies--wheezy adults? Review on long-term outcome until adulthood after early childhood wheezing. *Acta Paediatr*. 2008; 97 (1): 5-11.

Piippo-Savolainen E, Remes S and Korppi M. Does early exposure or sensitization to inhalant allergens predict asthma in wheezing infants? A 20-year follow-up. *Allergy Asthma Proc*. 2007; 28 (4): 454-461.

Plint AC, Johnson DW, Patel H, Wiebe N, Correll R, Brant R, Mitton C, Gouin S, Bhatt M, Joubert G, Black KJ, Turner T, Whitehouse S and Klassen TP. Epinephrine and dexamethasone in children with bronchiolitis. *N Engl J Med*. 2009; 360 (20): 2079-2089.

Prabhu N, Smith N, Campbell D, Craig LC, Seaton A, Helms PJ, Devereux G and Turner SW. First trimester maternal tobacco smoking habits and fetal growth. *Thorax*. 2010; 65 (3): 235-240.

Rakes GP, Arruda E, Ingram JM, Hoover GE, Zambrano JC, Hayden FG, Platts-Mills TA and Heymann PW. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. *Am J Respir Crit Care Med*. 1999; 159 (3): 785-790.

Ralston SL, Lieberthal AS, Meissner HC, Alverson BK, Baley JE, Gadomski AM, Johnson DW, Light MJ, Maraqa NF, Mendonca EA, Phelan KJ, Zorc JJ, Stanko-Lopp D, Brown MA, Nathanson I, Rosenblum E, Sayles S, 3rd, Hernandez-Cancio S and American Academy of P. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. *Pediatrics*. 2014; 134 (5): e1474-1502.

Rappo U, Schuetz AN, Jenkins SG, Calfee DP, Walsh TJ, Wells MT, Hollenberg JP and Glesby MJ. Impact of Early Detection of Respiratory Viruses by Multiplex PCR Assay on Clinical Outcomes in Adult Patients. *J Clin Microbiol*. 2016; 54 (8): 2096-2103.

Rautava S, Luoto R, Salminen S and Isolauri E. Microbial contact during pregnancy, intestinal

colonization and human disease. *Nat Rev Gastroenterol Hepatol*. 2012; 9 (10): 565-576.

Rautava S, Salminen S and Isolauri E. Specific probiotics in reducing the risk of acute infections in infancy--a randomised, double-blind, placebo-controlled study. *Br J Nutr*. 2009; 101 (11): 1722-1726.

Reddel HK, Bateman ED, Becker A, Boulet LP, Cruz AA, Drazen JM, Haahtela T, Hurd SS, Inoue H, de Jongste JC, Lemanske RF, Jr., Levy ML, O'Byrne PM, Paggiaro P, Pedersen SE, Pizzichini E, Soto-Quiroz M, Szefler SJ, Wong GW and FitzGerald JM. A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J*. 2015; 46 (3): 622-639.

Reijonen TM and Korppi M. One-year follow-up of young children hospitalized for wheezing: the influence of early anti-inflammatory therapy and risk factors for subsequent wheezing and asthma. *Pediatr Pulmonol*. 1998; 26 (2): 113-119.

Renwick N, Schweiger B, Kapoor V, Liu Z, Villari J, Bullmann R, Miething R, Briese T and Lipkin WI. A recently identified rhinovirus genotype is associated with severe respiratory-tract infection in children in Germany. *J Infect Dis*. 2007; 196 (12): 1754-1760.

Rollinger JM and Schmidtke M. The human rhinovirus: human-pathological impact, mechanisms of antirhinoviral agents, and strategies for their discovery. *Med Res Rev*. 2011; 31 (1): 42-92.

Rossi GA and Colin AA. Infantile respiratory syncytial virus and human rhinovirus infections: respective role in inception and persistence of wheezing. *Eur Respir J*. 2015; 45 (3): 774-789.

Royston L and Tapparel C. Rhinoviruses and Respiratory Enteroviruses: Not as Simple as ABC. *Viruses*. 2016; 8 (1):

Rubner FJ, Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Pappas TE, Gern JE and Lemanske RF, Jr. Early life rhinovirus wheezing, allergic sensitization, and asthma risk at adolescence. *J Allergy Clin Immunol*. 2017; 139 (2): 501-507.

Ruokolainen L, von Hertzen L, Fyhrquist N, Laatikainen T, Lehtomaki J, Auvinen P, Karvonen AM, Hyvarinen A, Tillmann V, Niemela O, Knip M, Haahtela T, Pekkanen J and Hanski I. Green areas around homes reduce atopic sensitization in children. *Allergy*. 2015; 70 (2): 195-202.

Russell CD, Unger SA, Walton M and Schwarze J. The Human Immune Response to Respiratory Syncytial Virus Infection. *Clin Microbiol Rev*. 2017; 30 (2): 481-502.

Rönmark E, Jönsson E, Platts-Mills T and Lundbäck B. Different pattern of risk factors for atopic and nonatopic asthma among children--report from the Obstructive Lung Disease in Northern Sweden Study. *Allergy*. 1999; 54 (9): 926-935.

Saglani S, Malmstrom K, Pelkonen AS, Malmberg LP, Lindahl H, Kajosaari M, Turpeinen M, Rogers AV, Payne DN, Bush A, Haahtela T, Makela MJ and Jeffery PK. Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. *Am J Respir Crit Care Med*. 2005; 171 (7): 722-727.

Saglani S, Payne DN, Zhu J, Wang Z, Nicholson AG, Bush A and Jeffery PK. Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. *Am J Respir Crit Care Med*. 2007; 176 (9): 858-864.

Savolainen-Kopra C, Korpela T, Simonen-Tikka ML, Amiryousefi A, Ziegler T, Roivainen M and Hovi T. Single treatment with ethanol hand rub is ineffective against human rhinovirus--hand washing with soap and water removes the virus efficiently. *J Med Virol*. 2012; 84 (3): 543-547.

Schroeder AR, Mansbach JM, Stevenson M, Macias CG, Fisher ES, Barcega B, Sullivan AF, Espinola JA, Piedra PA and Camargo CA, Jr. Apnea in children hospitalized with bronchiolitis. *Pediatrics*. 2013; 132 (5): e1194-1201.

Schuttelaar ML, Kerkhof M, Jonkman MF, Koppelman GH, Brunekreef B, de Jongste JC, Wijga A, McLean WH and Postma DS. Filaggrin mutations in the onset of eczema, sensitization, asthma, hay fever and the interaction with cat exposure. *Allergy*. 2009; 64 (12): 1758-1765.

Scottish Intercollegiate Guidelines Network (SIGN). Bronchiolitis in children. A national clinical guideline. 2006.

Sigurs N, Aljassim F, Kjellman B, Robinson PD, Sigurbergsson F, Bjarnason R and Gustafsson PM. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. *Thorax*. 2010; 65 (12): 1045-1052.

Sigurs N, Bjarnason R, Sigurbergsson F and Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med*. 2000; 161 (5): 1501-1507.

Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B and Bjorksten B. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective

cohort study with matched controls. *Pediatrics*. 1995; 95 (4): 500-505.

Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F and Kjellman B. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med*. 2005; 171 (2): 137-141.

Simoes EA, Carbonell-Estrany X, Rieger CH, Mitchell I, Fredrick L, Groothuis JR and Palivizumab Long-Term Respiratory Outcomes Study G. The effect of respiratory syncytial virus on subsequent recurrent wheezing in atopic and nonatopic children. *J Allergy Clin Immunol*. 2010; 126 (2): 256-262.

Simoes EA, Groothuis JR, Carbonell-Estrany X, Rieger CH, Mitchell I, Fredrick LM, Kimpen JL and Palivizumab Long-Term Respiratory Outcomes Study G. Palivizumab prophylaxis, respiratory syncytial virus, and subsequent recurrent wheezing. *J Pediatr*. 2007; 151 (1): 34-42, 42 e31.

Smedberg J, Lupattelli A, Mardby AC and Nordeng H. Characteristics of women who continue smoking during pregnancy: a cross-sectional study of pregnant women and new mothers in 15 European countries. *BMC Pregnancy Childbirth*. 2014; 14 213.

Smyth RL and Openshaw PJ. Bronchiolitis. *Lancet*. 2006; 368 (9532): 312-322.

Spergel JM and Paller AS. Atopic dermatitis and the atopic march. *J Allergy Clin Immunol*. 2003; 112 (6 Suppl): S118-127.

Stein RT and Martinez FD. Respiratory syncytial virus and asthma: still no final answer. *Thorax*. 2010; 65 (12): 1033-1034.

Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, Wright AL and Martinez FD. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet*. 1999; 354 (9178): 541-545.

Stellato C. Glucocorticoid actions on airway epithelial responses in immunity: functional outcomes and molecular targets. *J Allergy Clin Immunol*. 2007; 120 (6): 1247-1263; quiz 1264-1245.

Stensballe LG, Simonsen J, Jensen SM, Bonnelykke K and Bisgaard H. Use of antibiotics during pregnancy increases the risk of asthma in early childhood. *J Pediatr*. 2013; 162 (4): 832-838 e833.

Stensballe LG, Simonsen JB, Thomsen SF, Larsen AM, Lysdal SH, Aaby P, Kyvik KO, Skytthe A, Backer V and Bisgaard H. The causal direction in the association between respiratory syncytial virus hospitalization and asthma. *J Allergy Clin Immunol*. 2009; 123 (1): 131-137 e131.

Stone CA, Jr. and Miller EK. Understanding the Association of Human Rhinovirus with Asthma. *Clin Vaccine Immunol*. 2015; 23 (1): 6-10.

Strina A, Barreto ML, Cooper PJ and Rodrigues LC. Risk factors for non-atopic asthma/wheeze in children and adolescents: a systematic review. *Emerg Themes Epidemiol*. 2014; 11 5.

Subramony A, Zachariah P, Krones A, Whittier S and Saiman L. Impact of Multiplex Polymerase Chain Reaction Testing for Respiratory Pathogens on Healthcare Resource Utilization for Pediatric Inpatients. *J Pediatr*. 2016; 173 196-201 e192.

Svanes C, Omenaas E, Jarvis D, Chinn S, Gulsvik A and Burney P. Parental smoking in childhood and adult obstructive lung disease: results from the European Community Respiratory Health Survey. *Thorax*. 2004; 59 (4): 295-302.

Sykes A, Edwards MR, Macintyre J, Del Rosario A, Bakhsoliani E, Trujillo-Torralbo MB, Kon OM, Mallia P, McHale M and Johnston SL. Rhinovirus 16-induced IFN-alpha and IFN-beta are deficient in bronchoalveolar lavage cells in asthmatic patients. *J Allergy Clin Immunol*. 2012; 129 (6): 1506-1514 e1506.

Szefler SJ. Advances in pediatric asthma in 2013: coordinating asthma care. *J Allergy Clin Immunol*. 2014; 133 (3): 654-661.

Söderlund-Venermo M, Lahtinen A, Jartti T, Hedman L, Kemppainen K, Lehtinen P, Allander T, Ruuskanen O and Hedman K. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. *Emerg Infect Dis*. 2009; 15 (9): 1423-1430.

Tal A, Levy N and Bearman JE. Methylprednisolone therapy for acute asthma in infants and toddlers: a controlled clinical trial. *Pediatrics*. 1990; 86 (3): 350-356.

Tantisira KG, Hwang ES, Raby BA, Silverman ES, Lake SL, Richter BG, Peng SL, Drazen JM, Glimcher LH and Weiss ST. TBX21: a functional variant predicts improvement in asthma with the use of inhaled corticosteroids. *Proc Natl Acad Sci U S A*. 2004; 101 (52): 18099-18104.

Tantisira KG, Lasky-Su J, Harada M, Murphy A, Litonjua AA, Himes BE, Lange C, Lazarus R, Sylvia J, Klanderman B, Duan QL, Qiu W, Hirota T, Martinez FD, Mauger D, Sorkness C, Szefler S, Lazarus SC, Lemanske RF, Jr., Peters SP, Lima JJ, Nakamura Y, Tamari M

and Weiss ST. Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. *N Engl J Med*. 2011; 365 (13): 1173-1183.

Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ and Martinez FD. Tucson Children's Respiratory Study: 1980 to present. *J Allergy Clin Immunol*. 2003; 111 (4): 661-675; quiz 676.

Teach SJ, Gill MA, Togias A, Sorkness CA, Arbes SJ, Jr., Calatroni A, Wildfire JJ, Gergen PJ, Cohen RT, Pongracic JA, Kercsmar CM, Khurana Hershey GK, Gruchalla RS, Liu AH, Zoratti EM, Kattan M, Grindle KA, Gern JE, Busse WW and Szefler SJ. Preseasonal treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma exacerbations. *J Allergy Clin Immunol*. 2015; 136 (6): 1476-1485.

Thomsen SF, van der Sluis S, Stensballe LG, Posthuma D, Skytthe A, Kyvik KO, Duffy DL, Backer V and Bisgaard H. Exploring the association between severe respiratory syncytial virus infection and asthma: a registry-based twin study. *Am J Respir Crit Care Med*. 2009; 179 (12): 1091-1097.

Thumerelle C, Deschildre A, Bouquillon C, Santos C, Sardet A, Scalbert M, Delbecque L, Debray P, Dewilde A, Turck D and Leclerc F. Role of viruses and atypical bacteria in exacerbations of asthma in hospitalized children: a prospective study in the Nord-Pas de Calais region (France). *Pediatr Pulmonol*. 2003; 35 (2): 75-82.

Tregoning JS and Schwarze J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clin Microbiol Rev*. 2010; 23 (1): 74-98.

Turner SW, Palmer LJ, Rye PJ, Gibson NA, Judge PK, Cox M, Young S, Goldblatt J, Landau LI and Le Souef PN. The relationship between infant airway function, childhood airway responsiveness, and asthma. *Am J Respir Crit Care Med*. 2004; 169 (8): 921-927.

Turner SW, Young S, Goldblatt J, Landau LI and Le Souef PN. Childhood asthma and increased airway responsiveness: a relationship that begins in infancy. *Am J Respir Crit Care Med*. 2009; 179 (2): 98-104.

Turunen R, Jartti T, Bochkov YA, Gern JE and Vuorinen T. Rhinovirus species and clinical characteristics in the first wheezing episode in children. *J Med Virol*. 2016a; 88 (12): 2059-2068.

Turunen R, Koistinen A, Vuorinen T, Arku B, Söderlund-Venermo M, Ruuskanen O and Jartti T. The first wheezing episode: respiratory virus etiology, atopic characteristics, and illness severity. *Pediatr Allergy Immunol*. 2014; 25 (8): 796-803.

Turunen R, Vuorinen T, Bochkov Y, Gern J and Jartti T. Clinical and Virus Surveillance After the First Wheezing Episode: Special Reference to Rhinovirus A and C Species. *Pediatr Infect Dis J*. 2016b, 10.1097/INF.0000000000001495

Valkonen H, Waris M, Ruohola A, Ruuskanen O and Heikkinen T. Recurrent wheezing after respiratory syncytial virus or non-respiratory syncytial virus bronchiolitis in infancy: a 3-year follow-up. *Allergy*. 2009; 64 (9): 1359-1365.

van der Hulst AE, Klip H and Brand PL. Risk of developing asthma in young children with atopic eczema: a systematic review. *J Allergy Clin Immunol*. 2007; 120 (3): 565-569.

van Gageldonk-Lafeber AB, Heijnen ML, Bartelds AI, Peters MF, van der Plas SM and Wilbrink B. A case-control study of acute respiratory tract infection in general practice patients in The Netherlands. *Clin Infect Dis*. 2005; 41 (4): 490-497.

Vandini S, Calamelli E, Faldella G and Lanari M. Immune and inflammatory response in bronchiolitis due to respiratory Syncytial Virus and Rhinovirus infections in infants. *Paediatr Respir Rev*. 2017, 10.1016/j.prrv.2016.11.006

von Hertzen L, Beutler B, Bienenstock J, Blaser M, Cani PD, Eriksson J, Farkkila M, Haahtela T, Hanski I, Jenmalm MC, Kere J, Knip M, Kontula K, Koskenvuo M, Ling C, Mandrup-Poulsen T, von Mutius E, Makela MJ, Paunio T, Pershagen G, Renz H, Rook G, Saarela M, Vaarala O, Veldhoen M and de Vos WM. Helsinki alert of biodiversity and health. *Ann Med*. 2015; 47 (3): 218-225.

von Mutius E. The shape of the microbiome in early life. *Nat Med*. 2017; 23 (3): 274-275.

Wahn U, Bergmann R, Kulig M, Forster J and Bauer CP. The natural course of sensitisation and atopic disease in infancy and childhood. *Pediatr Allergy Immunol*. 1997; 8 (10 Suppl): 16-20.

Waris M. Pattern of respiratory syncytial virus epidemics in Finland: two-year cycles with alternating prevalence of groups A and B. *J Infect Dis*. 1991; 163 (3): 464-469.

Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, Holgate ST and Davies DE. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med*. 2005; 201 (6): 937-947.

Wawrzyniak P, Akdis CA, Finkelman FD and Rothenberg ME. Advances and highlights in mechanisms of allergic disease in 2015. *J Allergy Clin Immunol*. 2016; 137 (6): 1681-1696.

Wennergren G, Hansson S, Engstrom I, Jodal U, Amark M, Brolin I and Juto P. Characteristics and prognosis of hospital-treated obstructive bronchitis in children aged less than two years. *Acta Paediatr*. 1992; 81 (1): 40-45.

Williams JV, Piedra PA and Englund JA. Respiratory syncytial virus, human metapneumovirus and parainfluenza viruses. In Clinical Virology, Fourth Edition, 873-902; Whitley RJ Ricman DD, Hayden FG. 2017. ASM Press, Washington DC. 10. 1128/9781555819439.ch27

Wilson NM, Lamprill JR, Mak JC, Clarke JR, Bush A and Silverman M. Symptoms, lung function, and beta2-adrenoceptor polymorphisms in a birth cohort followed for 10 years. *Pediatr Pulmonol*. 2004; 38 (1): 75-81.

Winther B, McCue K, Ashe K, Rubino J and Hendley JO. Rhinovirus contamination of surfaces in homes of adults with natural colds: transfer of virus to fingertips during normal daily activities. *J Med Virol*. 2011; 83 (5): 906-909.

Wisniewski JA, Agrawal R, Minnicozzi S, Xin W, Patrie J, Heymann PW, Workman L, Platts-Mills TA, Song TW, Moloney M and Woodfolk JA. Sensitization to food and inhalant allergens in relation to age and wheeze among children with atopic dermatitis. *Clin Exp Allergy*. 2013; 43 (10): 1160-1170.

Wolsk HM, Harshfield BJ, Laranjo N, Carey VJ, O'Connor G, Sandel M, Strunk RC, Bacharier LB, Zeiger RS, Schatz M, Hollis BW, Weiss ST and Litonjua AA. Vitamin D supplementation in pregnancy, prenatal 25(OH)D levels, race, and subsequent asthma or recurrent wheeze in offspring: Secondary analyses from the Vitamin D Antenatal Asthma Reduction Trial. *J Allergy Clin Immunol*. 2017, 10.1016/j.jaci.2017.01.013

Wongtrakool C, Roser-Page S, Rivera HN and Roman J. Nicotine alters lung branching morphogenesis through the alpha7 nicotinic acetylcholine receptor. *Am J Physiol Lung Cell Mol Physiol*. 2007; 293 (3): L611-618.

Wu P, Dupont WD, Griffin MR, Carroll KN, Mitchel EF, Gebretsadik T and Hartert TV. Evidence of a causal role of winter virus infection during infancy in early childhood asthma. *Am J Respir Crit Care Med*. 2008; 178 (11): 1123-1129.

Wu P, Feldman AS, Rosas-Salazar C, James K, Escobar G, Gebretsadik T, Li SX, Carroll KN, Walsh E, Mitchel E, Das S, Kumar R, Yu C, Dupont WD and Hartert TV. Relative Importance and Additive Effects of Maternal and Infant Risk Factors on Childhood Asthma. *PLoS One*. 2016; 11 (3): e0151705.

APPENDICES

Appendix 1. PARENTAL QUESTIONNAIRE ON ADMISSION FOR VINKU-STUDY

1. Does your child have an out-patient family paediatrician?
2. 1) No 2) Yes, Dr _____ practicing in _____
3. Day care?
   Home Family day care Day care center
   Other, what? _____
4. Home
   House Apartment building Row house
   Farm Other, what? _____
5. Number of children in the family _____
6. Parents´ smoking? 1) No 2) Yes if they smoke, do they smoke
   inside the home 1) No 2) Yes
   in the car 1) No 2) Yes
7. Is there at home
   dog 1) No 2) Yes
   cat 1) No 2) Yes
   other animals 1) No 2)Yes,
   what?_____
   feather pillows/blankets 1) No 2) Yes
   fitted carpet 1) No 2) Yes
8. Are there at day care?
   pets/animals 1) No 2) Yes what?_____
   smoking 1) No 2) Yes
9. Does the child visit weekly places where there are
   animals 1) No 2) Yes
   smoking 1) No 2) Yes
10. Are there other family members with allergic symptoms?
    eczema 1) No 2) Yes
    mother/ father/ sisters
    rhinitis 1) No 2) Yes
    mother/ father/ sisters
    asthma 1) No 2) Yes,
    mother/ father/ sisters
11. Does your child (the one in this study) have allergic symptoms? Please, mark the <u>suspected</u> source on the reverse side.
    eczema 1) No 2) Yes
    rhinitis 1) No 2) Yes
    asthmatic 1) No 2) Yes
12. Does your child have an "allergy diet"?1) No 2)Yes. Please, specify the diet to study nurse.
13. Have the skin prick tests been done to your child?
    1) No 2) Yes
    when __/___(mo/yr),
    where _____
14. <u>Information about **allergies** (please circle the suspected sources):</u>
    Dietary; chocolate, cocoa, citrus, egg, fish, tomato, strawberry, pea, apple, carrot, nuts, pear, peach, cow´s milk, breast milk substitute, rye, barley, oats, wheat
    other _____
    Animals; dog, cat, horse, cow, guinea pig, feather
    other _____
    Pollen; birch, alder, conifer, hay, mugworth
    other _____
    Other causes; room dust, fungal spore
    other _____
15. <u>Information about the child´s **respiratory infections**:</u>
    During the **<u>last 12 months</u>**:
    "common cold" _____ times
    antibiotic prescription _____ times
    pneumonias _____ times
    bronchitis _____ times
    otitis _____ times
    parasenthesis _____ times
    other, what? _____
    Adenoidectomy 1) No 2) Yes,
    when __/___ (mo/yr), where _____
    Parasenthesis 1) No 2) Yes,
    when __/___ (mo/yr), where _____

16. Information about **breathing difficulty symptoms**:
Were there "common cold" symptoms during the **current** difficulty in breathing?
1) No 2) Yes 3) I can not say
If you suspect other causes, please name them;
_____ _____ _____
How long was the difficulty in breathing before admission to hospital acute care?_____ hours.
Have other family members had "common cold" symptoms?
1) No 2) Yes

17. How many difficulties in breathing has your child had during the life (**including the current one**) ___
The first difficulty was __/___ (mo/yr)

During the **last 12 months**:
How many times has your child had difficulties in breathing? _____ times
How many times has the child been to hospital because of breathing difficulties?
at the hospital acute care _____ times
on the hospital ward _____ times
Breathing difficulties have previously appeared only with the "common cold"? 1) No 2) Yes
If you suspect other causes, please name them; _____, _____, _____
Does your child have a booked appointment to a pediatrician for his/hers breathing difficulty?
Date__/___/____, where _____

18. Does your child have the daily/ regular asthma medication?
1) No 2) Yes, what?

medicine dose/day divice (f ex Spira, Babyhaler) Started (mo/yr) Where
_____ _____ _____ __/___ _____
_____ _____ _____ __/___ _____

19. Information about the **prolonged cough/ asthma**:
How old was your child when the symptoms started? _____ years.
The child has had prolonged (=over 4 weeks)
cough or coughing attacks 1) No 2) Yes
wheezy breathing 1) No 2) Yes
difficulty in breathing 1) No 2) Yes
nocturnal cough 1) No 2) Yes

The child has symptoms
during the "common cold" 1) No 2) Yes
in contact with animals/pollen 1) No 2) Yes
in contact smoking 1) No 2) Yes
in exercise 1) No 2) Yes
when laughing/crying 1) No 2) Yes
when cold outside 1) No 2) Yes

How many times has your child had symptoms during the last 12 months?
1-4 times 1) No 2) Yes
4-6 times 1) No 2) Yes
monthly 1) No 2) Yes
weekly 1) No 2) Yes
daily 1) No 2) Yes
often nightly 1) No 2) Yes

20. Information about **rash/eczema**:
Eczema started at (age) _____.
Eczema appeared first time in
face 1) No 2) Yes
bends 1) No 2) Yes
other location, where? _____
Eczema is currently situated in
bends 1) No 2) Yes
other location, where? _____
Eczema appears year-round? 1) No 2) Yes
Eczema gets worse in certain time-of-year? 1) No 2) Yes
Eczema disappears periodically? 1) No 2) Yes
Eczema gets worse with
swetting 1) No 2) Yes
food 1) No 2) Yes
colourants in clothing 1) No 2) Yes
sauna 1) No 2) Yes
other, what? _____
Eczema is itching at nighttime? 1) No 2) Yes

Appendix 2. PARENTAL QUESTIONNAIRE ON ADMISSION FOR VINKU2-STUDY

The key questions*
To be filled by study physician at parental interview
Name: _____
Social security number: _____
Names of the parents / guardians: _____
Address:_____
Phone: _____
Email: _____
Does the child fulfill inclusion criteria of the study: age 3-23 months, ≥h37+0, first episode of breathing difficulty and written informed consent from the parents? Yes □ No □
Does the child fulfill inclusion criteria of the intervention trial: rhinovirus PCR positive and still signs of lower respiratory infection (breathing difficulty, noisy breathing or cough)?
Yes □ No □
Randomized to receive the study drug: Yes □ No □
If yes, when (day, time) _____
Any exclusion criteria: chronic other than atopy related illness, previous systemic or inhaled corticosteroid treatment, participation to another study (excluding long-term follow-up studies in childhood), varicella contact if previously intact, need for intensive care unit treatment, or poor understanding of Finnish No □
Parents / guardians have received routine hospital wheezy questionnaires (2 forms) and symptom diaries (3 forms): Yes □
Height _____ cm and weight _____ kg
Still breastfeeding Yes □ No □
Duration of breastfeeding ___ months
Duration of exclusive breastfeeding ___ months
Does the child have doctor-diagnosed atopic eczema: Yes □ No □

	Mother	Father
Doctor-diagnosed asthma:	Yes □ No □	Yes □ No □
Allergic rhinitis:	Yes □ No □	Yes □ No □
Smoking:	Yes □ No □	Yes □ No □
Furry pets:		Yes □ No □

Number of children in the family: ___ children
Daycare: Home □ Small group □ Kindergarden □

Wheezy questionnaire*
To be filled by a parent/guardian

1. Does your child have a family doctor? No □ Yes □,
Dr _____ practicing in _____

2. Type of daycare?
1) Home □ 2) Family day care □ 3) Day care center □ 4) Other □, what? _____

3. Type of home?
1) Apartment building □ 2) House □ 3) Row house □ 4) Farm □ 5) Other □, what? _____

4. Number of children in the family? ____

5. Parental smoking? No □ Yes □, if yes, smoking:
   1) inside No □ Yes □
   2) in the car No □ Yes □

6. Pets at home?
   dog No □ Yes □
   cat No □ Yes □
   other animals No □ Yes □,
   what? _____

7. Other allergen sources at home?
   feather pillows/blankets No □ Yes □
   fitted carpet No □ Yes □

8. At day care
   pets/animals? No □ Yes □,
   what? _____
   smoking? No □ Yes □

9. At other places, weekly exposure to
   animals? No □ Yes □
   smoking? No □ Yes □

10. Are there allergic symptoms in the family?
    eczema No □ Yes □, underline: mother / father / sibling
    rhinitis No □ Yes □, underline: mother / father / sibling
    asthma No □ Yes □, underline: mother / father / sibling

11. Does the child have allergic symptoms? Please, mark the suspected source on the reverse side.
    eczema No □ Yes □
    rhinitis No □ Yes □
    asthma No □ Yes □

12. Does your child have an "allergy diet"?
    No □ Yes □ . Please, specify the diet to the study nurse.

13. Has your child ever undergone skin prick tests?No □ Yes □ , when ___/___(month/year), where _____

14. Information about **allergies** (please circle the suspected sources):

1) Dietary: chocolate, cocoa, citrus, egg, fish, tomato, strawberry, pea, apple, carrot, nuts, pear, peach, cow´s milk, breast milk substitute, rye, barley, oats, wheat, other_____
2) Animals: dog, cat, horse, cow, guinea pig, feather, other _____
3) Pollen: birch, alder, conifer, hay, mugworth, other _____
4) Other causes: room dust, fungal spore, other _____

15. Information about the child´s **respiratory infections**:
    During the last 12 months:
    1) "common cold" ___ times
    2) antibiotic prescription ___ times
    3) pneumonias ___ times
    4) bronchitis ___ times
    5) otitis ___ times
    6) parasenthesis ___ times
    7) other, what? _____

16. Adenoidectomy No □ Yes □ ,
when ___/___ (month/year), where _____

17. Maxillary sinus puncture No □ Yes □ ,
when ___/___ (month/year), where _____

18. Information about breathing difficulty symptoms:
Were there "common cold" symptoms during the current difficulty in breathing? No □ Yes □ I can't say □
If you suspect other causes, please name them: _____
19. The duration of respiratory symptoms before study entry?
    1) rhinitis _____ days
    2) cough _____ days
    3) rhinitis _____ days

20. Have other family members had "common cold" symptoms?
    No □ Yes □

21. Is this your child's first episode of breathing difficulties?
    No □ Yes □

22. Does your child have any regular medication?
No □ Yes □,
what? _____
*The key questions are directly translated from Finnish study form. The wheezy questionnaire contains selected questions from 2 page standard wheezy questionnaire and 7 page standard allergy questionnaire used at Turku University Hospital

Appendix 3. PARENTAL QUESTIONNAIRE AT THE 7-YEAR FOLLOW-UP VISIT FOR VINKU-STUDY

1. Has a doctor ever diagnosed **asthma** in your child ? 1) No 2) Yes If yes When (month/year)? Where? By whom?
Has the dyspnoea been relieved by *quick-relief medication* (such as Foradril, Formoterol, Oxis, Airomir, Buventol, Salbuvent, Ventoline, Serevent, Bricanyl, Seretide, Symbicort)? 1) No 2) Yes
Has the *long-term control medication* ever been started continuing for >4 weeks (such as Aerobec, Beclomet, Busonid, Pulmicort, Flixotide, Asmanex, Seretide, Symbicort)? 1) No 2) Yes
When? What preparates? How long did the regular daily long-term control therapy continue? How long did the long-term therapy continue regularly/intermittently?

2. After the study entry has your child ever had **cough/dyspnoea with wheezing**? 1) No 2) Yes
If yes How many times to eventual asthma diagnosis?
Where were they diagnosed if some of them where doctor-confirmed?
Has the dyspnoea been relieved by *quick-relief medication* (please see the list above)?
Has the *long-term control medication* ever been started continuing for <4 weeks for wheezing (please see the list above)?
When? What preparates? How long did the regular daily long-term control therapy continue? How long did the long-term therapy continue regularly/intermittently?

3. After the study entry has your child ever had **prolonged cough contiunuing >4 weeks**?1) No 2) Yes
If yes How many times to eventual asthma diagnosis?
Where were they diagnosed if some of them where doctor-confirmed?
Has the cough been relieved by *quick-relief medication* (please see the list above)?
Has the *long-term control medication* ever been started continuing for <4 weeks for the cough (please see the list above)? When? What preparates? How long did the regular daily long-term control therapy continue? How long did the long-term therapy continue regularly/intermittently?

4. **What factors caused the wheezing or cough**?
Flu/cold?
Allergies? What allergy?
Exercise?
Cold air?
Other? What?

5. Has your child ever had **itching rash** that has been called **eczema, dermatitis, atopic dermatitis**?
1) No 2) Yes
If yes Was the rash/eczema doctor-confirmed?
On what areas it appeared? How long did eczema continue regularly/intermittently?

6. Has your child ever had **hay fever, pollen allergy or some other allergic rhinitis** (sneezing, itching nose, rhinitis caused by pollen/animals)? 1) No 2) Yes
If yes Was it doctor-confirmed?

7. Has your child ever had **allergic conjunctivitis**? 1) No 2) Yes
If yes Was it doctor-confirmed?

8. Has your child had **wheezing or asthma attack during the preceding 12 months**? 1) No 2) Yes
If yes How many times totally? How many times it required a doctor-admission? How many times it required a hospitalization?
Has the *long-term control medication* ever been started continuing for >4 weeks?When? Who prescribed? What preparates?
How long did the regular daily long-term control therapy continue? How long did the long-term therapy continue regularly/intermittently?

9. Has your child had **prolonged cough continuing >4 weeks during the preceding 12 months**? 1) No 2) Yes
If yes How many coughing periods totally?
How many times it required a doctor-admission?
Has the *long-term control medication* ever been started continuing for >4 weeks? When? Who prescribed? What preparates? How long did the regular daily long-term control therapy continue? How long did the long-term therapy continue regularly/intermittently?

10. What factors caused the wheezing or cough **during the preceding 12 months**?
Flu?
Allergies? What allergy?
Exercise?
Cold air?
Other? What?

11. Has your child neede *quick-relief medication* **during the preceding 12 months** (please see the list above)? 1) No 2) Yes
If yes Weekly? Monthly? More seldom?

12. Has your child needed cortisone tablets per oral or intravenously **during the preceding 12 months?** 1) No 2) Ye
If yes How many?

13. Has the mother of your child ever had **hay fever, pollen allergy or some other allergic rhinitis** (sneezing, itching nose, rhinitis caused by pollen/animals)? 1) No 2) Yes
If yes Was it doctor-confirmed? Was it confirmed with PRICK or blood testing? What allergens were positive?

14. Has the father of your child ever had **hay fever, pollen allergy or some other allergic rhinitis** (sneezing, itching nose, rhinitis caused by pollen/animals)? 1) No 2) Yes
If yes Was it doctor-confirmed? Was it confirmed with PRICK orblood testing? What allergens were positive?

15. Has the mother of your child ever had **doctor-diagnosed asthma**? 1) No 2) Yes
If yes Was it as a child, but no longer as an adult (> 16 years)? Are there still on-going symptoms without doctor-comfirmation? Are there stillasthma symptoms and a need for doctor-prescriped asthma therapy?

16. Has the father of your child ever had **doctor-diagnosed asthma**? 1) No 2) Yes
If yes Was it as a child, but no longer as an adult (> 16 years)? Are there still on-going symptoms without doctor-comfirmation? Are there stillasthma symptoms and a need for doctor-prescriped asthma therapy?

17. Have you ever had a **pet indoor**? 1) No 2) Yes
If yes What animals? Were they before your child was born?
Totally how long?

18. Has the mother ever **smoked (**inside and/or outside)? 1) No 2) Yes
If yes Has she smoked inside? Does she still smoke **daily (**inside and/or outside)? Does she still smoke **occasionally (**inside and/or outside)? How many years has she totally been smoking (daily or occasionally)? How many cigarettes/day she smokes/smoked?

19. Has the father ever **smoked (**inside and/or outside)? 1) No 2) Yes
If yes Has he smoked inside? Does he still smoke **daily (**inside and/or outside)? Does he still smoke **occasionally (**inside and/or outside)? How many years has he totally been smoking (daily or occasionally)? How many cigarettes/day he smokes/smoked?

20. How many hours/day your child stays indoors where others smoke?

21. Was your child breast fed? 1) No 2) Yes
If yes How long?

22. Has there been problems with mould or humidity at the child´s home or day care? 1) No 2) Yes If yes Only a mild problem (eg. only seldom, mild odour mainly in living rooms or in cellar)? A significant problem (often a mild or occasionally obvious odour when coming from outdoor to indoor)? How long your child was exposed to the mould or humidity problem?

Appendix 4. PARENTAL QUESTIONNAIRE AT THE 7-YEAR FOLLOW-UP VISIT FOR VINKU2-STUDY
*** RISK FACTORS FOR ASTHMA**

1) Has a parent of your child ever had doctor-diagnosed asthma?
1) yes ☐ 2) no ☐
2) Has your child ever had doctor-diagnosed eczema?
1) yes ☐ 2) no ☐
b) If yes: Where and when was it diagnosed?_____
3) Has your child ever had wheezing without cold/flu symptoms?
1) yes ☐ 2) no ☐
4) Has your child ever had pet allergy?
1) yes ☐ 2) no ☐
b) If yes: Where and when was it diagnosed? _____
5) Has your child ever had, pollen allergy?
1) yes ☐ 2) no ☐
b) If yes: Where and when was it diagnosed? _____
6) Has your child ever had dust mite allergy?
1) yes ☐ 2) no ☐
b) If yes: Where and when was it diagnosed? _____
7) Has your child ever had doctor-diagnosed food allergy?
1) yes ☐ 2) no ☐
If yes:
b) What allergies? _____
c) Where and when was it diagnosed? _____
8) Has your child ever been tested for allergy blood tests (outside this research)?
1) yes ☐ 2) no ☐
b) If yes: Where have the tests been taken? _____

*** CHILD HEALTH DURING THE LAST MONTH**

9) How many times did your child suffer from breathing diffidulty such as wheezing, cough or dyspnoea?
1) never ☐ 2) 1-3 times ☐ 3) once a week ☐
4) 2-3 times a week ☐ 5) 4 or more times a week ☐
6) I don't know ☐
10) How often did your child wake up in the night due to breathing difficulty (wheezing, cough or dyspnoea)?
1) never ☐ 2) 1-3 times ☐ 3) once a week ☐
4) 2-3 times a week ☐ 5) 4 or more times a week ☐
6) I don't know ☐
11) How much did the breathing difficulties, such as wheezing, cough or dyspnoea, restrict your child's normal life (playing, kindergarten, other)?
1) not at all ☐ 2) a little ☐ 3) to some extent ☐
4) quite lot ☐ 5) very much ☐
12) How many days a week on average your child needed inhaled bronchodilating medication (for example Airomir, Bricanyl, Buventol, Fomeda, Foradil, Formoterol, Oxis, Salbumatol, Serevent, Symbicort (as an quick-relief medicine), Ventilastin, Ventoline) **for his/hers breathing difficulty?**
1) never ☐ 2) less often than once a week ☐ 3) once a week ☐4) twice a week ☐ 5) three times a week ☐ 6) 4-6 times a week ☐
7) daily ☐8) many times a day ☐

*** CHILD HEALTH DURING THE LAST 12 MONTHS**

13) Has your child had expiratory breathimg difficulty or asthma attack?
1) yes ☐ 2) no ☐
b) If yes: How many times? _____
c) Was there expiratory wheezing? 1) yes ☐ 2) no ☐
14) Has your child had tight coughing (outside the question 13 expiratory breathimg difficulties)?
1) yes ☐ 2) no ☐
b) If yes: How many times? _____
15) Has your child benefitted from *quick-relief medication* (for example Airomir, Bricanyl, Buventol, Fomeda, Foradil, Formoterol, Oxis, Salbumatol, Serevent, Symbicort (as an quick-relief medicine), Ventilastin, Ventoline) **during the expiratory breathimg difficulties or asthma attack?**
1) yes ☐ 2) no ☐
b) If yes: During how many periods? _____
c) What product?: _____
16) Has your child benefitted from *quick-relief medication* (for example Airomir, Bricanyl, Buventol, Fomeda, Foradil, Formoterol, Oxis, Salbumatol, Serevent, Symbicort (as an quick-relief medicine), Ventilastin, Ventoline) **during the tight coughing periods (outside the question 15 expiratory breathimg difficulties)?**
1) yes ☐ 2) no ☐
b) If yes: During how many periods? _____
c) What product?: _____
17) Has your child had expiratory breathimg difficulties or asthma attacks that lasted longer than 24 hours and affected his/hers sleep? 1) yes ☐ 2) no ☐
b) If yes: During how many periods? _____
18) Has your child had periods of tight cough that lasted longer than 24 hours and affected his/hers sleep (outside the question 17 expiratory breathimg difficulties)? 1) yes ☐ 2) no ☐
b) If yes: During how many periods? _____

19) Has your child needed repeatedly (≥2 times a week) inhaled bronchodilating medication for a prolonged expiratory breathimg difficulty, tight cough or asthma attack for over a month?
1) yes ☐ 2) no ☐

20) Has your child needed systemic cortisone (intramuscular, tablets per oral or intravenously; Prednison, Prednisolon, Dexametason or Oradexon) **for an expiratory breathimg difficulty, tight cough or asthma attack?** 1) yes ☐ 2) no ☐

b) If yes: During how many periods? _____

21) Has your child needed doctor-appointments for his/hers expiratory breathimg difficulty, tight cough or asthma attack during the previous 12 months (excluding the times he/she was hospitalized)? 1) yes ☐ 2) no ☐

If yes:

b) How many times? _____

c) At which health centre or hospital? _____

22) Has your child been hospitalized for his/hers expiratory breathimg difficulty, tight cough or asthma attack during the previous 12 months? 1) yes ☐ 2) no ☐

If yes:

b) How many times? _____

c) At which hospital? _____

23) Has your child been described regular daily asthma controler therapy (inhaled or per oral, for example Aerobec, Astecon, Beclomet, Budesonid, Dexas, Depo-Medrol, Dexametason, Flixotide, Lomudal freoniton, Medrol, Montelukast, Novopulmon, Prednisolon, Prednison, Pulmicort, Seretide, Singulair, Solomet, Solu-medrol, Symbicort, Tilade freoniton, Xolair) **during the previous 12 months for his/hers repeated breathimg difficulty, prolonged cough or asthma?**

1) yes ☐ 2) no ☐

If yes:

b) What product/s? _____

c) When was it started (mo/y)? /

d) Where was it started? _____

e) How many months was the therapy in use? _____months

f) Has the therapy been in use **during the previous 4 weeks**?

1) yes ☐ 2) no ☐

24) Has a doctor called the breathing difficulty "asthma" during the previous 12 months?

1) yes ☐ 2) no ☐

25) Has your child had itching rash (eczema, dermatitis, atopic dermatitis**) during the previous 12 months?**

1) yes ☐ 2) no ☐

If yes:

Was the rash in these locations: inside of the elbows or knees, front of the ankles, gluteals, neck, or around the ears or eyes?

1) yes ☐ 2) no ☐

26) Has your child had allergic rhinitis (sneezing, itching nose, rhinitis) **or conjunctivitis due to aeroallergens, such as pollen, room dust or animals during the previous 12 months?**

1) yes ☐ 2) no ☐

If yes:

b) When was it started (mo/y)? /

c) What was the possible cause? _____

*** PREVIOUS HEALTH**

Has your child ever, earlier than the previous 12 months had

27) Acute wheezing or bronchiolitis? 1) yes ☐2) no ☐

b) If yes, when last time (mo/y)? /

28) Has a doctor diagnosed asthma in your child?

1) yes ☐ 2) no ☐

b) If yes, when was the diagnose set (mo/y)? /

c) Where? _____

29) Has your child been described regular daily asthma controler therapy (inhaled or per oral, for example Aerobec, Astecon, Beclomet, Budesonid, Dexas, Depo-Medrol, Dexametason, Flixotide, Lomudal freoniton, Medrol, Montelukast, Novopulmon, Prednisolon, Prednison, Pulmicort, Seretide, Singulair, Solomet, Solu-medrol, Symbicort, Tilade freoniton, Xolair) **for his/hers repeated breathimg difficulty, prolonged cough or asthma?** 1) yes ☐ 2) no ☐

If yes:

b) What product/s? _____

c) When first time (mo/y)? /

d) Where was it started(mo/y)? /

e) When was it ended (mo/y)? /

30) If your child had asthma, has the symptoms relieved?

1) yes ☐ 2) no ☐

b) If yes, when did it happen? (kk/v) /

31) Has your child any other chonic disease, what?_____

32) Has you child any other regular medication (>1 months) than the above asked?

1) yes ☐ 2) no ☐

If yes:

b) What? _____

c) When started? _____

d) How long did it last (months)? _____.