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THE FIRST WHEEZING EPISODE IN SMALL CHILDREN: VIRUS ETIOLOGY, CLINICAL CHARACTERISTICS AND ONE-YEAR OUTCOME

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(née Nieminen)

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To my family

Abstract

Riitta Turunen

The first wheezing episode in small children: virus etiology, clinical characteristics and one-year outcome

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Background: Early wheezing induced by rhinovirus infection has been recognized as an important risk factor for recurrent wheezing and asthma. Different rhinovirus species may also vary in their pathogenicity to cause severe illness. Oral corticosteroid has been shown to decrease the risk for recurrent wheezing episodes after the early rhinovirus induced wheezing. However, limited data exist on the first wheezing episode induced by rhinovirus.

Aims and methods: The aim of this thesis was to study the virus etiology of the first wheezing episode in small children by using PCR methods, as well as to specify the role of rhinoviruses in wheezing. Rhinovirus species were analyzed using sequencing and their association with atopic characteristics in small children was studied. The one-year virus surveillance was conducted focusing on rhinoviruses. The efficacy of oral corticosteroid, prednisolone, on short- and long-term outcomes was investigated using randomized controlled trial conducted in children of 3-23 months of age.

Results: Rhinovirus infection was found in 76% of children aged 3-23 months experiencing their first wheezing episode. Rhinovirus induced first wheezing episode was positively associated with atopic characteristics and prolonged cough. Of the rhinovirus species, rhinovirus C (58%) was the most common, followed by rhinovirus A (21%) and B (1.2%). Atopic characteristics and illness severity factors were more common with rhinovirus A and C than with other respiratory infections. Children with rhinovirus A or C infection had an increased risk for recurrent wheezing episodes and the initiation of regular controller medication for asthma symptoms. Children with high rhinovirus load benefitted from prednisolone in long- and short-term outcomes.

Conclusions: Rhinovirus is a common pathogen in causing the first wheezing episodes in 3 -23 months old children. In wheezing children, the most important rhinovirus species are A and C. These species are associated with a high recurrence rate. Prednisolone might be effective in a subgroup of first-time wheezing children with a high rhinovirus load.

Keywords: children, clinical diagnosis, infection, oral corticosteroid, polymerase chain reaction, prednisolone, rhinovirus, rhinovirus species, sequencing, wheezing

Tiivistelmä

Riitta Turunen

Pikkulasten ensimmäinen akuutti uloshengitysvaikeus: virusetiologia, kliininen taudinkuva ja yhden vuoden seuranta

Turun Yliopisto, Lääketieteellinen tiedekunta, Lastentautioppi ja Virusoppi, Turun yliopiston kliininen tohtoriohjelma (TKT),
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Tausta: Varhainen rinoviruksen aiheuttama akuutti uloshengitysvaikeus on merkittävä riskitekijä toistuville uloshengitysvaikeuksille ja astmalle. Eri rinovirusgenotyypit voivat myös vaikuttaa taudin vaikeusasteeseen. Suun kautta otetun kortikosteroidin on osoitettu vähentävän riskiä uloshengitysvaikeuksien toistuvuudelle rinoviruksen aiheuttaman kohtauksen jälkeen. Ensimmäistä akuuttia uloshengitysvaikeutta on kuitenkin tutkittu vielä vähän.

Tavoitteet ja menetelmät: Väitöskirjatutkimuksessa tutkittiin ensimmäisen akuutin uloshengitysvaikeuden virusetiologiaa pienillä lapsilla ja rinoviruksen merkitystä akuutissa kohtauksessa. Rinoviruksen genotyypit analysoitiin sekvensoimalla ja niiden yhteyttä atooppisiin piirteisiin analysoitiin. Virusetiologia tutkittiin vuoden seurannassa keskittyen erityisesti rinovirusinfektioiden toistuvuuteen. Prednisolonin teho lyhyen ja pitkän aikavälin tuloksiin analysoitiin käyttäen randomisoitua kontrolloitua tutkimusta 3-23 kuukauden ikäisillä lapsilla.

Tulokset: Rinovirus löytyi 76 %:lta ensimmäistä infektioon liittyvää uloshengitysvaikeutta sairastavalta lapselta, jotka olivat iältään 3-23 kuukautta. Rinoviruksen aiheuttama ensimmäinen akuutti uloshengitysvaikeus oli yhteydessä atooppisiin piirteisiin ja pitkittyneeseen yskään. Yleisimmin löytyi rinovirus C ryhmän viruksia (58%), toiseksi yleisimmin A ryhmän viruksia (21%) ja kolmanneksi rinovirus B ryhmän viruksia (1.2%). Atooppiset piirteet ja vaikeampi taudinkuva olivat yhteydessä rinovirus A ja C aiheuttamaan infektioon. Niillä lapsilla, joilla ensimmäisen uloshengitysvaikeusepisodin aiheuttajana oli rinovirus A tai C, olin enemmän uusitumisia kuin muilla lapsilla, joilla aihettajana oli joku muu virus. Lapset, joilla rinoviruksen määrä hengitystie-eritteessä oli suuri, hyötyivät prednisolonista.

Johtopäätökset: Rinovirus on merkittävä patogeeni ensimmäisessä akuutissa uloshengitysvaikeudessa 3 – 23 kuukauden ikäisillä lapsilla. Näillä lapsilla yleisimmät rinoviruksen genotyypit ovat A ja C, jotka ovat yhteydessä myös riskiin kohtauksien toistumiselle. Prednisolonista hyötyvät lapset, joilla viruksen määrä on suuri ensimmäisessä akuutissa uloshengitysvaikeuskohtauksessa.

Avainsanat: kliininen diagnoosi, kortikosteroidi, lapset, polymeraasi ketju reaktio, prednisolone, rinovirus, rinovirus genotyyppi, sekvensointi, tulehdus, uloshengitysvaikeus

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Abbreviations

AdV	adenovirus
ANOVA	one-way analysis of variance
BLAST	Basic Local Alignment Search Tool
BP	base pair
CDHR3	cadherin-related family member 3
cDNA	complementary DNA
CI	confidence interval
CV	coronavirus
EIA	enzyme immune assay
EV	enteroviruses
Flu	influenza virus
HBoV	human bocavirus 1
HR	Hazard ratio
ICS	inhaled corticosteroid
IFN	interferon
Ig	immunoglobulin
IL	interleukin
IP-10	interferon-gamma inducible protein 10
IQR	interquartile range
MDA	melanoma differentiation-associated gene
MIP-1α	macrophage inflammatory protein 1α
MPV	metapneumovirus
NCR	non-coding region
NPA	nasopharyngeal aspirate
OR	odds ratio
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PIV	parainfluenza virus
RANTES	Regulated on Activation, Normal T Cell Expressed and Secreted
RCT	randomized controlled trial
RIG-1	retinoic acid inducible gene 1

RSV	respiratory syncytial virus
RT	reverse transcriptase
RV	rhinovirus
RV-A	rhinovirus A
RV-B	rhinovirus B
RV-C	rhinovirus C
SD	standard deviation
TLR3	Toll-like receptor 3
TLR7/8	Toll-like receptor 7/8
VP	viral protein

List of original publications

This thesis is based on the following publications, which are referred to in the text by the Roman numerals I-IV. The original publications have been reprinted with the kind permission of the copyright holders.

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

II Turunen R, Jartti T, Bochkov Y, Gern J, Vuorinen T. Rhinovirus species and clinical characteristics in the first wheezing episode in children. J Med Virol. 2016; 88: 2059-2068.

III Turunen R, Vuorinen T, Bochkov YA, Gern JE, Jartti T. Clinical and virus surveillance after the first wheezing episode: special reference to rhinovirus A and C species. *Submitted*

IV Jartti T, Nieminen R, Vuorinen T, Lehtinen P, Vahlberg T, Gern J, Camargo CA Jr, Ruuskanen O. Short- and long-term efficacy of prednisolone for first acute rhinovirus-induced wheezing episode. J Allergy Clin Immunol. 2015; 135: 691-8.e9.

Turunen (née Nieminen)

1. Introduction

Early wheezing episodes are very common in small children and in approximately 95% of the cases the cause is a virus infection (Jackson et al. 2008, Marguet et al. 2009, Table 1). Up to half of the children in general suffer from wheezing illnesses before school-age (Martinez et al. 1995). Of them, 30-40% of children will develop recurrent wheezing (Kurukulaaratchy et al. 2003, Matricardi et al. 2010). The prevalence of asthma is approximately 7-9% among children younger than 16 years of age (von Hertzen et al. 2006, Lai et al. 2009, Global Asthma Network 2012). There are no efficient ways to predict the development of childhood asthma, however, the recognized traditional risk factors such as atopic characterisrics (aeroallergen sensitization, increased blood eosionophil count, or atopic eczema), parental asthma or factors related to parental atopy may help to predict the development of asthma.

Virus infections, especially caused by rhinovirus (RV) and respiratory syncytial virus (RSV) infections, are recognized as early risk factors for recurrent wheezing. Virus-induced wheezing typically occurs earlier than the development of allergen sensitizations (Jackson et al. 2008, Wu et al. 2011, Kusel et al. 2012, Nissen et al. 2013). An early wheezing episode induced by RV has been notified to be an important risk factor for recurrent wheezing (Lemanske et al. 2005, Kusel et al. 2007, Jackson et al. 2008, Lukkarinen et al. 2013). It has been associated with atopic characteristics and poor lung function which further increases the risk for recurrencies (Malmström et al. 2006, Guilbert et al. 2011, Jackson et al. 2012). The predisposing factors of RV-induced wheezing have been shown to be low interferon (IFN) responses, pre-existing airway inflammation, and damage in bronchial epithelium and genetics (Jakiela et al. 2008, Baraldo et al. 2012, Carroll et al. 2012, Calıskan et al. 2013, Contoli et al. 2015, Bochkov et al. 2015). Also, RV species might have an effect on the severity of the infections and wheezing illnesses, since RV-A and RV-C have been known to cause illnesses more severe than RV-B (Miller et al. 2009, Arden et al. 2010, Bizzintino et al. 2011). However, data concerning the first wheezing episode are still rather limited. Understanding the different early life risk factors of atopic and non-atopic asthma of later childhood, could provide a novel approach into early intervention strategies for asthma prevention (Gern et al. 2010).

This study was launched in order to determine the aspect of the first wheezing episode in small children in the development of recurrent wheezing focusing on RV etiology. The aim was to study the virus etiology of the first wheezing episode in order to better understand the role of viruses, RV in particular, in early wheezing and how they are related to atopic characteristics and illness severity. Moreover, the prevalence and characteristics of different RV genotypes were assessed. The virus etiology and the occurrence of RV reinfection was also investigated during a 12-month follow-up period after the first wheezing episode. How RV-A and RV-C induced first wheezing episodes were linked to recurrent wheezing was also investigated. In addition to affore mentioned, the short- and long-term efficacy of oral corticosteroid, prednisolone, in RV-induced first wheezing episode was evaluated.

2. Review of the literature

2.1. Definitions of bronchiolitis, wheezing and asthma

Bronchiolitis is an acute respiratory illness of small bronchioles and an inflammation of the surrounding tissue that affects infants and young children and is caused by a virus infection. It is a clinical diagnosis based on typical characteristics and clinical findings. Bronchiolitis is characterized by rhinitis, dry wheezy cough, tachypnea, low-grade fever, hyperinflation, chest retraction, and widespread crackles and wheezing (Smyth et al. 2006). Typical pulmonary findings on auscultation include inspiratory crackles and/or a high-pitched expiratory wheeze (Jartti et al. 2009). Bronchiolitis begins with upper respiratory infection, which develops into breathing difficulty approximately in 3-5 days. The cause of bronchiolitis in up to 80% of the cases is RSV (Henrickson et al. 2004, Marguet et al. 2009, Hall et al. 2009, Hall et al. 2013, Meissner et al. 2016). Other common viruses causing bronchiolitis are metapneumovirus (MPV), influenza viruses (Flu), parainfluenza viruses (PIV), RV and adenoviruses (AdV) (Boivin et al. 2003, Korppi et al. 2004, Williams et al. 2004, Mansbach et al. 2012).

Infants who develop bronchiolitis severe enough to require hospitalization are at increased risk of developing recurrent wheezing or childhood asthma (Singh et al. 2006). Signs of a severe illness are dehydration, cyanosis, recurrent apnea periods and eating problems (Smyth et al. 2006, Scottish Intercollegiate Guidelines Network 2006). Small children may also suffer from breathing insufficiency, electrolyte disruption, secondary bacterial infections and heart and circulation problems (Smyth et al. 2006, Scottish Intercollegiate Guidelines Network 2006). The typical length of illness is 2 weeks. The risk factors for bronchiolitis include young age, prematurity (gestational age < 28 weeks), chronic pulmonary disease, for example bronchopulmonary dysplasia, congenital heart disease, immunodeficiences, or muscular disease and early exposure to tobacco smoke (Scottish Intercollegiate Guidelines Network 2006, Ralston et al. 2014, Meissner et al. 2016).

The definitions of bronchiolitis vary around the world. In USA, bronchiolitis is defined as an expiratory breathing difficulty caused by a virus infection in children less than 2 years of age (Ralston et al. 2014). In Europe, bronchiolitis is defined as an illness which typically manifests in <12 months old children and it is the first expiratory breathing difficulty caused by a virus infection (Scottish Intercollegiate Guidelines Network 2014, Smyth et al. 2006, Ralston et al. 2014). Typically, bronchiolitis is the most prevalent in children less than12 months of age (Meissner et al. 2016, Sigurs et al. 2005, Wu et al. 2008, Jartti et al. 2009).

Wheezing is a descriptive term. It is defined as a whistling sound during expirium with acute respiratory breathing difficulty (National Asthma Education and Prevention Program (NAEPP) guidelines 2007, Brand et al. 2008, Scottish Intercollegiate Guidelines Network 2014, Ralston et al. 2014). The international terminology, however, varies in many ways.

The terms used include: acute wheezing, obstructive bronchitis, recurrent wheezing, pre-asthma, viral wheeze and wheezy bronchitis have been used. The line between obstructive bronchitis and asthma is sliding. The diagnosis of wheezing can be set if the illness does not fulfill the diagnosis of bronchiolitis or asthma and there is a transient smooth muscle contraction in the airways. Majority of the children without allergic sensitization will have a relinquishing tendency, which is caused by the tightness of small bronchioles during virus infection (Jackson et al. 2008). Wheezing illness is typical in toddlers. From 30% to 50% of children have at least one wheezing episode before school age, but many children outgrow their disease (Martinez et al. 1995, Bisgaard et al. 2007). RV-induced wheezing has been separated as an own entity. It has also been recognized as a strong predictor of childhood asthma (Gern et al. 2009, Rubner et al. 2016). Data on the role of the first wheezing episode, especially caused by RV infection in young children less than two years of age, are still limited.

Most children, especially infants and children in pre-school, have episodes of wheezing, cough and difficulty of breathing without persisting symptoms (Martinez et al. 1995, Kurukulaaratchy et al. 2003, Lau et al. 2003). They will stop having recurrent lung symptoms by school age. Only a minority of children who wheeze in early life due to viral infections will go on to develop persistent wheezing and futher asthma (Martinez et al. 1995, Kurukulaaratchy et al. 2003, Matricardi et al. 2010, Ducharme et al. 2014). The ones who do, are usually sensitized to allergens, for example pollen and animal dust, which are both significant risk factors for asthma (NAEPP guidelines 2007, Chang et al. 2013, Scottish Intercollegiate Guidelines Network 2014, Rubner et al. 2016). They may also have low lung function, high airway resistence and genetic variants in the 17q21 locus of GSDMB and ORMDL3 genes (Brussee et al. 2004, Lowe et al. 2005, Guilbert et al. 2011, Caliskan et al. 2013). Furthermore, persistent wheezing has been associated with eczema, maternal asthma and blood eosinophilia (Midulla et al. 2012, Carroll et al. 2012, Lukkarinen et al. 2013). Children with recurrent wheezing most likely avail from therapeutic interventions (Bisgaard et al. 1999,

Scottish Intercollegiate Guidelines Network 2014). Inhaled corticosteroids are consistently the most used treatment among these children (NAEPP guidelines 2007). The diagnosis of asthma is based on the characteristics of symptoms and signs (Scottish Intercollegiate Guidelines Network 2014).

Asthma is a chronic disorder characterized by underlying inflammation in the airways, airflow obstruction, bronchial hyperresponsiveness and increased mucus secretion that causes repetitive breathing difficulties, recurrent wheezing and cough (NAEPP guidelines 2007, Scottish Intercollegiate Guidelines Network 2014, Global Initiative for Asthma 2015). The episodes are characterized by squashing of the airways, which is either recovered by itself or with the help of medication. The chronic inflammation sensitizes airways to many environmental stimuluses. Atopy is the strongest identifiable risk factor for developing asthma (Sly et al. 2008, Jackson et al. 2008, Simpson et al. 2010, Jackson et al. 2012, Stoltz et al. 2013). A raised specific immunoglobulin E (IgE) to wheat, egg,

and inhalant allergens such as house dust mite and cat dander predict also later childhood asthma (Sears et al. 1989, Kotaniemi-Syrjänen et al. 2003). Since lung function tests are difficult to perform in small children, is the diagnosis of asthma in small children based on symptoms (NAEPP guidelines 2007, Scottish Intercollegiate Guidelines Network 2014, Global Initiative for Asthma 2015). National Heart, Lung and Blood Institute has published diagnostic criteria for the initiation of regular controller medication (most often inhaled corticosteroids) among children younger than 4 years of age. These criteria also contribute to asthma inception in children with susceptible genetic background. Although > 90% of asthma exacerbations in children are linked to virus infections, it is important to also recognize the predisposing factors of asthma, most importantly undertreated airway inflammation and sensitization to allergens (Global Initiative for Asthma 2015, NAEPP guidelines 2007, Scottish Intercollegiate Guidelines Network 2014).

2.2. Epidemiology

A third of children suffer from wheezing illnesses during respiratory infections before the age of three (Rhodes et al. 2001, Taussig et al. 2003, Matricardi et al. 2008). The most common reason for hospitalization in small children is bronchiolitis. Approximately 2-3% of all infants under the age of 1 year (most of them aged <6 months) are admitted to hospital due to bronchiolitis (Smyth et al. 2006, Hall et al. 2009). The most common pathogen causing bronchiolitis is RSV, especially in children < 6 months of age (Hall et al. 2009). In children 6-12 months of age, RSV and RV are the two most common pathogens (Kotaniemi-Syrjänen et al. 2003, Jartti et al. 2009, Midulla et al. 2010). In Finland, according to the epidemiologic surveillance and response unit of National Institute for Health and Welfare large and small RSV epidemics have occurred in alternate years beginning in November-December (National Institute for Health and Welfare 2016).

Of the wheezing children, approximately 30-40% develop recurrent wheezing before school-age (Martinez et al. 1995, Kurukulaaratchy et al. 2003, Matricardi et al. 2008). About 50% of them are also sensitized to inhaled allergens before school age (Kurukulaaratchy et al. 2003, Illi et al. 2006). Viral etiologies have been reported approximately in 95% of the wheezing illnesses (Jackson et al. 2008, Marguet et al. 2009, Table 1). Recent studies have also shown that RV-induced wheezing before school-age is a strong predictor (e.g. Odds ratio (OR) 9.8 with 95% confidence interval (CI) 4.3, 22 in high risk cohort) of recurrent wheezing before school age (Lemanske et al. 2005, Kusel et al. 2007, Jackson et al. 2008, Lukkarinen et al. 2013).

Asthma is the most common chronic disease in children. Overall, the prevalence of childhood asthma is 11.7% in 6–7-year age group and 14.1% in 13-14-year age group (Mallol et al 2013). According to International Study of Asthma and Allergies in Childhood (ISAAC), in USA the prevalence of childhood asthma is approximately 14% in 6–7-year age group and 22% in 13-14-year age group n USA (Asher et al. 2006). In Finland, the overall prevalence of childhood asthma is 7-9% (Lai et al. 2009, Asthma: Current Care

Guidelines 2012). Childhood asthma is more common among boys than girls (9.3% vs 3.8%) (Hugg et al. 2008).

2.3. The virus etiology of wheezing

As mentioned earlier, of the wheezing episodes up to 95% are triggered by viral respiratory infections during the first three years of life (Jartti et al. 2004, Sears et al. 2007, Jackson et al. 2008, Jartti et al. 2009, Marguet et al. 2009, Busse et al. 2010, Forno et al. 2012). RV and RSV are the most frequent and important triggers of wheezing illnesses (Kotaniemi-Syrjänen et al. 2003, Jartti et al. 2004, Fujitsuka et al. 2011, Mansbach et al. 2012, Midulla et al. 2010). In recent years though, human bocavirus (HboV) has also been found to be an important pathogen causing respiratory infections and wheezing (Söderlund-Venermo et al. 2009). The other important respiratory viruses are MPV, PIV, Flu, AdV, coronavirus (CV) and certain enterovirus types (EV). Nowadays, the association between virus infections and wheezing illness is clear (Meissner et al. 2016, Gern et al. 2015).

Table 1. Virus etiology of the first wheezing episode in hospitalized children aged under 3 years.

1st author, publication year	N	Age (mo)	Atopy	Virus etiology (%)										
				RV	RSV	EV	HboV	MPV	PIV	Flu	AdV	CV	≥1 virus	≥2 viruses
Bosis, 2008	85	0-12	-	4.7	74	1.2	1.2	2.5	1.2	8.2	3.5	7.1	89	18
Marguet, 2009	209	1-12	+†	27	64	4.3	–	7.7	1.9	<1	–	<1	95	23
Jartti, 2009	166	3-35	+	31	47	17	23	7	6	2	5	3	93	30
Antunes, 2010	253	0-24	-	3.2	67	–	4.2	<1	8.3	2.8	10	–	78	13
Nascimento, 2010	77	0-24	+*	34	64	21	16	12	7.8	3.9	–	2.6	94	44
Calvo, 2010	370	0-24	-	17	53	1.4	3.5	11	3.2	1.4	7.6	1.1	74	21
Midulla, 2010	182	0-12	+*	8.8	41	–	12	1.7	1.7	0.7	–	0.7	57	15
Pientong, 2011	170	1-24	+*	–	65	–	–	3.5	–	17	–	–	85	–
Flores, 2011	143	0-24	+†	–	52	–	–	–	6.3	4.2	1.4	–	64	–
Ricart, 2012	484	0-12	+	19	43	2.2	18	3.9	4.9	3.4	7.8	3.4	85	46

N, number of patients; Mo, months; RV, rhinovirus; RSV, respiratory syncytial virus; EV, enteroviruses; MPV, metapneumovirus; HboV, human bocavirus 1; PIV, parainfluenzavirus; Flu, influenzavirus; AdV, adenovirus; CV, coronavirus. Atopy, + defined, - not defined, *family history of atopy only defined, †Personal atopy and family history of atopy defined. PCR was used as a method in all references. Immunofluorecent assay was used in articles from Antunes et al, Marguet et al, and Flores et al. Immunochromatography was used in the article from Ricart et al. All articles which did not include viral etiology of the first wheeze and age <36 months were excluded. All of the studies used bronchiolitis and wheezing for the first time as inclusion criteria except articles from Bosis et al and Jartti et al, which used only wheezing as an inclusion criterion.

2.3.1. Rhinovirus

Rhinoviruses are small, single-stranded, positive sense RNA viruses with a 7200 base pair (bp) genome. They belong to the *Enterovirus* genus in the Picornaviridae family. They are heterogeneous genetically and antigenetically. The first RV was discovered in 1953, and by 1987 RVs were classified into 100 distinct serotypes based on their antigenic

crossreactivity in neutralization tests (Hamparian et al. 1987). Further, full-length and partial genome sequencing of prototype strains and clinical isolates showed that RVs cluster genetically into two groups namely rhinovirus A (RV-A) and rhinovirus B (RV-B) (Horsnell et al. 1995, Savolainen et al. 2002, Savolainen et al. 2002, Ledford et al. 2004). For entering cells, RV-A and RV-B exploit either intercellular adhesion molecule 1 (ICAM-1) or low-density lipoprotein receptor (LDLR) (Andries et al. 1990, Uncapher et al. 1991). Later, the improvement of polymerase chain reaction (PCR) method led to the discovery of a third RV species, rhinovirus C (RV-C) (Palmenberg et al. 2009, Simmonds et al. 2010, McIntyre et al. 2013). RV-C does not grow in conventional cell culture, which has most probably postponed its discovery until recently (Lee et al. 2007, Lau et al. 2007, McErlean et al. 2007). Bochkov et al showed proliferation of RV-C isolates in organ culture of nasal epithelial cells predicting unique cell attachment cite for RV-C (Bochkov et al. 2011). Recently, it was also discovered that expression of human cadherin-related family member 3 (CDHR3) enables RV-C binding and replication in cells (Bochkov et al. 2015). To date, altogether more than 160 RV types have been identified (Simmonds et al. 2010, McIntyre et al. 2013, Bochkov et al. 2014).

RV has an icosahedral structure, which contains 60 structural proteins named VP1, VP2, VP3 and VP4 forming a viral capsid that encases the RNA genome. VP1 works as the site of attachment to cell surface receptors. The remaining nonstructural proteins are involved in viral genome replication and assembly. Of proteins, VP1, VP2 and VP3 account for the antigenic diversity of the virus, whereas VP4 attaches the RNA core to the capsid. RV-A, RV-B and RV-C have a similar genomic organization. The classification is based on the sequence divergence (Simmonds et al. 2010, Bochkov et al. 2012, McIntyre et al. 2013).

A large number of distinct RV strains circulate throughout the year (Rollinger et al. 2011, van der Zalm et al. 2011). Higher prevalence of RV infections has been described from September to November and from April to May (Rollinger et al. 2011). RV-A and RV-C are the principal virus types circulating among individuals which makes them more common than RV-B (Miller et al. 2009, Marcone et al. 2014). RV-A and RV-C typically peak during different seasons, which suggests that they might interfere each other's ability to cause a disease or that there is cross-protection from a previous infection (Lau et al. 2009, Miller et al. 2013). RV diagnosis relies almost entirely on PCR-based tests because of the challenges of viral culture, and the limited availability of the serological and the antigen detection tests (Kieninger et al. 2012, Jartti et al. 2013).

Since their discovery, RVs have been known to be the most common viruses causing the common cold (Andrewes et al. 1953). RVs were thought to replicate best at 33°C and 35°C being consistent with their role as upper airway pathogens (Stott et al. 1972). Later, it has also been discovered that RVs frequently infect also lower airways thus causing infants and young children to suffer from lower respiratory infections and precipitating wheezing symptoms (Gern et al. 1997, Papadopoulos et al. 2000, Miller et al. 2007). Although lung parenchyma is at 37°C, airways are cooler and therefore the

temperature in medium and large-sized airways is optimal for RV replication (Gern et al. 1997, Papadopoulos et al. 2000). RV-C seems to replicate equally well at 33 °C and 37 °C (Ashraf et al. 2013). To support the ability to infect both upper and lower airways, RV has been detected in sputum and bronchial biopsy specimens of the upper airway and in lower airway biopsies from infants with recurrent wheezing after experimental inoculation (Gern et al. 1997, Papadopoulos et al. 2000, Malcolm et al. 2001, Mosser et al. 2005, Malmström et al. 2006).

RV infections can cover a broad range of infections all the way from asymptomatic condition to a severe disease leading to hospitalization. To endorse the role in severe lower respiratory tract infections, RV has been found to cause symptomatic respiratory infections in 23 – 41% in children (Blomqvist et al. 2002, van Benten et al. 2003, Fry et al. 2011, Marcone et al. 2013, Marcone et al. 2014). On the contrary, RV has beenfrequently been detected in 8 – 40% of the children's asymptomatic infections as well (Rakes et al. 1999, Nokso-Koivisto et al. 2002, van Benten et al. 2003, Jartti et al. 2004, Singleton et al. 2010, Iwane et al. 2011). Moreover, RV has been found to be the most common pathogen causing wheezing among >12 months old children, whereas among children <12 months of age the most common pathogen is RSV (Jartti et al. 2004, Mansbach et al. 2008, Jartti et al. 2009, Midulla et al 2010). The prevalence of RV in the first wheezing episode has been shown to vary from 5% to 34% (Table 1). When the number of previous wheezing episodes has not been specified, the prevalence of RV infections in connection to wheezing has varied between 7% - 81% (Table 2). Besides the common cold and wheezing, RVs have been found to play a critical role in exacerbations of asthma and other lung disease, as well as bronchiolitis and pneumonia.

RV has been found in bronchial biopsies of infants with recurrent respiratory symptoms (Malmström et al 2006). RV-induced wheezing episode is an increased risk factor for developing asthma and when asthma has been established, RV infections are, in children especially, the most common cause of acute exacerbations, especially in children (Jackson et al 2008, Arden et al 2010, Bizzintino et al 2011). RV-A and RV-C are the most common RV species in acute asthma, wheezing and lower airway illnesses (Arden et al. 2010, Bizzintino et al. 2011, Miller et al. 2011, Lee et al. 2012, Martin et al. 2015, Fawkner-Corbett et al. 2016, Müller et al. 2015).

The severity of infections caused by different RV species has become of interest lately. RV-A and RV-C infections are more likely than RV-B infections to lead to moderate-to-severe respiratory illnesses (Lee et al. 2012, Miller et al. 2009). RV-B has also been found to be associated with a lower cellular cytotoxicity and cytokine production when compared to RV-A or RV-C, thus indicating a less severe illness (Nakagome et al. 2014). Additionally, some studies have reported results signifying that RV-C are in fact more likely to lead to severe illness, even to hospitalization, than RV-A or RV-B infections (Linder et al. 2013, Cox et al. 2013). The mechanism underlying the enhanced severity of RV-C infection remains still unclear.

2.3.2. Respiratory syncytial virus

Respiratory syncytial virus is an enveloped, negative stranded, non-segmented RNA virus, which belongs to the *Pneumovirinae subfamily in the Paramyxoviridae* family. RSV has two antigenic groups, RSV A and RSV B. RSV genome includes 10 genes, encoding seven structural and four non-structural proteins (Le Souef et al. 2009). Two viral glycoproteins, designated as G (large glycoprotein) and F (fusion glycoprotein), are involved in virus-host cell attachment and cell fusion. RSV uses Annexin II as a receptor on airway epithelial cells and CX3CR1 receptors for G-protein on leucocytes and immune-effector cells (Malhotra et al. 2003). The epidemics of RSV are seasonal. The peak epidemic occurs typically from late autumn or winter to early spring in the northern hemisphere (Lanari et al. 2002, Medici et al. 2006, Hall et al. 2009, Jackson et al. 2010). Furthermore, RSV infections are universal in infancy but large and small RSV epidemics occur in alternate years.

Today, RSV diagnosis mainly relies on PCR based tests, although antigen detection and virus cultivation are also used in clinical laboratories. Yet, the interpretation of positive PCR test results may be complicated due to the high sensitivity of the test, as very small amounts of virus RNA may not prove causality of the symptoms (Jansen et al 2010). On the other hand, there are case-control studies performed among asymptomatic and symptomatic young children that have concluded that a positive RSV PCR test result is almost always clinically relevant (Kumar et al. 2008, Jansen et al. 2011). RSV replicates in epithelial cells lining the upper airways and bronchioles, as well as in type I pneumocytes. RSV infection causes airway obstruction in lower airways by inducing epithelial cell necrosis, inflammation and mucus hypersecretion (Habibi et al. 2012).

Respiratory syncytial virus is the most common pathogen causing severe lower respiratory tract illnesses leading to hospitalization during infancy, especially during the first 6 months of life (Mansbach et al. 2012). RSV infections are the most common cause of bronchiolitis during the first year of life (Hall et al. 2009). In addition, RSV has been detected in up to 85% of the wheezing in children (Table 2) (Mak et al. 2011). Moreover, it has been prevalent in 41% - 74% of the first wheezing episodes (Table 1). Also, the RSV-induced wheezing episodes have been associated with the development of recurrent wheezing episodes and asthma in early childhood (Stein et al. 1999, Sigurs et al. 2005, Wu et al. 2008, Sigurs et al. 2010, Korppi et al. 2004, Hyvärinen et al. 2005). However, no link between allergic sensitization and RSV-induced lower respiratory infection has been found as has been found in RV associated wheezing episodes (Stein et al. 1999, Henderson et al. 2005). The association between recurrent wheezing and RSV infection is the strongest in children with severe RSV illnesses leading to hospitalization (Carroll et al. 2009).

Primary RSV infection is mainly symptomatic, but the disease severity varies (Carroll et al. 2009, Mansbach et al 2012). Only subset of children develops severe disease (Malhotra et al. 2003, Medici et al. 2006, Kumar et al. 2008, Jansen et al. 2011, Jackson et al. 2010). In addition to the severity of different RSV seasons, a number

of host-related characteristics, for example prematurity, low birth weight, male sex, day care attendance, the number of siblings living in child's household and tobacco exposure, may affect the disease severity, (Simoes et al. 2003). Furthermore, infants hospitalized for RSV-induced lower respiratory tract infection tend to be younger than those hospitalized for another respiratory virus infection (Rossi et al. 2007). The severity of RSV infection in young infants may be explained by the incomplete development of the respiratory system, small airway diameters, and the immaturity of the immune system for which reason the first RSV infections tends to be the most severe one (Murphy et al. 1986, Yamazaki et al. 1994, Delgado et al. 2009). The natural differences in the innate immune response has also shown to predispose children for severe RSV infection rather than the infection adjusting immune responses in childhood (Juntti et al. 2009).

2.3.3. Human bocavirus

Human bocavirus was discovered in 2005 in the respiratory secretions of children with respiratory infection (Allander et al. 2005). HBoV belongs to the *Parvovirinae* subfamily of the *Parvoviridae* family (Allander et al. 2005). The first new pathogen was named HBoV1, and later three additional types were discovered and named as HBoV2-4 (Arthur et al. 2009, Kapoor et al. 2009). HBoV is a single-stranded, 5200 nucleotides long DNA virus with a negative-polarity. HBoV genome contains three open reading frames between 5' and 3' terminus: first two encode NS1 and NP1 (nonstructural proteins) and the third the capsid proteins VP1 and VP2. The capsid proteins bind to surface cell receptors, transport the genome into the nucleus and are targets of immune response (Luo et al. 2013, Chiu et al. 2014, Deng et al. 2014). The infections of HBoV occur throughout the year, with peak epidemics appearing during winter and spring (Bastien et al. 2006, Esposito et al. 2008).

The diagnosis of HBoV is based on serology and virus detection by PCR (Söderlund-Venermo et al. 2009, Kantola et al. 2011). For the diagnosis of primary infection, it has been suggested that at least two markers must be present: positive IgM, seroconversion or a four-fold increase of IgG titer, low IgG avidity and medium-high viral load in serum sample by PCR (Söderlund-Venermo et al. 2009, Kantola et al. 2011). For diagnosis of an acute HBoV infection, it is necessary to analyze either respiratory or stool samples as well as serum samples (Söderlund-Venermo et al. 2009, Don et al. 2010, Hedman et al. 2010, Kantola et al. 2008). As DNA virus, detection of virus in respiratory sample alone is not diagnostic enough of acute infections, and hence the blood sample is needed to confirm the acute infection (Kantola et al. 2008, Söderlund-Venermo et al. 2009, Don Pediatr et al 2010, Hedman et al. 2010).

HBoV1 causes mainly upper and lower respiratory tract infection and pneumonia as primary infections whereas other HBoV species are found from stool samples causing gastroenteritis (Söderlund-Venermo et al. 2009, Don et al. 2010, Anders et al. 2015, Berry et al. 2015, Alam et al. 2015). The prevalence of HBoV in children's respiratory tract infections has ranged from 1.5% - 27% (Bosis et al. 2008, Söderlund-Venermo et al. 2009,

Jartti et al. 2012). HBoV has also been recognized as a common pathogen for wheezing causing up to 25% of infections in children (Table 1, Table 2) (Deng et al. 2012, Meriluoto et al. 2012). Moreover, HBoV can also be detected from nasopharyngeal aspirates (NPA) of asymptomatic patients (Jartti et al. 2012). In up 44% of respiratory samples from asymptomatic individuals, high viral load of HboV1 has been detected (Longtin et al. 2008, Blessing et al. 2009, Martin et al. 2010).

2.3.4. Other respiratory viruses

Enteroviruses

Enteroviruses belong to *Picornaviridae* family and are small, non-enveloped, positive-stranded RNA viruses. Human EVs were originally classified as polioviruses (3 different serotypes), coxsacie A- and B-viruses, echoviruses and enteroviruses. Currently they are classified to EV species A-D according to their genetic relatedness. A total of 116 different EV types have been discovered (Knowles et al. 2012). EVs are common pathogens circulating world-wide and the incidence is highest during summer and in autumn (Byington et al. 1999, Lee et al. 2006). Currently, PCR is the most sensitive method of detecting EVs from clinical samples (Vuorinen et al. 2003, Rittichier et al. 2005, Benschop et al. 2008). EVs are transmitted through the fecal-oral and transplacental routes as well as by respiratory droplets (Bendig et al. 2003, Konstantinidou et al. 2007).

Enteroviruses cause both upper and lower respiratory infections and gastrointestinal symptoms, as well as infections of central nervous system, paralysis, hand-foot-and-mouth disease, myocarditis, herpangina and rashes (Singer et al. 1980, Sawyer et al. 2002, de Crom et al. 2012, Tapparel et al. 2013). EVs have also been found to cause bronchiolitis (8-12%), acute expiratory wheezing (25%) and asthma exacerbations (16%) (Andreoletti et al. 2000, Thumerelle et al. 2003, Jartti et al. 2004, Jacques et al. 2008). When it comes to the first wheezing episode, EV has been present in 1.2% - 21% of the respiratory samples of the first time wheezing children (Table 1). Recently, it has been suggested that in susceptible individuals, enterovirus infection together with specific genetic host factors can trigger an insulin dependent type I diabetes in susceptible individuals (Tauriainen et al. 2011, Oikarinen et al. 2012, Holm-Hansen et al. 2016).

Human metapneumovirus

MPV is a single-stranded negative-sense RNA virus, belonging to the family of *Paramyxoviridae* in the subfamily *Pneumovirinae*, genus *Pneumovirus* (van den Hoogen et al. 2001). Virus has two major antigenic groups (A and B) and four minor subgroups (A1, A2, B1 and B2) (Feuillet et al. 2012). MPV has been detected on all continents and outbreaks occur most commonly during winter and spring months (Choi et al. 2006). One study has reported that seasonality of MPV and RSV infections overlaps (Chan et al. 2007). MPV is transmitted by airborne droplets causing a respiratory infection (Kahn et al. 2006). PCR is most commonly used for MPV detection, because the cell culture

techniques are laborious and the virus grows slowly (Maertzdorf et al. 2004, Kuypers et al. 2005).

MPV has been found to be a common pathogen in respiratory tract infection in infants and children (Jartti et al. 2002, van den Hoogen et al. 2003, Boivin et al. 2003, Freymouth et al. 2003, Mullins et al. 2004, Chano et al. 2005, Wiliams et al. 2005). The most common diagnosis of hospitalized children with MPV infection are bronchiolitis, wheezing, and pneumonia (García-García et al. 2006, Jartti et al. 2002, Williams et al. 2005). In wheezing illnesses, MPV has been detected in 1% - 31% (Camps et al 2008, Antunes et al. 2010, Table 1, Table 2) Upper respiratory infections, such as coryza, conjunctivitis, pharyngitis, and otitis media also occur in connection to MPV (Williams et al. 2006). Asymptomatic infections of MPV seem to be uncommon (Williams et al. 2004).

Coronavirus

CVs are part of the *Coronaviridae* family. Altogether 4 different CVs are known: 229E and OC43 are alfa CVs and HKU1 and NL63 are beta CVs (Fouchier et al. 2004, van der Hoek et al. 2004, Woo et al. 2005, Esper et al. 2005,). CVs are single-stranded, enveloped RNA viruses. They have four structural proteins: spike, envelope, membrane and nucleocapsid proteins. CVs can be found worldwide, epidemics peaking mainly in winter (Chiu et al. 2005, Sloots et al. 2006, Gerna et al. 2006, Esposito et al. 2006). CVs are recognized as viruses most often responsible for upper and mild respiratory tract infections, for example common cold and tonsillitis (Esper et al. 2005, Fouchier et al. 2004). They have also been detected in connection to wheezing, bronchiolitis and pneumonia (Jartti et al. 2004, van Elden et al. 2004, Woo et al. 2005, Talbot et al. 2009). CVs have been found in 1% - 13% of children with early wheezing episodes (Marguet et al. 2009, Bisgaard et al. 2010, Table 1, Table 2).

Influenza viruses

Influenza viruses are negative-stranded, enveloped RNA viruses belonging to *Orthomyxoviridae* family. They are classified in influenza A, B and C viruses. Influenza A viruses are further subtyped based on the antigeninicity of hemagglutinin (HA) and neuraminidase (NA) glycoproteins located on the external layer of the virus. Influenza A is causing the majority of the seasonal epidemics as well asbeing able to cause pandemics. Compared to Influenza A and B, influenza C has minor relevance, is studied less, and is causing only small epidemics with mild symptoms, although, in children it has been shown to have similar clinical presentation as influenza A and B (Matsuzaki et al. 2006). The genome of influenza A and B viruses includes 8 negative-stranded RNA segments. The role of each segment is to code one or two proteins with the total of 10 and 11 different proteins in influenza A and B viruses. The proteins act as structural proteins or they have different roles in viral replication. Influenza infections cause a substantial number of hospitalizations each year (Yu et al. 2014, Heikkinen et al. 2016). Nowadays, the diagnosis of influenza infections is based on reverse transcriptase (RT) – PCR and/or antigen detection. However, the antigen tests have poorer sensitivity than (RT) – PCR and viral culture (Weinberg et al. 2004). Before, the golden standard for diagnosis was viral culture.

Non-complicated influenza infection is characterized by respiratory symptoms, for example cough, rhinitis, pharyngitis, fever, myalgia, and headache (Cox et al. 1999, Heinonen et al. 2012). Fever has been shown to be the most common symptom (Silvennoinen et al. 2009). The degree of fever correlates positively with the viral load, as well as, nasal and plasma IL-6 levels (Kaiser et al. 2001). In wheezing illnesses, the prevalence of influenza virus infection has varied between 2 – 17% (Pientong et al. 2011, Calvo et al. 2010, Table 1, Table 2). The most typical complications of an influenza infection are pneumonia, acute otitis media, and laryngitis; however, the complications outside of the respiratory symptoms are rare (Winther et al. 2010, Ruuskanen et al. 2011, Kuiken and Taubenberger et al. 2008, Peltola et al. 2002).

Parainfluenzaviruses

PIVs belong to the *Paramyxoviridea* family, *Paramyxovirinae* subfamily, genus *Rubulavirus*. They are enveloped, single-stranded, negative-sense RNA viruses and classified into subtypes 1 – 4 (Hall et al. 2001). The PIV4 is further divided into A and B subtypes on the basis of their antigenic differences (Canchola et al. 1964). Seasonalities of different PIVs differ. PIV 1 typically occurs every 2 years, peaking in the autumn. PIV 3 causes infections annually in spring-summer time. Seasonality of parainfluenza type 2 is somewhat erratic. Seasonality of PIV4 infections is difficult to define, because these infections are only seldom diagnosed (Hall et al. 2001). The fastest and most sensitive ways for PIV diagnosis are antigen tests and PCR methods (Aquilar et al. 2000, Kuypers et al. 2006). The most common illnesses caused by PIV infection are upper respiratory tract infections, lower respiratory tract infections, pneumonia, and bronchiolitis. The PIVs have been detected in 1-30% of children with bronchiolitis, in 28% of children with pneumonia, 15% of children with upper respiratory tract infection and up to 14% in early wheezing (Kotaniemi-Syrjänen et al. 2003, Weinberger et al. 2012, Nascimento et al. 2010).

Adenoviruses

AdVs are non-enveloped, double-stranded linear DNA viruses that belong to the *Adenoviridae* family, *Mastadenovirus* genus. There are known at least 68 known virus types which are classified into seven species (A-G) according to their biophysical, biochemical and genetic characteristics (Harrach et al. 2011). The species correlate with antigenecity, epidemiologic characteristics, clinical manifestations of infection, and also correlate to some antivirals like cidofovir and ganciclovir (Morfin et al. 2009, Lion et al. 2014,). There is no peak season for AdV infections, for they occur evenly worldwide year-round. Earlier studies have frequently shown that recombination can occur between the members of the same species and different AdV species (Lukashev et al. 2008). This means that certain new types of AdVs may have different pathogenicity than old types as well as strong potential towards causing epidemic outbreaks. AdV plays an important role in upper and lower respiratory tract infections. In early wheezing episodes, AdV has been present from 1% to 29% of children (CAmara et al. 2004, Chung et al. 2008). In a recent study AdV was shown to most commonly cause recurrent wheezing which occures in

52% of the children < 14 years with an AdV-induced respiratory tract disease (Calvo et al. 2015). Most common illnesses caused by AdV infections are tonsillitis and bronchitis (Lin et al. 2015). Besides respiratory tract infections, AdVs type 40 and 41 typically viruses cause gastroenteritis through fecal-oral route (Khoshdel et al. 2015).

2.3.5. Coinfections

The development of molecular diagnostic techniques has improved the identification of different pathogens and led to the detection of more than one pathogen in a sample. In the first wheezing episode, virus coinfections have been shown to occur in 13 – 46 % of the cases (Table 1, Jartti et al. 2009, Nascimento et al. 2009). However, one study has found as high as 51% coinfection rate among wheezing children aged <24 months (Miron et al. 2010). In children with lower respiratory tract infection, coinfections with two or more viruses have been found in 45% of the children (Cebey-Lopez et al. 2015). Earlier studies have shown that virus coinfections are especially common in children aged 12-24 months, which might be explained by a slower viral clearance after the primary infection due to an immature immune system (van der Zalm et al. 2009, Huijskens et al. 2012, Cebey-Lopez et al. 2015). By using PCR, as many as four different respiratory viruses have been detected simultaneously in one sample. This makes the clinical interpretation of the results difficult, since the presence of the viral nucleic acids in the sample may be originated from an on-going, past or up-coming infection (Brand et al. 2012). On the other hand, as recently has been shown to happen between HBoV and RV, the virus–virus cross talk may also modulate the immune responses of the viruses (Lukkarinen et al. 2014). In addition, the viral interference has also been observed between RV and RSV (Wisdom et al. 2009).

Some viruses occur more ofte than others in coinfections. RSV and RV have been found frequently in coinfections in wheezing and there is evidence that coinfection with RV and RSV can lead to particularly severe illness (Papadopoulos et al. 2002, Marguet et al. 2009, Garcia-Garcia et al. 2006). Recently found HBoV has also commonly been detected in coinfections. Even up to 88% of the HBoV positive samples have been coinfections with another virus (Brieu et al. 2008, Allander et al. 2007, Schildgen et al. 2008). This might be due to a prolonged shedding without being the causative agent of primary infection. It has been suggested that RV coinfection may increase the risk of recurrent wheezing, however, RV infection alone is the most significant risk factor for recurrent wheezing (Jackson et al. 2008, Jartti et al. 2008). The impact of coinfections on illness severity is varying. There are studies that have shown coinfections to be associated with severity of wheezing illness and respiratory tract infection (Papadopoulos et al. 2002, Lemanske et al. 2005, Cilla et al. 2008, Esposito et al. 2008). Yoshida et al have shown that virus coinfections also increase the risk for lower respiratory tract infection (Yoshida et al. 2013). One earlier study has shown that risk for hospitalization is also increased (Kouni et al. 2012). On the contrary, few recent studies have shown that coinfections are associated with children having a lower respiratory infection (Aberle et al. 2005, Mansbach et al. 2008, Brand et al. 2012).

Table 2. Virus etiology of acute wheezing or wheezing/bronchiolitis in children aged less than 3 years.

1st author, publication year	N	Age (mo)	Atopy	Virus etiology (%)										
				RV	RSV	EV	HBoV	MPV	PIV	Flu	AdV	CV	≥1 virus	≥2 viruses
Rakes, 1999	22	0-24	-	50	83	5.5	-	-	-	-	-	-	81	-
Papadopoulos, 2002	118	0-18	-	21	53	-	-	-	2.5	2.5	7.6	2.5	73	14
Kotaniemi-Syrjänen, 2003	81	1-23	-	33	26	12	-	-	14	-	6.2	-	73	15
Heymann, 2004	79	0-36	-†	33	46	1.3	-	-	13	19	7.6	5.0	84	29
Camara, 2004	74	0-24	+†	20	39	-	-	-	-	-	29	4.1	61	6.8
Bouschambert-Duchamp, 2005	94	0-24	-*	31	51	3	9	-	4.5	1.5	-	-	72	33
Kusel, 2007	236	0-12	+†	69	46	16	-	1.4	-	8.1	4.1	2.7	69	2.7
Jackson, 2008	442	0-36	+†	48	21	2	-	7	12	4	4	5	90	11
Camps, 2008	74	0-12	-*	81	28	-	9.5	31	-	2.7	2.7	1.4	81	15
Chung, 2008	308	0-24	-	-	36		5.8	12	6.2	1.3	<1	<1	63	2.6
Jaques, 2008	192	0-36	-	24	31	9	4	12	2	6	1.5	-	72	5.2
Helminen, 2008	139	0-6	-	6.5	70	-	1.4	-	-	4.3	<1	-	87	4.3
Bisgaard, 2010	481	0-36	-	-	17		3.7	8.3	8.1	6.4	1	13	65	18
Miron, 2010	465	0-24	-	28	76	-	5.4	6.7	-	2.8	2	-	91	51
Laham, 2010	98	0-24	-*	19	66	-	-	-	-	-	-	-	84	16
Smuts, 2011	163	0-24	-	56	-	-	4.9	3.7	-	-	-	2	66	9.2
Fujitsuka, 2011	115	0-36	-	43	53	-	<1	-	<1	-	-	-	86	18
Brand, 2011	142	0-24	+†	30	73	7.0	6.3	4.2	4.2	4.9	9.2	5.6	56	41
Kim, 2015	70	0-24	-	17	56	-	-	20	11	5.7	1.4	2.9	100	13

N, number of patients; Mo, months; RV, rhinovirus; RSV, respiratory syncytial virus; EV, enterovirus; MPV, metapenumovirus; HBoV, human bocavirus; PIV, parainfluenzavirus; Flu, influenzavirus; AdV, adenovirus; CV, coronavirus. Atopy, + defined, - not defined, *family history of atopy only defined, †Personal atopy and family history of atopy defined. Search terms "wheeze", "wheezing" or "bronchiolitis" and virus. Search criteria: bronchiolitis or wheezing, major studies were included, the amount of wheezing episode was not specified. Limits published in English, from year 1993 (Hits 1696).

2.4. Pathogenesis of rhinovirus

RV-A and RV-B replicate mostly in airway epithelial cells, however, the replication of RV RV-A and RV-B has also been found tot ake place in the middle ear and sinuses (Arruda et al. 1995, Bochkov et al. 2011). However, RV-C replication in sinus tissue has been limited to the epithelium but RV-C has been able to grow in cultures of epithelial cells isolated from airway tissue that have been re-differentiated under air-liquid interface conditions (Hao et al. 2012, Ashraf et al. 2013, Bochkov et al. 2011). In contrast to the majority of respiratory viruses, the exposure of airway epithelial cells to RV normally induces a virus-specific cytopathic effect rather than a cytotoxic effect which increases levels of interleukins (IL) (Papadopoulos et al. 2000).

RV deposits nasal or conjunctiva mucosa and is transported to posterior nasopharynx by mucociliary action of epithelial cells (Harris 1996). When RV is attached to the cell surface receptors (ICAM-I, LDLR, CDHR3), it is internalized into an endosome. The subsequent drop in pH causes the virus's positive-sense strand of RNA to uncoat. Virus

RNA is then released to the cytoplasm which leads to the translation of viral proteins. The replication of viral RNA forms negative-sense complementary strands that allow for the transcription of mRNA-like positive strands and an assembly of new viral particles. The new virus particles are released through epithelial cell lysis for which reason the viral particles shed into the neighboring cells (Jacobs et al. 2013). Uncoated viral RNA is recognized by toll-like receptor 3 (TLR3) and toll-like receptor 7/8 (TLR7/8). RV infection enhances the expression of TLR3, which increases the IL-8 production (Figure 1) (Hewson et al. 2005). At the same time, the activation of TLR3 leads to the induction of a retinoic acid inducible gene 1 protein (RIG-I) and a melanoma differentiation-associated gene 5 (MDA-5) which upregulate the innate IFN responses to the RV infection as well as increase the production of T cell and neutrophil cytokines, including the regulated, normal T cell expressed, and secreted (RANTES) (Slater et al. 2010, Triantafilou et al. 2011). RIG-I and MDA-5 recognize the newly synthetized viral RNA in the cytoplasm. The double-stranded RNA produced during viral replication activates TLR3 on the endosomal membrane, which ultimately induces the nuclear factor κB (NF-κB). The sum result of the activation of the RNA sensing proteins is the production of IFNs and other effector molecules with antiviral properties as defense mechanism towards infection (Papadopoulos et al. 2004).

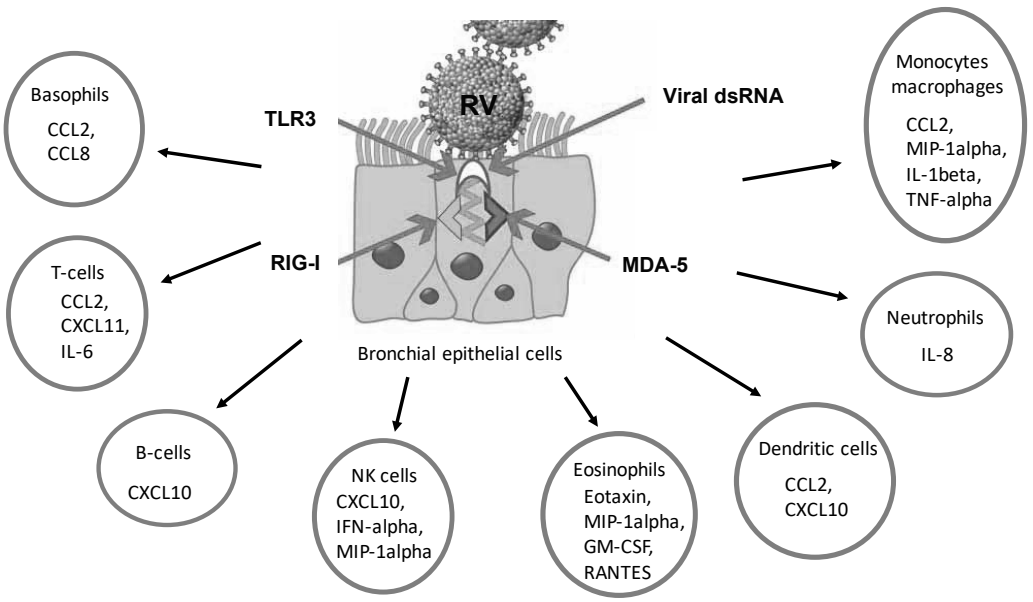

Figure 1. Pathogenesis of rhinovirus infections. RV RNA is recognized by Toll-like receptor (TLR)-3 in airway epithelial cells. This upregulates the retinoic acid-inducible gene (RIG-I), melanoma-associated gene (MDA)-5 and other pattern recognition receptors. Following infection induces the production of a variety of cytokines and chemokines. These recruit and activate inflammatory and immune-effector cells. Basophils, T- and B-cells, NK cells eosinophils, dendritic cells, neutrophils, monocytes and macrophages. IL, interleukin; IFN, interferon; MIP, macrophage inflammatory protein; TNF, tumour necrosis factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; RANTES, regulated on activation normal T-cells expressed and secreted (Modified from Rossi et al. 2013).

RV-infected epithelial cells secrete a variety of acute phase cytokines (TNF-α, IL-6), chemokines (IL-8, RANTES, macrophage inflammatory protein 1α (MIP-1α), and interferon-gamma induced protein 10 (IP-10)) and growth factors. These initiate the respiratory tract inflammatory response, and recruit and activate cellular inflammatory responses to infection presenting with clinical symptoms (Figure 1) (Nurani et al. 2003, Gern et al. 2003, Korpi-Steiner et al. 2006, Proud et al. 2008, Lau et al. 2008, Wang et al. 2009). The production of growth factors in epithelial cells infected by RV supports the role of RV in airway remodeling which is typical for asthma (Leigh et al. 2008). Furthermore, RV has developed a mechanism in order to inhibit antiviral responses by cleaving proteins such as RIG-I and IPS-1, which are involved in virus recognition pathways (Barral et al. 2009, Drahos et al. 2009). However, the specific mechanism is still unclear whether RVs act directly in the pathogenesis of asthma by triggering the destroying inflammatory responses or if they act as a catalyst in stimulating the disease in the genetically predisposed individuals.

2.5. The risk factors for recurrent wheezing and asthma

It has been stated that half of the infants hospitalized for wheezing go on to develop recurrent wheezing. This has led to a speculation that early viral respiratory illnesses promote asthma (Piippo-Savolainen et al. 2007, Gern et al. 2010), which is a reasonable hypothesis because lungs and immune system are developing and vulnerable during infancy (Gern et al. 2005). Several studies have shown that RV-induced wheezing, family history of asthma or allergy, allergic sensitization, decreased IFN responses and genetic predisposition are risk factors for recurrent wheezing and asthma. Early-life aeroallergen sensitization and viral wheezing illnesses are independent and synergistic risk factors for asthma inception.

2.5.1. Virus etiology

RV-induced wheezing has been concluded to be a risk factor for recurrent wheezing and subsequent asthma (Kotaniemi-Syrjänen et al. 2003, Hyvärinen et al. 2005, Matricardi et al. 2008, Kusel et al. 2012, Midulla et al. 2012, Jackson et al. 2008, Jackson et al. 2012, Lukkarinen et al. 2013). In a high-risk cohort study an RV-induced wheezing episode was markedly associated with increased incidence of wheezing at the age of three years (OR 10, 95% CI 4.7, 23) (Lemanske et al. 2005). In this same outpatient cohort, infants with moderate-to-severe RV-induced wheezing were 3 times more likely to experience wheezing during the third year when compared to infants who suffered from RSV-induced wheezing (Lemanske et al. 2005). According to Childhood Origins of Asthma (COAST) high risk birth cohort study, RV-induced wheezing illness during the first three years of life was significantly associated with the development of asthma at age 6 (OR 9.8, 95% CI 4.3, 22) (Jackson et al. 2008). Australian birth cohort study of outpatient children has shown that wheezing caused by either RSV or RV was associated with asthma at the age 5 (Kusel et al. 2007). Interestingly though, this could only be seen in children who had

developed allergic sensitization by the age of 2 years. According to Tucson birth cohort study, children who experienced a RSV-induced lower respiratory tract infection during the first year of their life were 4- and 2- times more likely to have frequent wheezing at the age of 6 and 11 than infection caused by other respiratory viruses (Stein et al. 1999). A prospective controlled study from Sweden showed that RSV bronchiolitis was associated with asthma at the age of 7. However, the RV etiology was not investigated within this study (Sigurs et al. 2000). Also in hospitalized cohorts, RV seems to be an early sign of asthma in atopic asthma-prone children by inducing expiratory wheezing (Kotaniemi-Syrjänen et al. 2003, Lehtinen et al. 2007, Lukkarinen et al. 2015). Even though RV has been demonstrated to be an important risk factor for recurrent wheezing, a recent high risk birth cohort, namely Copenhagen Prospective Studies of Asthma in $Childhood_{2000}$ ($COPSAC_{2000}$), showed that the number of wheezing illnesses during the first year of life was associated with asthma development independent of virus etiology (Bønnelykke et al. 2015).

RV-associated asthma risk has been explained by an increased susceptibility to lower airway RV infection in children with pronounced atopic characteristics (allergen specific IgE sensitization, blood eosinophilia, eczema, maternal atopic eczema, and increased IL-4, -5, and -13 responses in airway seceretions), damaged airway epithelium, decreased IFN α/β/γ/λ and IL-10 responses in airway secretions and genetic factors (Papadopoulos et al. 2002, Wark et al. 2005, Contoli et al. 2006, Johston et al. 2007, Holt et al. 2012). Since aeroallergen sensitization develops slowly, it thereby decreases its value for asthma risk indices and calls for better early risk factors such as RV infections (Illi et al. 2006, Jackson et al. 2009, Jartti et al. 2009). RSV-bronchiolitis has also been shown to be a risk factor for childhood asthma (OR 2.5) (Henderson et al. 2005, Gern et al. 2009). However, wheezing caused by RV is a stronger predictor of recurrent wheezing and asthma than wheezing caused by RSV. COAST study showed that RSV was associated with increased risk of asthma at the age of 6 compared to non-RSV (OR 2.6, 95% CI 1.0, 6.3), and RV together with RSV (OR 10, 95% CI 4.5, 22.2) increased the risk (Jackson et al. 2008). An Australian study showed that RSV and RV coinfection during the first year of life was a risk factor for a recurrent wheezing at the age of 5 (Kusel et al. 2007). The risk for asthma is further increased by allergic sensitization (Kusel et al. 2007, Holt et al. 2011).

2.5.2. Atopic characteristics

Atopic illnesses in early life and viral triggers, especially RV, are true asthma risk factors and the interaction between allergic sensitization and virus infections is possibly involved (Illi et al. 2006, Matricardi et al. 2008, Holt et al. 2011, Durrani et al. 2012, Jackson et al. 2012, Jackson et al. 2016). Several studies have addressed that allergies and asthma may be associated with more-severe viral respiratory illnesses and impaired antiviral responses. It has been shown that children with atopic features are prone to develop additional wheezing episodes. Atopy is one of the strongest risk factors for developing childhood asthma after virus-induced wheezing episodes in infancy (OR 4.6) (Martinez et

al. 1995, Sly et al. 2008). Moreover, both food and aeroallergen sensitizations are major risk factors of asthma, and their early appearance serves as a feasible tool in predicting asthma in young wheezing children (Kusel et al. 2007, Nissen et al. 2013, Chiu et al. 2014, Lukkarinen et al. 2015).

The COAST study has shown the causality of an early-life aeroallergen sensitization preceding an RV illness and asthma (Jackson et al. 2008, Jackson et al. 2012). The COAST study presented the synergistic effect of allergic inflammation and RV infection. The study showed that the children who has the greatest risk for asthma inception were those who had suffered from aeroallergen sensitization (OR 3.6) and RV-induced wheezing (OR 2.8) during the first 3 years of their life (Jackson et al. 2008). There is also evidence that early life allergic sensitization has its strongest effect on recurrent wheezing and asthma if the process occurs during the first few years of the child's life (Kusel et al. 2007, Sly et al. 2008, Simpson et al. 2010, Stoltz et al. 2013). Children with RV infections both sensitized and exposed to aeroallergens are the most prone to have recurrent wheezing and asthma exacerbations (Heymann et al. 2004, Soto-Quiros et al. 2012). However, RV-induced wheezing does not increase the risk for subsequent allergic sensitization but the early appearance of both food and aeroallergen sensitization is high in asthma prone children (Kusel et al. 2007, Jackson et al. 2012, van der Gugten et al. 2013). Allergic inflammation in the airways lead to impaired barrier function, increased airway responsiveness and enhanced mucous production (Kloepfer et al. 2010). In relation to this, allergic asthma has been associated with deficient innate immune responses to RV (Figure 2) (Wark et al. 2005, Contoli et al. 2006, Durrani et al. 2012, Sykes et al. 2012, Baraldo et al. 2012).

As a mechanism for synergistic effect of allergic sensitization and virus infection, it has been proposed that allergens could damage the air epithelium and enhance the absorption of virus replication, thus increasing the susceptibility to inflammation which in turn may lead to a severe infection (Lopez-Souza et al. 2004, Jakiela et al. 2008, Lachowicz-Scroggins et al. 2010). Also, RV infection stimulates cytokines, for example TLR3 dependent secretion of thymic stromal lymphopoietin (TSLP), that enhance allergic inflammation (Kato et al. 2007). This leads to the counteraction of Th1 (IFN-γ and IL-10) and Th2 cytokines (IL-4, IL-5, IL-13), which may explain the tendency for RV infection in allergen sensitized children. Hence, the allergic sensitization upregulates Th2-type cytokines, which increases the expression of ICAM-1 favoring the RV infection (Figure 2) (Martinez et al. 1995, Sigurs et al. 2000). The RV infection then further embellishes the ICAM-1 expression amplifying the inflammatory response to allergens (Sethi et al. 1997, Papi et al. 1999). A vicious circle of events is thus created. It may modify the host antimicrobial defence and weaken the ability to fight against virus infections via immune alteration (Xatzipsalti et al. 2008). Moreover, RV infections and allergens have been found to enhance the airway epithelial cell production of IL-33, which also promotes type 2 airway inflammation and compresses the induction of Th1 cytokines (Message et al. 2008, Mehta et al. 2016). IL-33 polymorphism has been linked to the intermediate and late-onset wheezing and allergic sensitization (Savenije et al. 2014). There is also evidence that allergic sensitization directly impairs

antiviral responses, as virus-induced type I and III IFN (IFN-β and –λ) production from plasmacytoid dendritic cell from patients with allergic asthma is impaired by cross linking of the high affinity IgE receptor FceRI (Gill et al. 2010, Durrani et al. 2012). The impaired pDC IFN production may also lead to an enhanced Th2 inflammatory response in the airways (Huber et al. 2010, Pritchard et al. 2012).

In addition to allergic sensitization, the presence of eosinophilic inflammation together with RV infection has been identified as a risk factor for acute wheezing episodes (Heymann et al. 2004, Soto-Quiros et al. 2012, Midulla et al. 2014). Midulla et al showed that blood eosinophilia together with RV infection associated with recurrent wheezing (Midulla et al. 2014). Furthermore, it has also been found to be associated with the persistence of asthma (Hyvärinen et al. 2010). Eczema is the earliest manifestation of atopic illness followed by food allergy, wheezing/asthma and allergic rhinitis. A meta-analysis of four birth cohorts and nine eczema cohorts have shown OR 2.1 for the risk of asthma after eczema (van der Hulst et al. 2007). It has been also shown that maternal atopic asthma is a risk factor for RV-induced but not for RSV-induced wheezing (Carroll et al. 2012).

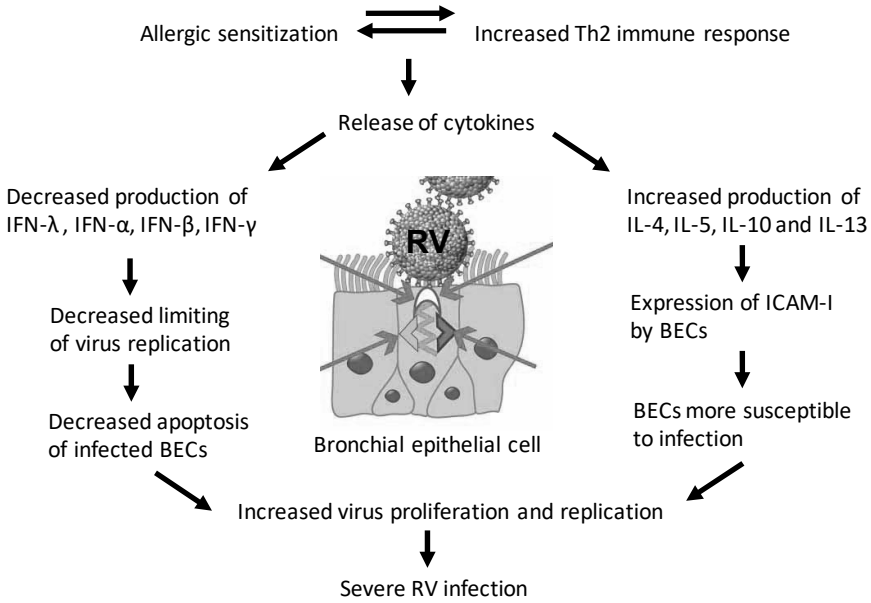

Figure 2. Factors contributing to the illness severity of RV infection in allergic subjects. BEC, bronchial epithelial cell; IL, interleukin; IFN, interferon (Modified from Rossi et al. 2013).

2.5.3. Changes in immune responses

Impaired innate immune response of the asthmatic airway epithelium is a result of deficiencies in antiviral response of both epithelial and immune cells. Deficiencies in innate and adaptive immunity are likely to increase the risk of viral lower respiratory tract infection especially in children with high risk for asthma and allergies (Tulic et al. 2007).

RV infections have shown to stimulate TLR3- dependent secretion of thymic stromal lymphpoietin (TSLP), a cytokine that can enhance allergic inflammation (Figure 2). There is also evidence that RV replication is enhanced in mucus-secreting goblet cells, which are present in greater numbers in the airways of individuals with asthma than in healthy persons (Lachowicz-Scroggins et al. 2010). Furthermore, weak blood cell IFN responses at the time of birth has been demonstrated to be a risk factor for more severe respiratory illnesses, including wheezing i (Copenhaver et al. 2004, Gern et al. 2006). The decreased IFN expression is associated with airway inflammation caused by RV which leads to slow viral clearence, improved virus replication and more severe airway inflammation (Contoli et al. 2006, Busse et al. 2010).

Severe RV infections has been associated with low IFN-γ levels (Aberle et al. 2004). RV is endocytosed by epithelial cells and is recognized primarily by TLR3 in early infection and by RIG-I and MDA-5 in later infection following upregulation of pattern recognition receptors (PRR), and further increasing RV replication and reducing the induction of IFN-λ (Wang et al. 2009, Holgate et al. 2011). It has been shown that in connection to RV infection, asthmatic patients develop less INF-β and –λ than non-asthmatic patients, which makes asthmatic patients more susceptible to infection facilitating viral replication as well as slower to clear the infection (Wark et al. 2005, Contoli et al. 2006). It has also been reported that RV has enhanced a deficient INF-λ production in asthmatic primary bronchial epithelial cells which brings forth the possibility of asthma being associated with a global defect in IFN production (Figure 2) (Contoli et al. 2006, Wark et al. 2005). Baraldo et al showed that the production of IFN-λ and IFN-β is impaired in RV infections independent of atopic status or asthma (Baraldo et al. 2012). Furthermore, decreased IFN-α and IFN-β production has been shown to also in connection to RV-induced wheezing (Figure 2) (Sykes et al. 2012). It has also been demonstrated that Th1 responses (production of IFN-λ and IL-10) are associated with fewer respiratory symptoms, whereas Th2 responses, such as IL-4, IL-5, and IL-13, are associated with an increase of respiratory symptoms in RV-induced lower respiratory illness (Message et al. 2008). This implicates that in connection to RV infection, Th2 immune responses are increased, whereas Th1 immune responses are decreased, thus shifting the balance of Th1/Th2 toward Th2. This in turn may lead to disrupted pulmonary functions, postnatal lung growth, and persistent asthma as the end result.

The association between RV wheeze and pre-existing, partly atopy related, inflammation in the airways is also supported by the efficacy of prednisolone in the treatment of early wheezing (Lehtinen et al. 2007, Lukkarinen et al. 2013). In asthmatic patients, the epithelium is exhibited with cellular damage. Broken epithelial cell surface has also shown to associate with a higher level of RV replication than intact cell surfaces, which may have an effect on illness severity (Jakiela et al. 2008). One study discovered that RV infection reduced the self-repairing capacities of bronchial epithelial cells in culture models of epithelial damage (Bossios et al. 2005).

2.5.4. Genetics

An intriguing gene-environment interaction related to asthma inception and early life RV infection was recently defined. Namely, it was shown shown that variants in the 17q21 locus of GSDMB and ORMDL3 genes are closely associated with RV-induced wheezing and subsequent asthma specifically in children who developed wheezing illnesses in connection to RV infections during early life (Caliskan et al. 2013). The effect of this genetic variant has been found to be larger in children exposed to environmental tobacco smoke suggesting gene-by-environment interaction (Bouzigon et al. 2008). Moreover, CDHR3 has been demonstrated to be a susceptible gene for wheezing illnesses and hospitalizations in children with asthma (Bonnelykke et al. 2014). As mentioned earlier, CDHR3 is also associated with increased RV-C binding and replication (Bochkov et al. 2015). Furthermore, maternal asthma has been associated with RV caused wheezing (Carroll et al. 2012).

2.5.5. Exposure to smoking

Parental smoking has been associated with poorer lung function and an increased risk for asthma in asthmatic children (Cook et al. 1998, Spanier et al. 2006). Children suffering from wheezing have also been shown to have poorer lung function and modified bronchial inflammation when maternal smoking was present (Kalliola et al. 2013). Furthermore, cigarette smoke extract in the epithelial cells suppresses RV-induced expression of a substantial number of epithelial cells that plays a role in direct host antiviral defence as well as molecules involved in innate immune responses that contribute to antiviral immunity (Proud et al. 2012).

2.5.6. Lung function

Research has revealed that by school-age the lung function of children with persistent wheezing has diminished (Morgan et al. 2005). Studies on lung function identify preschool years as the critical window for intervention in order to prevent loss of function of the lung over time. Low lung function may work as a risk factor for wheezing. RV-induced wheezing is associated with deficient immune responses, which may lead to lower lung function by increasing bronchial responsiveness (Wark et al. 2005, Contoli et al. 2006, Malmström et al. 2006). It has been discovered that young wheezing children have an increased number of proinflammatory cells in bronchoalveolar lavage (BAL) (Krawiec et al. 2001). Another study showed by the age of 3, children with recurrent wheezing had developed a reticular basement membrane thickening and an eosinophilic inflammation, both common features of childhood asthma (Saglani et al. 2007). Illi et al have concluded that there is an association between reduced lung function and children with early allergic sensitization and allergen exposure (Illi et al. 2006). Children with RV-related wheezing before the age of three have been shown to more likely suffer from reduced lung function by school age than children whose wheeze is caused by RSV or any other virus (Guilbert et al. 2011). Earlier findings have suggested that children who have bronchiolitis and

subsequent wheezing illnesses are genetically predisposed to also have diminished lung function (Young et al. 1995, Turner et al. 2002). One study showed that the increased total lung resistance measured at the age of 2 months was associated with subsequent RV-induced wheezing (van der Zalm et al. 2011).

2.6. Diagnosis of wheezing illnesses

2.6.1. Clinical diagnosis

Wheezing is a clinical diagnosis based on symptoms and signs. It is based on the pulmonary auscultation findings. High-pitched whistling sound can be heard in expiration, and sometimes also in inspiration. The work of breathing is increased, and subcostal, intercostal and supraclavicular recessions may be seen (Lakhanpaul et al. 2009). In addition to lung auscultation, oxygen saturation is commonly used to predict the need for supplementary oxygen. Low oxygen saturation levels (<92%) predict a more severe disease and longer length of stay than high oxygen saturation levels (Pruikkonen et al. 2014). Fever may be present, but high fever (≥39 °C) is not common (Scottish Intercollegiate Guidelines Network 2014). Rhinorrhoea preexists the onset of other symptoms, for example tachypnea, cough, and respiratory distress (Fitzgerald et al. 2004, Rakshi et al. 1994). Dry cough and increased respiratory rate are typical in wheezing and might be the earliest symptoms. Chest radiography is needed if the course of disease is atypical, there is no response to treatment, or there is diagnostic uncertainty.

The clinical history of the patient is important because a history of eczema and rhinitis as atopic conditions increases the probability of asthma. A raised specific immunoglobulin E (IgE) to wheat, egg, inhalent allergens such as house dust mite and cat dander predict later childhood asthma (Sears et al. 1989, Kotaniemi-Syrjänen et al. 2003). Positive skin prick tests and raised blood eosinophil count are related to the severity of current asthma and its persistence through childhood. Information concerning family history regarding atopy indicates the risk for recurrent wheezing. Maternal atopy is a strong risk factor for the onset of childhood asthma and recurrent wheezing (Martinez et al.1995, Rona et al. 1997, Tariq et al. 1998, Rusconi et al. 1999). Abnormal lung function is always examined when the test can be performed, since abnormal lung function detected by spirometry or bronchial hyperresponsiveness, and an increased airway responsiveness during childhood are associated with recurrent wheezing (Toelle et al. 2004).

2.6.2. Laboratory tests and rhinovirus diagnostics

From blood samples, C-reactive protein, leukocyte level and blood gas analyses are not routine practice but may be measured for differential diagnosis and for prediction of illness severity in wheezing illnesses (Scottish Intercollegiate Guidelines Network 2006). Virus specific diagnosis is not routinely performed except the rapid RSV test which is used to allocate patients into cohorts in hospitals and guiding isolation during epidemic in Finland. However, depending on country, some clinics use full respiratory virus panel including RV,

RSV, Flu, PIV, MPV, Mycoplasma, AdV for children seen in emergency clinic or admitted to the ward. For studying the upper respiratory tract infections, nasopharyngeal swabs or aspirates are preferred (Tapiainen et al. 2016). The virus etiology is more thoroughly analyzed when the patient is severely ill and intensive care unit treatment is needed (Tapiainen et al. 2016).

The first PCR-based assays able to detect RV in respiratory samples were reported in the late 1980s. The detection of virus genome without virus cultivation resulted into an the increase of the RV detection rate. The new method also enabled the identification of virus types and discovery of new virus types. Currently, PCR is the method of choice in diagnosing entero- and RVs. PCR primers designed for the conserved 5' untranslated region of the virus genome enables the detection of virtually all entero- and rinovirus strains (Hyypiä et al. 1989, Torgersen et al. 1989, Gama et al. 1988, Lönnrot et al. 1999, Kares et al. 2004).

Since the discovery of diagnostic picornavirus RT-PCR tests various applications have been published (Lönnrot et al. 1999, Volle et al. 2012, Kares et al. 2004). A common method for differentiating RVs and EVs is to use virus-spesific probes or primers (Kares et al. 2004, Tapparel et al. 2009, Lönnrot et al. 1999). Virus identification is also possible by for example melting curve analysis, sequencing or mass spectrometry (Österback et al. 2012, Piralla et al. 2009). Since it has been reported that higher RV loads correlate with symptomatic disease (Gerna et al. 2009, Utokaparch et al. 2011). Therefore, the use of quantitative RT-PCR assays may help in the interpretation of the clinical significance of the PCR positive results. However, the collection of samples and method used should be standardized. No commercial quantitative PCR assays are available at the moment.

Currently, the sequencing of 5' NCR is commonly used in RV diagnostics. However, a capsid protein sequence is needed in order to determine the genetic type of RV strains (Savolainen-Kopra et al. 2009, McIntyre et al. 2013). The 5' NCR sequencing can be used used for typing rhinoviruses into species level (A, B and C) when the nucleotide homology achieves 96% (Miller and Mackay 2013). The nucleotide sequence identity should exceed 90% to identify the RV type from VP4/VP2 region of the genome and 87% when VP1 region is used (McIntyre et al. 2013). Several methods for RV typing from 5' NCR have been developed and validated with RV prototypes and clinical isolates (Bochkov et al. 2014, Lee et al 2007, Peltola et al. 2008). However, the VP4/VP2 genome region is widely used for typing RVs in clinical specimens (Savolainen et al. 2002, Jin et al. 2009; Linsuwanon et al. 2009). VP1 genome is the formal way of determining the genetic type pf RV types and is also used for identifying and analyzing new RV-C (and other RV) types (McIntyre et al. 2013).

Cell culture has been the standard method for RV isolation (Bochkov et al. 2011). Fetal embryonic lung or human foreskin fibroblast cell lines and HeLa cell lines are most commonly used for culturing RVs. Inoculated cultures are maintained at a neutral pH

because RVs are acid sensitive (Laundry 2011). The optimal temperature for RV growth is 33°C – 34°C, although some strains also grow well at 37°C (Papadopoulos et al. 2000, Ashraf et al. 2013). RV-C types are unable to grow in standard cell lines used for virus cultivation due to the absence of the cellular receptor needed for virus internalization (Bochkov et al. 2011).

The existence of over 160 RV types and the considerable antigen diversity between viruses have hampered the development of antigen dectection and serologic tests. Virus neutralization has been the method of choice for RVs. Complement fixation has low sensitivity (Blomqvist et al. 2002). Recently, cross-reactive VP1 and VP4 antigens between RVs have been successfully raised of serological tests for RVs (Katpally et al 2009, Niespodziana et al. 2015). There are promising results in that the discrimination of infections caused by different RV groups could be done by different antibody reactivity against VP1 epitopes (Niespodziana et al. 2015). In practice, serological tests are used for seroepidemiological studies and they do not have a role in the diagnosis of acute RV infections. Currently, no antigen detection tests are available for clinical use.

2.7. Treatment of wheezing and asthma

Acute virus induced wheezing is treated using bronchodilator (such as inhaled short-acting β2 agonists) as needed for relieving the symptoms during the wheezing episodes (Global initiative for asthma 2015). If the bronchodilatator is not effective enough in the acute wheezing episodes, ipratropiumbromide can be used in acute wheezing episodes (Global initiative for asthma 2015). It is recommended to use supplementary oxygen if the oxygen saturation drops too low (≤92%) (Global initiative for asthma 2015, Asthma: Current Care Guidelines 2012, Scottish Intercollegiate Guidelines Network 2014). For intermittent asthma symptoms the bronchodilator treatment is the recommended course of action (Global initiative for asthma 2015).

Regular controller medication for childhood asthma is indicated to be initiated when a child has had >3 wheezing episodes lasting more than 1 day during the course of one last year (Asthma: Current Care Guidelines 2012, Global Initiative for Asthma 2015, NAEPP guidelines 2007). The criteria for initiation of regular controller medication are that wheezing episodes have affected sleep and a child has a positive asthma risk profile. A positive asthma risk profile consists of either one of the following: parental history of asthma, physician diagnosed atopic dermatitis, or evidence of sensitization to aeroallergens or 2 of the following: sensitization to foods, ≥4 percent blood eosinophilia, or wheezing apart from colds. Regular controller therapy for 3 months may be considered when a child requires bronchodilator treatment for more than 2 days per week or more than four weeks (Global Initiative for Asthma 2015, NAEPP guidelines 2007, Asthma: Current Care Guidelines 2012). Also therapy should be considered if a child has 2 exacerbations requiring oral corticosteroid treatment during last 6 months or a new severe wheezing episode in 4-6 weeks (Chang et al. 2013, NAEPP guidelines 2007).

When a child is having persistent symptoms of asthma, the recommendation for the treatment is to use inhaled corticosteroid (ICS) or montelukast given orally or inhaled cromolyn as alternative medications (Global Initiative for Asthma 2015, NAEPP guidelines 2007, Asthma: Current Care Guidelines 2012). A short course of systemic corticosteroid can be considered if the symptoms are severe or the patient has a history of previous severe exacerbations of asthma or wheezing (Global Initiative for Asthma 2015, NAEPP guidelines 2007, Asthma: Current Care Guidelines 2012).

2.8. Prevention and treatment of RV induced wheezing

There is substantial evidence of the role of RVs role in early wheezing and asthma inception. Preventing RV infection and keeping an eye out early on in childhood for all the known risk factors of wheezing and asthma and are therefore important targets for clinical work and research. Understanding the host-pathogen interactions that determine the severity of respiratory illnesses and long-term sequel of respiratory illnesses, would be of great help in identifying the high-risk children and in designing new and effective treatments and preventive strategies (Gern et al. 2010).

A recent study has shown that during the first 3 years of life the timing of sensitization to aeroallergens and RV-induced wheezing has a strong influence on asthma risk in adolescence (Rubner et al. 2016). Based on the clear associations between allergic sensitization, RV-induced wheezing and asthma inception and exacerbation, it is logical to focus interventions on the prevention of allergic sensitization and allergic inflammation. In USA, the use of Omalizumab, a monoclonal antibody directed at IgE, has had a significant benefit on the care of asthma symptoms by blocking IgE-mediated inflammation in patients who are sensitized and exposed to allergens and augmenting type I IFN response (Busse et al. 2011, Teach et al. 2015). This could be a potential approach to prevent asthma in high risk children. There is also a great interest in the role of early microbial exposures in the development of allergic sensitization, viral wheezing and asthma (Han et al. 2012). It has been suggested that early life microbial exposures, such as early life at farm and pet exposure, may decrease the risk for the development of allergic diseases (Ownby et al. 2002, Remes et al. 2005, Ege et al. 2011). Analysis of gene markers can also be a potential mechanism for the prevention, since the 17q21 locus in genes GSDMB and ORMDL3 and CDHR3 gene are associated with RV-induced wheezing (Caliskan et al. 2013, Bonnelykke et al. 2014). Interestingly, a recent study reported that independent from virus etiology the high MIP-1α in the first wheezing episode may predict recurrent wheezing episodes in the first wheezing episode in children with virus infection (Sugai et al. 2016).

Currently, there is no available therapeutics that could prevent or treat RV infection (Bardin et al. 2004, Gern et al. 2010). Because of the high number of different RV types, high rates of virus infections in early life, and relative immaturity of the infant immune system, the development of vaccine has remained challenging, albeit attempts in producing an effective vaccine are ongoing (McLean et al. 2012, Hansbro et al. 2008, Rosenthal et al.

2010, Bizzintino et al. 2011). Furthermore, recently discovered RV-C may have been the obstacle preventing the development vaccinations. Because of the correlation between RV-C and illness severity, it would seem important to aim toward RV-C in therapeutics (Miller et al. 2009). Also, it needs to be determined whether certain subtypes of RV are more asthmagenic than others, as the development of therapeutics needs to focus on those (Stone et al. 2015). Because of the challenges in vaccine development, specific antiviral drugs designed to inhibit RV attachment, entry to the cell, viral uncoating, and RVA and protein synthesis have been considered to be more effective in preventing RV infection than vaccination (Rollinger et al. 2011). Specific anti-RV neutralizing antibodies, anti-receptor antibodies, and soluble receptor molecules can prevent virus attachment. In theory, the prevention of RV to adhere the binding sites, ICAM1 and CDHR3, would be an effective way to stop RV infection (Charles et al. 2003, Fang et al. 2004). Pleconaril, a capsid binding protein, is one example which lead to decreased cold symptom scores and shortened duration of illness. The further development was stopped, however, because of the side effects and medication interactions (Hayden et al. 2003).

The efficacy of systemic corticosteroid in viral wheezing episodes has been conflicting when virus specific analyses are not carried out (Horowitz et al. 1994, Oommen et al. 2003, Csonka et al. 2003, Jartti et al. 2006, Jartti et al. 2007, Panickar et al. 2009). In RV specific study performed among children with RV-inuduced wheezing, the length of hospitalization was shorter in prednisolone group than in placebo group among children with RV-induced wheezing (Jartti et al. 2006). Furthermore, in a post-hoc study, prednisolone reduced the recurrent wheezing after RV-induced first wheezing episode (Lehtinen et al. 2007, Lukkarinen et al. 2013). The efficacy of prednisolone in RV affected children might be explained by the pre-existing, partly atopy related, inflammation in the airways and that prednisolone might downregulate this inflammation when administered early (Stellato et al. 2007, Holt et al. 2012, de Benedictis et al. 2012). An important mechanism of the drug might be its ability to decrease the transcription of many inflammatory genes and their transcription factors and to induce the expression of a number of anti-inflammatory genes (Stellato et al. 2007, de Benedictis et al. 2012). Furthermore, oral glucocorticoids suppress RV-induced up-regulation of its receptor ICAM-I in pulmonary mucosa (Papi et al. 2000). Short-term benefits of prednisolone have been varying (Alansari et al. 2013, Jartti et al. 2006, Plint et al. 2009, Bisgaard et al. 2006, Busse et al. 2011). However, the lack of rapid bed side test for RV is a challenge for clinical trials involving treatments for RV infection (Jartti et al. 2013).

3. Aims of the study

The specific aims for this study were:

1. To study the virus etiology of the first wheezing episode in children aged 3 – 23 months and how the virus etiology is associated with clinical characteristics and illness severity (*Study I*)

2. To analyze underlying RV species and their clinical characteristics of children with the first wheezing episode (*Study II*)

3. To examine the clinical and virus surveillance after the first wheezing episode with special focus on RV-A and RV-C species (*Study III*)

4. To determine the short- and long-term efficacy of oral corticosteroid, prednisolone, in the first RV-induced wheezing episode in children aged 3 – 23 months (*Study IV*)

4. Materials and methods

Details of the material and methods are presented in the original publications.

4.1. Enrollment and intervention

All four studies were based on Vinku2 (vinku means wheeze in Finnish) prospective study for which the patients were consecutively recruited in Finland between June 2007 and March 2010 from the outpatient clinic of the Department of Pediatrics (21%), or the Pediatric Infectious Diseases Ward in the Turku University Hospital (79%). The inclusion criteria were the age of 3-23 months, delivery at 36 gestational weeks or later, the first wheezing episode based on parental report and confirmed from medical records, and an informed consent from a parent or a guardian. The child was excluded from the study if a chronic illness other than atopy was present, a history of previous inhaled or systemic corticosteroid treatment, varicella contact in a patient without a previous varicella illness, the child's caretaker had a poor understanding of Finnish and/or there was a need for intensive care. Study physician/nurse or on-duty physician/nurse told the guardian about the study and gave a written information leaflet concerning the study. The study was commenced once the guardian had signed the informed consent form (Appendix 1).

The study physician or on-duty physician clinically examined the study subject, and verified wheeze and breathing difficulty. Every new patient was examined between 8 am and 10 pm 7 days per week. Thereafter, the physician examined and recorded symptoms, medications, and the use of supplementary oxygen daily at the ward (Appendix 2). NPA sample was taken for virological analyses and blood was drawn at the study entry. A total of 125 children were eligible for the study. Of them, 12 declined and 2 were excluded from the analysis because of laryngomalasia and foreign body in the airways. Finally, 111 children aged 3-23 months with first wheezing episode were included.

To be eligible for *Study IV*, children had to have with RV infection and on-going signs of lower respiratory tract symptoms (cough, noisy breathing, or wheezing) at the time when PCR results were available. It was a randomized, placebo-controlled, double-blind trial that investigated the efficacy of the systemic corticosteroid treatment. A total of 74 children were randomized for the study treatment. They received either oral prednisolone or a matching placebo. The first dose of the study drug was 2 mg/kg, followed by 2 mg/kg per day in 2 divided doses for 3 days. Maximum dose was 60 mg per day. Prednisolone 5 mg tablets were used. Hospital nurses independent of the study administered the drug while children were hospitalized. The randomly assigned drug was initiated immediately when the RV etiology was confirmed and the child fulfilled the study criteria. The oral prednisolone and placebo were both obtained from Leiras Takeda (Helsinki, Finland). The study physician arranged scheduled follow-up visit after 2 weeks, 2 months and 12 months. A double blind randomized controlled trial (RCT) design was used for this study.

The study protocol was registered at ClinicalTrials.gov in August 2008 (ClinicalTrials.gov number NCT00731575).

4.2. Monitoring

The guardian was interviewed using a standardized health questionnaire including questions on the host and environmental risk factors for asthma (Appendix 1). The respiratory symptom score was calculated each day if the child stayed in a hospital. The respiratory symptom score was the sum of scores for the degree of dyspnea (0 non, 1 mild, 2 moderate, 3 severe), type of breathing (0 normal, 1 use of stomach muscles, 2 use of intercostal muscels, 3 nasal flaring), severity of auscultation findings on wheezing (0 none, 1 expiratory, 2 inspiratory and expiratory, 3 audible without a stethoscope) and assessment of expiratory : inspiratory time ratio (0 = 1:2; 1 = 1:1; 2 = 2:1; 3 = 3:1) (Appendix 2). After discharge, the guardian recorded daily respiratory symptoms (rhinitis, cough, expiratory breathing difficulty, noisy breathing, and nocturnal wakening because of breathing difficulties) and medication in a diary for two weeks. The symptom severity was assessed on a 4-graded scale for the next 2 weeks as well (Appendix 3). Patients were discharged from the hospital when the difficulty of breathing had subsidedd.

Blood eosinophil counts, C-reactive protein and leukocyte levels, and serum levels of allergen specific IgE were analyzed from blood samples by routine diagnostic procedures at the Central Laboratory of Turku University Hospital.

For *Study III*, follow-up visits were arranged at 2-weeks, 2-months and 12-months after the enrollment. The guardians were asked to fill out a symptom and medication diary for the first 2 months of the follow-up period. For the first two weeks they were asked to assess the symptom severity on a 4-graded scale and thereafter to fill in the dates of expiratory breathing difficulties, respiratory medications, and visits to health care providers (Appendix 3, Appendix 4). Blood samples were taken also at 12-months visit. Nasopharyngeal swab samples were also taken at each follow-up visit. During the follow-up period the guardian was instructed to bring the child to the hospital each time the child had breathing difficulties. At these acute visits the study physician confirmed the diagnosis, filled out a symptom diary and an NPA sample was taken.

4.3. Definitions

Wheezing was defined as an expiratory breathing difficulty with a high-pitch sound by auscultation during expiration. Atopy was defined as a positive IgE antibody result (≥0.35 kU/L) to any of the following allergens: codfish, cow's milk, egg, peanut, soybean, wheat, cat, dog, horse, birch, mugwort, timothy grass, *Cladosporium herbarum*, and *Dermatophagoides pteronyssinus* (Phadiatop Combi®, Phadia, Uppsala, Sweden). Aeroallergen referred to apositive IgE antibodies to any of the last 8 allergens. Perennial aeroallergen sensitization was defined as a positive IgE antibody result to dog, cat, or

Dermatophagoides pteronyssinus. Birch, mugwort, timothy grass, and *Cladosporium herbarum* were considered as seasonal allergens. Atopic eczema was defined as a physician diagnosed eczema with typical symptoms including pruritus, typical morphology, and chronic type of illness and child being atopic. The term 'atopic characteristics' included specific sensitization as demonstrated by positive IgE antibody, eczema and/or blood eosinophil count ≥0.4 x 10^9/l.

4.4. Sampling

The NPA sample was taken through a nostril with a disposable catheter connected to a mucus extractor. A nasopharyngeal swab (nylon flocked dry swab, 520CS01; Copan, Brescia, Italy) was dipped into the NPA, transported to the laboratory during the same day and stored at -70°C. For follow-ups, nasopharyngeal swab samples were collected using a sterile cotton swab which was placed into dry and sterile vials and transported at room temperature to the laboratory and stored at -70°C. Serum samples were collected at study entry, 2 weeks' and 12 months follow-up visits and stored at -70°C.

4.5. Laboratory analyses

Prior to the extraction of nucleic acids, 1 ml of phosphate-buffered saline was added to the nasopharyngeal swabs and mixed. For "in-house" RT-PCR detecting simultaneously RV, EV and RSV, nucleic acids were isolated using a commercial kit (High Pure Viral Nucleic Acid Kit, Roche Diagnostics, Mannheim, Germany) according to manufacturer's instructions. For the other PCR analyses, 550 µl of naspharyngeal sample in phosphate-buffered saline (PBS) was added to lysis buffer and mixed. The nucleic acids were extracted from 550 µl to elution volume of 55 µl using or NucliSens EasyMag automated extractor (bioMèrieux, Boxtel, the Netherlands) according to the manufacturer's instructions. The extracted nucleic acids were stored at -70°C.

At study entry, NPAs were analyzed within 3 days for RV, EV and RSV. RT-PCR amplification was performed with picornavirus primers (4- and 3+) from the conserved 5' NCR of the genome (Lönnrot et al. 1999). cDNA reaction was carried out in a mixture of 8,9 µl sterile water, 8 µl M-MLV RT 5X buffer (Promega), 8 µl 100mM dNTP (Fermentas), 0,1 µl RNase-inhibitor (RiboLock™ 40u/µl, Fermentas), 0,1 µl RT-enzyme (RevertAid™ H Minus M-MuLV Reverse Transcriptase 200u/µl) and 4,9 µl reverse primer 4-. The cDNA reaction was performed with 30 µl of cDNA reaction mixture and 10 µl of RNA at 42 °C for 1 hour using Perkin Elmer Cetus DNA Thermal Cycler –machine.

PCR reaction mixture contained 4,5 µl sterile water, 1,5 µl of primers PR3+ and PR4-, and 12,5 µl of SybrGreen (Maxima™ SybrGreen qPCR Master Mix). "In-house" PCR was performed with Rotor-Gene 6000 (Corbett Research) from 5 µl of cDNA and 20 µl of PCR reaction mixture. The amplification conditions were 95 °C 15 min, followed by 45 cycles at 95 °C for 15 min, 65 - 55 °C for 30s (touchdown 1°C/cycle for the first 10 cycles), and

72 °C for 40s (melt 72 - 95 °C, 0,5°C/s). RV1B was used as a positive control. Positive amplicons were identified as RV, EVs and RSV according to melting temperatures.

A multiplex PCR (Seeplex RV12 ACE Detection; Seegene, Seoul, Korea) test was done for all nasopharyngeal samples. It was used for the detection of RV, RSV, PIV1-3, MPV, AdV, CV (229E, NL63, OC43, and HKU1), and influenza virus A and B according to manufacturer's instructions. PCR products were analyzed by Screentape machine (Lab901 ScreenTape®System).

HBoV was analyzed using PCR and serology. HBoV PCR was carried out at the Department of Virology in University of Turku (Allander et al. 2007). HBoV serology was analyzed from paired serum samples collected 2-3 weeks apart at the Haartman Institute, Helsinki, Finland as previously described (Söderlund-Venermo et al. 2009). All serum samples were analyzed for IgG and IgM antibodies against HBoV1 using enzyme immunoassay (EIA). Diagnostic criteria for acute HBoV1 infection were seroconversion or a $\geq$4-fold increase in virus specific IgG antibody levels in paired serum samples taken 2-3 weeks apart, and a positive IgM result.

Quantitative RT-PCR

The RV viral load was analyzed from RNA of RV positive samples by a quantitative RT-PCR using known concentrations of RV-B14 plasmid. As a standard, the range of the assay was 10^1 – 10^5 viral genome copies / 5 µl. For quantitative RT-PCR, the same PCR reaction mixture and amplification conditions were used as in the "in-house" RT-PCR. The plasmid with known quantity was received from Glyn Stanway at the University of Colchester (Essex, United Kingdom).

RV sequencing

RV genotypes were analyzed from the 5' NCR and partial VP4/VP2 region of the genome using primers shown in Table 3. The sequencing of 5' NCR was performed as earlier described (Peltola et al. 2008). The reverse primer was the same as used in "in-house" RT-PCR. The cDNA was produced as described above and the same PCR reaction mixture was used.

Three sets of primers were used for sequencing VP4/VP2 region (Wisdom et al. 2009, Linsuwanon et al. 2009, Bochkov et al. 2014) (Table 3). The cDNA reaction mixture contained 5 µl of RNA, 4.0 µl of 5xBuffer, 4.0 µl of dNTP, 2.0 µl of primer, 0.1 µl of Rnasin inhibitor and RT enzyme and 4.8 µl of nuclease free water adding the total volume of 20 µl. (Bochkov J Clin Microbiol 2014, Wisdom et al. 2009, Linsuwanon et al. 2009). In cDNA reaction, the primer used was 5'-AGGCCGGTGAAGGGDATNGTRAA-3' when using primers from Bochkov et al in PCR (Bochkov et al. 2014). A random primer from Thermo Fisher Scientific was used for in cDNA reaction when using primers Linsuwanon and Wisdom et al (Linsuwanon et al. 2009, Wisdom et al. 2009).

The PCR reaction mixture consisted of 5 µl of cDNA, 12.5 microL of QuantiTect SYBR Green PCR mix (Qiagen, Venlo, Netherlands), 1.0 µl of each VP4/VP2 region specific primers (Table 3) and 5.5 µl of nuclease free water adjusted to final volume of 25 µl (Bochkov et al. 2014, Wisdom et al. 2009, Linsuwanon et al. 2009). Each round of RT-PCR was carried out in a RotorGene 3000 instrument (Corbett Life Sciences, Sydney, Australia). PCR conditions for VP4/VP2 sequencing are listed in Table 4.

Table 3. Primers used for RV sequencing.

Primers	Length (bp)	Reference
5' NCR		
Forward primer U2+ 5´- CAAGCACTTCTGTTTCCCC -3´	397	Peltola et al. 2009
Reverse primer 4- 5'- GAAACACGGACACCCAAAGTA -3'		
VP4/VP2		
Forward primer OS 5'- CCGGCCCCTGAATGYGGCTAA -3'	629	Wisdom et al. 2009
Reverse primer IAS 5'- TCWGGHARYTTCCAMCACCANCC -3'		
Forward primer F587 5'- CTACTTTGGGTGTCCGTGTTTC -3'	540	Linsuwanon et al. 2009
Reverse primer RV 5'- ATCHGGHARYTTCCAMCACCA -3'		
Forward primer R848f 5'- ACTACTTTGGRTGTCCGTGT -3'	330	Bochkov et al. 2014
Reverse primer VP2-C252-5r 5'- AGTGATTTGYTTIAGCCTATC -3'		

Table 4. PCR conditions for VP4/VP2 region sequencing

Primers used	PCR conditions
Bochkov et al. 2014	denaturation at 95°C for 10 min, followed by 45 cycles at 95°C for 10 s; 50°C for 30 s and 72°C for 30 s and melt rising by 1°C from 72-92°C for 60 s for the first step and then for 5 s for next steps
Wisdom et al. 2009 Linsuwanon et al. 2009	denaturation at 95°C for 10 min, followed by 45 cycles at 95°C for 15 s; 55°C for 30 s and 72°C for 45 s melt rising by 0.2°C from 72-95°C for 45 s for the first step and then for 5 s for next steps

For the purification of the products sequenced with VP4/VP2 regions primers, Exonuclease I (Thermo Fisher Scientific, Waltham, MA, USA) was used. The products of 5' NCR were purified by the QIAquick PCR purification kit (Qiagen, Venlo, Netherlands). The sequencing of VP4/VP2 was performed at GATC Biotech AG, Konstanz, Germany and of 5' NCR at the Turku Centre for Biotechnology's sequencing services, Turku, Finland. The edition of sequences was carried out using FinchTV® (FinchTV 1.5.0, Geospiza, Inc. Seattle, WA, USA). The edited VP4/VP2 and 5' NCR sequences were analyzed by multiple sequence alignment CLUSTALW2 method in SeaView editor (Qouy et al. 2010). Phylogenetic trees were constructed and visualized using the neighbor-joining method and Kimura's two-

parameter with 1000 bootstrapping method implemented in the MEGA5.5® version 5.0 program (Tamura et al. 2011). RV species were assigned by comparing each RV strains with all of the available RV reference sequences encoding either VP4/VP2 region or 5' NCR in Basic Local Alignment Search Tool (BLAST) program available in GenBank (http://www.ncbi.nlm.gov/BLAST).

The type was assigned by the ≥96% identity with the reference sequence when analyzed from 5' NCR and ≥90% from VP4/VP2 region in BLAST (Miller and Mackay 2013). Pairwise method was used to determine the similarity between sequences, for which all the strains were aligned in BLAST. The sequence similarity of ≥98% identity was considered to represent the same type (Peltola et al. 2008). All consecutive strains during the follow-up period were further pairwise aligned in BLAST in order to determine their similarity.

4.6. Outcomes, data analysis and statistical methods

Statistical power calculations were carried out for *Study IV*, but not for *Studies I, II and III*. Two-sided p-values less than 0.05 were considered statistically significant. Data were analyzed using JMP software (Version 8.0.2, SAS Institute, Gary, NC, USA) for *Study I*, SPSS software (Version 23, SPSS Inc, Chicago, Ill, USA) for *Studies II and III*, and SAS/STAT version 9.1.3 SP4 software for the SAS System for Windows (SAS Institute, Cary, NC) for *Study IV*. Basic statistics were analyzed using t-test or one-way ANOVA to compare the differences in means, Mann–Whitney U-test or Kruskall-Wallis test to compare the differences in medians, and Pearson's chi-square test or Fischer's exact test were used to compare the differences in proportions as appropriate. Moreover, in- and outpatients and atopic and non-atopic patients were compared with each other's *in Study I*. Basics statistics included the information collected at the study entry from the guardian via a questionnaire covering on host and environmental risk factors for asthma. The information concerning daily symptoms and medication was also included.

For *Study I*, univariate and multivariate logistic regression analyses were used to investigate the associations patient characteristics and virus etiology as well as the severity of infection and virus etiology. Illness severity variables included inpatient status (inpatient vs. outpatient), severity score (score ≥6 vs. <6), duration of hospitalization (≥24 h vs. <24 h), and total duration of wheezing (≥3 days vs. <3 days) and cough (≥14 days vs. <14 days). Patient characteristics consisted of age, sex, atopic characteristics, sensitization to food, aeroallergen and perennial, total IgE level ≥45 kU/l, blood eosinophil count ≥ 0.4 9 10^9/l, eczema, atopic eczema and history of parental rhinitis, asthma and smoking. Virus etiology contained RV, RSV, HBoV and coinfection. Multivariate analyses were adjusted for age and sex when relevant. Patients receiving prednisolon (n = 38) were excluded from the illness severity analyses at the time point of study drug initiation because prednisolon is associated with short-term outcomes of acute wheezing. Logistic regression results were expressed as OR and 95% CI.

For *Study II* the data were the same as for Study I. The children who had a RV infection and received prednisolone were censored from the analysis at the initiation of the drug. The type assignments based on 5' NCR and VP4/VP2 region were merged and processed as equal. Normality of data distribution was tested using Kolmogorov-Smirnov test. Children infected with non-typeable RV species were excluded. The single RV-B was also omitted from the statistical analysis. Patient characteristics and illness severity were compared between RV-A, RV-C and non-RV (other virus than RV) infections using Pearson's chi square test or Fischer exact test for categorical variables and one-way analysis of variance (ANOVA) and Kruskall-Wallis test for continuous variables. Comparisons between two groups were tested using Bonferroni corrections for skewed data and Tukey's test for normal distributed data for continuous variables.

In *Study III*, symptomatic infections included cough and rhinitis as symptoms. Parametric variables were expressed as mean and standard deviation (SD) and nonparametric variables as median and interquartile range (IQR). Univariate Cox regression analysis was used to analyze the risk factors, including viral etiology (RV-A, RV-C and non-RV), any sensitization, eczema, parental asthma, parental smoking, age and sex, for the primary outcomes. The three primary outcomes were time to a new physician-confirmed wheezing episode during one-year follow-up, time to a new RV-associated wheezing episode during follow-up and time to an initiation of regular controller medication. Non-typeable RV cases (17 of 84 RV infections) and the single RV-B case were excluded from the analyses. Of non-typeable RVs, 11 could not be typed because of low sensitivity of the primers used in sequencing reactions and six due to a poor quality of the amplicons. The multivariable Cox regression was carried out including the covariates (any sensitization) that were significantly ($p < 0.05$) associated with the primary outcomes in univariate Cox regression analysis. The modifying effect of RV-A or RV-C induced first wheezing episode at study entry on the effect of prednisolone was tested including the RV-A and RV-C infection as interaction effect on Cox regression model. Cox regression results were expressed as hazard ratios (HR) and 95% CIs.

In *Study IV*, the three primary outcomes were the occurrence of a new physician-confirmed wheezing episode during the 2-month follow-up period, the number of physician confirmed wheezing episodes during the 12-month follow-up period and the initiation of regular controller medication for asthma symptoms during the latter follow-up period. The time to a new physician-confirmed wheezing episode was extended up to the 12-month follow-up as an exploratory outcome. Both in Study *III* and Study *IV*, secondary outcomes were the occurrence and severity of respiratory symptoms (cough, expiratory breathing difficulty, noisy breathing, rhinitis, and nocturnal wakening for breathing difficulties) displayed on a 4-point scale, as well as medications and unscheduled doctor's appointments recorded by the parents on a 2-week daily symptom diary.

The regular controller medication was initiated according to 2007 guidelines for initiating daily long-term control therapy for 0- to 4-year-old children. RV load was defined as the primary interaction analysis. It was used in order to investigate whether the effects of

prednisolone vs placebo on the 3 primary outcomes were dependent on the RV copy number. Baseline differences between groups were analyzed using Pearson's chi-square test or Fischer's exact test for categorical variables and for normally and non-normally distributed data 2-sample t-test and Wilcoxon rank sum tes were usedt. Cox regression analysis was used to analyze the difference between prednisolone and placebo groups in primary outcomes. Time to event was defined as the time from study entry to the time of occurrence of wheezing or the initiation of regular controller medication for asthma symptoms. Tin order to investigate the difference between the prednisolone group and placebo groups regarding the number of physician-confirmed wheezing episodes within 12 months, Poisson regression analysis was used. The modifying effect of dichotomized RV load at study entry was studied including an RV load-group interaction effect in the Cox and Poisson models. No statistically significant differences in baseline characteristics were found between prednisolone and placebo groups so Cox and Poisson regression models did not include any covariates.

4.7. Ethics

Written informed consent was obtained from the parents of the participating children. The study protocols were approved by the Ethics Committee of the Hospital District of Southwest Finland, Turku, Finland.

5. Results

5.1. Study population and patient characteristics

For *Studies I, II and III*, there were 125 consecutive children that were eligible of which 12 children were declined by parents and 2 children were excluded because of misdiagnoses. Hence, 111 children were enrolled and included in the analyses (Figure 3). Of those 111 children, 21% were aged 3-5 months, 30% 6-11 months, and 50% 12-23 months. Of the children enrolled, 79% were hospitalized and 67% were male.

During the 12-month follow-up period, 96% (107/111) participated the 2-week follow-up visit, 95% (105/111) the 2-month follow-up visit and 83% (92/111) the 12-month follow-up visit. First recurrent wheezing episode was recorded in 65 of the children (Figure 3). Of the 65 first recurrent wheezing episodes, 92% were seen by the study physician, and 8% were reviewed from the medical records. During the 12-months follow-up period, the regular controlled medication for asthma symptoms was initiated for 38% (35/92) of the children.

For *Study IV,* 61 inpatients and 18 outpatients with a RV infection were randomized using a separate list. All 79 patients were able to take at least half of the 3-day course of the study drug. During the 12-month follow-up period, 5 children from the prednisolone group and none from the placebo group dropped out yielding an analytic sample of 74 patients (80% inpatients and 20% outpatients). Of the 74 children, 15% were aged 3 to 5 months, 45% were 6-11 months and 55% were aged 12-23 months. Seventy-seven percent were male.

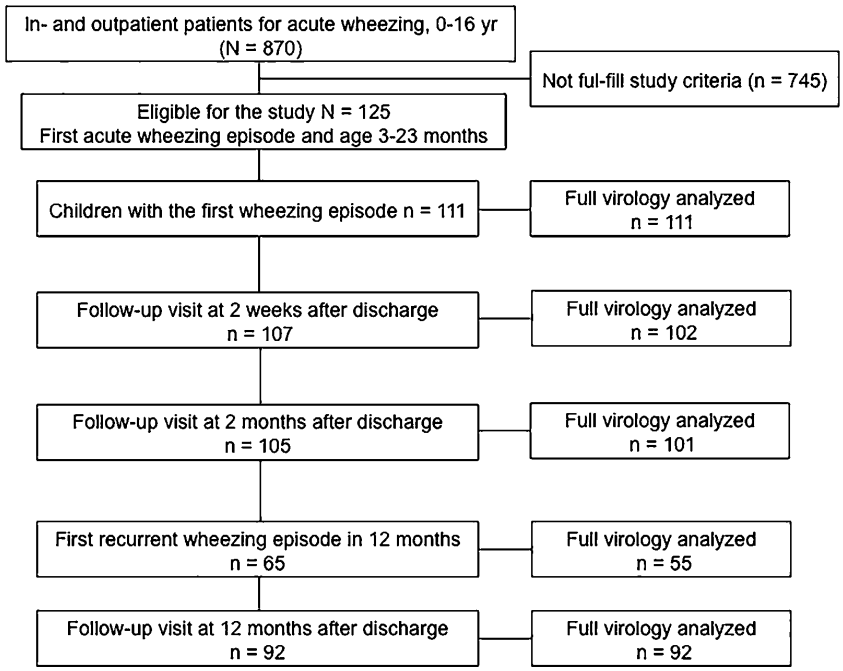

Figure 3. Simplified study flow chart for Studies I-III.

At least one atopic characteristic was present in 56% (62/111) of the children (Table 5). Eczema was present in 29% (32/111) of the children, atopic eczema in 16% (17/108), allergen specific IgE sensitization in 23% (25/107), blood eosinophilia ≥0.4 x10^9/l 41% (45/107) and parental asthma in 20% (22/111) (Table 5). The median durations of symptoms before recruitment were: wheezing 1 day (IQR 1, 2), cough 3 days (IQR 2, 5), rhinitis 3 days (IQR 2,6) and fever 1 day (IQR 0,2). Hospitalized patients had longer median duration of fever (1 [IQR 0, 2] vs 0 day 52 [respectively, 0, 2], p = 0.012), lower median O_2 saturation (97 % [95, 98] vs 98 % [97, 99], p = 53 0.012), higher median body temperature (37.5 °C [37.1, 38.0] vs 37.0 °C [37.0, 37.5], p = 0.012) and higher mean respiratory frequency (51 breaths per minute [sd 13] vs 43 breaths per minute [respectively, 10], p = 55 0.031) than outpatients. Atopic children were older (means [sd], 16 months [5.4] vs 11 months 56 [5.7], p = 0.0002) and had more often dermatitis (68% vs 32%, p = 0.0001) than the non-atopic 57 children. No other differences were found between in- and outpatients and atopic and non-atopic patients. The patient characteristics at study entry and during the visits are shown in Table 5.

Table 5. Simplified patient characteristics

Factor	
At study entry	**N = 111**
Age	12 (SD 6.0)
Male sex	74 (67%)
Any atopic characteristic	62 (56%)
Any sensitization (atopy)	25/108 (23%)
Food	24/108 (22%)
Aeroallergen	12/108 (11%)
Perennial	11/107 (10%)
Blood eosinophilia ≥0.4 x10^9/l	45/107 (41%)
Parental asthma	22 (20%)
Parental rhinitis	66 (59%)
Parental smoking	45 (41%)
At 2 weeks follow-up visit	**N = 107**
Wheezing	7 (6.3%)
Rhinitis / cough	64 (60%)
At 2 months follow-up visit	**N = 105**
Wheezing	8 (7.6%)
Rhinitis / cough	57 (54%)
At 12 months follow-up visit	**N = 92**
Male sex	61 (67%)
Any atopic characteristic	50 (45%)
Any sensitization (atopy)	32 (29%)
Food	36 (32%)
Aeroallergen	16 (14%)
Perennial	16 (14%)
Blood eosinophilia	26 (23%)

Of the 74 randomized children in *Study IV*, 30% had allergen specific IgE sensitization, 38% had eczema, 21% has atopic eczema, and 23% had parental asthma. There were no differences between the two randomly assigned groups, prednisolone vand placebo. Neither was there any difference in the delay of prednisolone or placebo initiation at the study entry between the two treatment groups (prednisolone vs placebo: mean, 45 [SD 22] vs 52 [SD 29] hours, p = 0.27).

5.2. Virus infections

At the first wheezing episode, one or more viruses were detected from NPA samples of all the children (111/111, 100%). A total number of positive PCR findings was 167 from 111 NPA samples. One virus was found in 62% of the samples and two or more viruses in 38%, of the samples.

The most common pathogen was RV which was detected in 76% (84/111) of the samples, followed by RSV in 28% (31/111) and HBoV (PCR or serodiagnosis) in 18% (20/111) of the samples. Other respiratory viruses were detected less than 10% of samples (Table 6). Furthermore, in single virus infection RV was detected in 72% of samples, followed by RSV in 16%. Other viruses were detected less than 10% of samples. Of the 42 virus coinfections, RV was detected in 81% of patients. All rhinovirus coinfections are shown in Table 7.

Table 6. Virus etiology of the first wheezing episode at study entry

Virus	Total (N = 111)
Rhinovirus	84 (76%)
Rhinovirus-A	17/84 (20%)
Rhinovirus-B	1/84 (1.2%)
Rhinovirus-C	49/84 (58%)
Respiratory syncytial virus	31 (29%)
Human bocavirus 1	20 (18%)
Parainfluenzavirus	10 (9.0%)
Metapneumovirus	7 (6.3%)
Adenovirus	4 (3.6%)
Coronavirus	5 (4.5%)
Enterovirus	4 (3.6%)
Influenzavirus	2 (1.8%)
1 virus	69 (62%)
2 viruses	31 (28%)
3 viruses	9 (8.1%)
4 viruses	2 (1.8%)
≥1 viruses/sample	111(100%)
≥2 viruses/sample	42 (38%)
≥3 viruses/sample	9 (8.1%)
≥4 viruses/sample	2 (1.8%)

Table 7. Rhinovirus coinfections (N = 34)

Coinfections with 2 viruses	**24 (71%)**
Rhinovirus+HBoV	10 (29%)
Rhinovirus+RSV	6 (18%)
Rhinovirus+MPV	3 (8.8%)
Rhinovirus+PIV	2 (5.9%)
Rhinovirus+CV	2 (5.9%)
Rhinovirus+EV	1 (2.9%)
Coinfections with 3 viruses	**8 (24%)**
Rhinovirus+RSV+EV	2 (5.9%)
Rhinovirus+RSV+HBoV	2 (5.9%)
Rhinovirus+RSV+Flu	1 (2.9%)
Rhinovirus+RSV+PIV	1 (2.9%)
Rhinovirus+MPV+PIV	1 (2.9%)
Rhinovirus+HBoV+CV	1 (2.9%)
Coinfections with 4 viruses	**2 (5.9%)**
Rhinovirus+AdV+HBoV+PIV	1 (2.9%)
Rhinovirus+AdV+EV+RSV	1 (2.9%)
Coinfections with RV species	**23/34 (68%)**
Coinfections with RV-A	**7 (21%)**
RV-A + HBoV	2 (29%)
RV-A + RSV	1 (14%)
RV-A + CV	1 (14%)
RV-A + MPV	1 (14%)
RV-A + PIV	1 (14%)
RV-A + RSV + HBoV	1 (14%)
Coinfections with RV-B	**1 (2.9%)**
RV-B + HBoV	1 (100%)
Coinfections with RV-C	**15 (44%)**
RV-C + HBoV	3 (20%)
RV-C + RSV	2 (13%)
RV-C + CV	1 (6.7%)
RV-C + EV	1 (6.7%)
RV-C + MPV	1 (6.7%)
RV-C + PIV	1 (6.7%)
RV-C + RSV + EV	2 (13%)
RV-C + RSV + Flu	1 (6.7%)
RV-C + HBoV + CV	1 (6.7%)
RV-C + MPV + PIV	1 (6.7%)
RV-C + RSV + EV+ AdV	1 (6.7%)

Adv, adenovirus; CV, coronavirus; EV, enterovirus; Flu, Influenza virus; HBoV, human bocavirus; MPV, metapneumovirus; PIV, parainfluenza virus; RV, rhinovirus; RSV, respiratory syncytial virus.

During the follow-up period, full respiratory virus test sets were analyzed from samples from 102 children at the 2-week visit, 101 children at the 2-month visit and 92 children at the 12-month visit. Of the first recurrent wheezing episode, respiratory viruses were analyzed from 94% patients (61/65). A total of 231 PCR positive virus detections were found from 258 follow-up samples. RV was the most common virus detected in 64% (148/231) of all virus detections during follow-up. RV occurred in 48% of all symptomatic infections and in 31% of all asymptomatic infections. Of recurrent wheezing episodes, RV was detected from 62% children with a new wheezing episode during follow-up. The second most common was

HBoVm detected in 19% of all infections, followed by RSV in 13%, respectively. Coinfections were detected in 28% of the children during the 12-month follow-up period.

5.3. RV genotypes

In the first wheezing episode, RVs were successfully typed from 75% (63/84) of the samples using the 5' NCR specific primers and from 40% (34/84) of the samples with VP4/VP2 primers. Altogether 58% (49/84) of the genotyped clinical isolates were identified as RV-C, 20% (17/84) as RV-A and 1.2% (1/84) as RV-B (Figure 4.). Other respiratory virus infections (non-RV) were present in 24% of the samples. A total of 67 clinical RV isolates represented 37 different genotypes of which 11 were identified as different A types, 1 B type and 24 different C types.

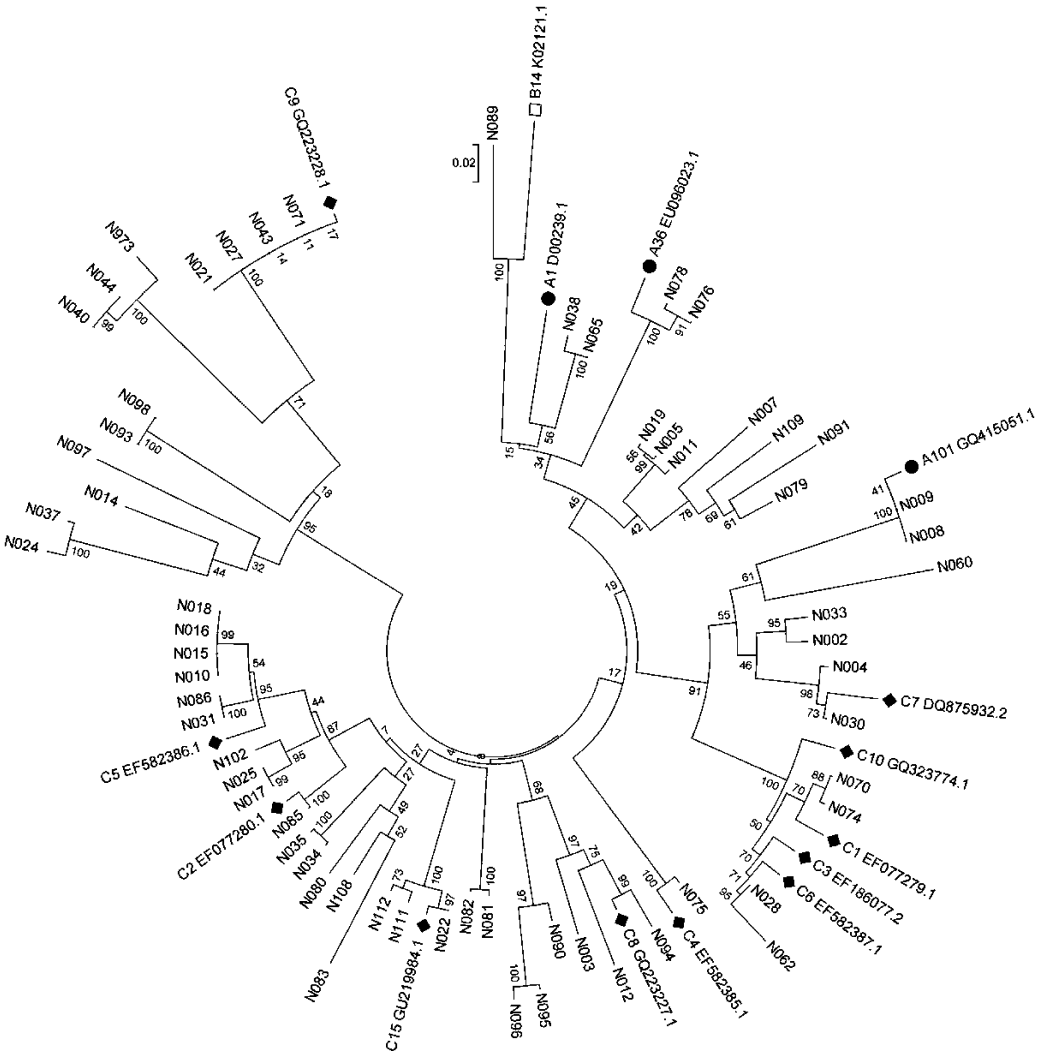

Figure 4. The phylogenetic tree of partial 5' non-coding region sequences of the rhinovirus clinical isolates and selected RV-A, RV-B, and RV-C reference types. Reference strains are identified by symbols (round, RV-A; square RV-B; quadrangle RV-C) and their GenBank accession number. This figure is printed with the permission of copyright holders (Turunen et al. 2016, Study II).

Rhinovirus typing from both 5' NCR and partial VP4/VP2 region was successful from 44 % (23/50) of patients taken at the 2 weeks follow–up visit, from 77% (20/26) at the 2-month follow-up visit, from 61% (23/38) at the 12-month follow-up visit and from 83% (33/40) of the first recurrent wheezing episodes. Pairwise comparison of the sequences from 5' NCR between consecutive samples taken at study entry, 2 weeks, 2 months, 12 months time points and at the time of a new physician-confirmed wheezing episode revealed that up to 97 % (67/69) of the patients had RV infection caused by a different virus strain of the same or distinct type. The sequence similarity between consecutive samples was less than 98%. Interestingly, only 2 children had RV infection caused by the same RV strain at 2 weeks' time point and at the study entry. Nevertheless, 36% (55/154) of patients were not successfully typed. RV-C dominated in the follow-up samples being detected in 29% of the samples. The second most common virus strainwas RV-A in 9.4% of samples and RV-B was rare in 1.7% of the samples. RV occurred simultaneously with another virus in 38% of the samples taken at the study entry. Of coinfections 44% were afflicted with RV-C, 21% with RV-A and 1.2 % with RV-B.

5.4. Signs and symptoms of RV-induced first wheezing episode

Children with RV infection at the study entry were associated with higher age (OR 1.1, 95% CI 1.0, 1.2), any atopic illness (OR 4.0, 95% CI 1.5, 12), blood eosinophil count ≥0.4 x 10^9/l (OR 11, 95% CI 2.9, 72), eczema (OR 4.2, 95% CI 1.3, 19), and atopic eczema (OR 6.1, 95% CI 1.1, 110). Children with RV induced first wheezing at study entry associated positively with parental smoking (OR 3.4, 95% CI 1.2, 10) and parental allergic rhinitis (OR 2.5, 95% CI 1.0, 6.5). Children with RSV infections were negatively associated with age (OR 0.90, 95% CI 0.83, 0.97), male sex (OR 0.33, 95% CI 0.14, 0.79), eczema (OR 0.23, 95% CI 0.050, 0.73), blood eosinophil count ≥0.4 x 10^9/l (OR 0.27, 95% CI 0.068, 0.63), and parental smoking (OR 0.38, 95% CI 0.14, 1.0). HBoV was associated with older age (OR 1.1, 95% CI 1.0, 1.2). Virus coinfections were associated with maternal allergic rhinitis (OR 2.3, 95% CI 1.0, 5.2). Of illness severity characteristics, RV was associated with prolonged cough (OR 3.5, 95% CI 1.2, 11), whereas RSV was associated positively with inpatient status (OR 9.5, 95% CI 2.2, 68) and longer duration of hospitalization (OR 2.8, 95% CI 1.1, 7.3). Virus coinfections independent of the virus etiology was associated with prolonged wheezing (OR 8.7, 95% CI 1.5, 160).

Children with RV-A and RV-C induced wheezing were older than children with non-RV induced wheezing (p = 0.014). Atopic characteristics were also differentially distributed between the children with RV-A, RV-C and non-RV infections (p = 0.001) (Figure 5). The most differentially distributed was food allergen sensitization (p = 0.029): RV-A 47%, RV-C 23% and non-RV 12%. Parental allergic rhinitis (p = 0.019), maternal allergic rhinitis (p = 0.032 and paternal smoking (p = 0.032) were also more common in children with RV-A and RV-C induced wheezing than in children with non-RV induced wheezing.

Illness severity was defined by the time of cough, wheezing and rhinitis before recruitment for the study, measured blood eosinophil count, vital functions and use of medication or supplemental oxygen at the ward. Children with RV-A and RV-C induced wheezing were associated with illness severity factors whereas children with non-RV induced wheezing were not. The blood eosinophil count was significantly higher in children with RV-A and RV-C infection than in children with non-RV ($p < 0.001$) (Figure 5). Indicating a rapid onset of illness, RV-A and RV-C infected children experienced significantly shorter time of wheezing and duration of fever before the recruitment than non-RV ($p = 0.040$). Children with RV-A (94%) and RV-C induced wheezing (87%) required the use of bronchodilatators more often than those infected with non-RV (94%, 87% and 63%, respectively ; p=0.012).

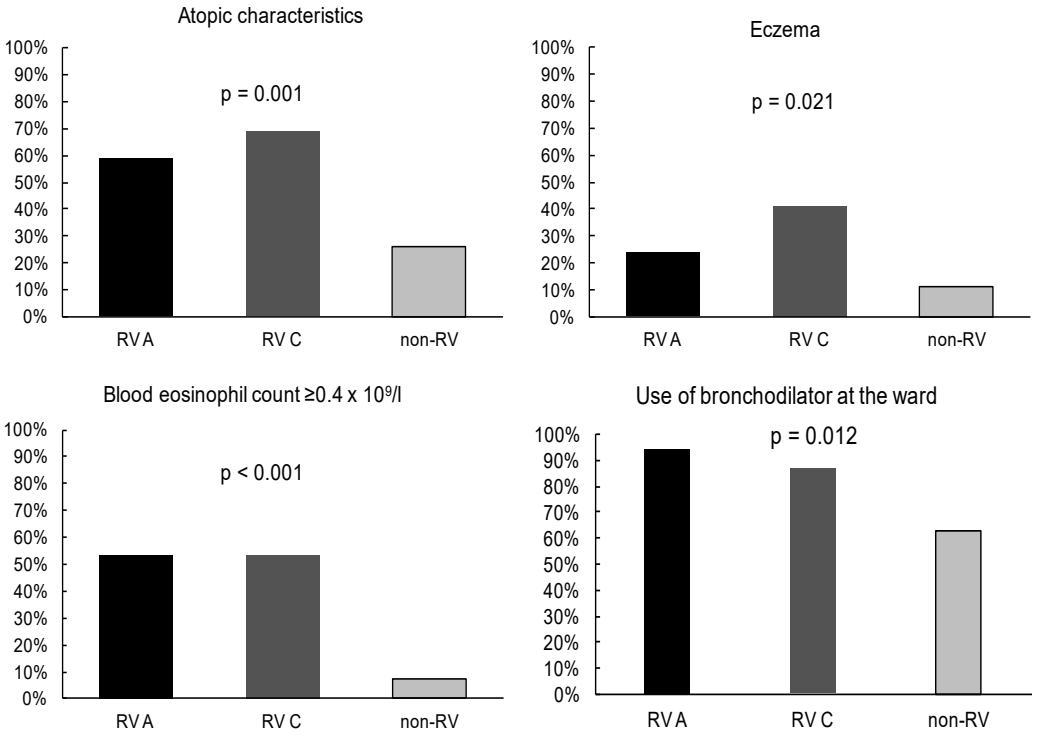

Figure 5. The association between blood eosinophilia, atopic characteristics, eczema, the use of bronchodilator at the ward and RV-A, RV-C and non-RV. P-value less than 5 indicates statistically significant result. Pearson's chi-square test or Fischer exact test were used to analyze the differences in proportions. One case with RV-B was excluded from the analysis. RV, rhinovirus.

5.5. Clinical characteristics during the 12-months follow-up after the first wheezing episode

At the 2-week follow-up visit, 6.3% (7/107) of the children had wheezing, 29% (32/107) had rhinitis, and 35% (39/107) had cough, respectively (Table 5). At the 2-month follow-up visit, 7.6% (8/105) of the children had wheezing, 44% (46/105) had rhinitis and 38%

(40/105) had cough, respectively. At the 12-month follow-up visit, 45% (50/92) of the children had any atopic characteristics, 23% (26/90) had blood eosinophilia, 14% (16/92) had aeroallergen sensitization, 32% (36/92) had food sensitization, 14% (16/92) had perennial sensitization, and 29% (32/92) were sensitized to any of the common allergens, respectively (Table 5).

5.6. Outcome of RV-A and RV-C induced first wheezing in 12-months follow-up

The time to a new recurrent wheezing episode was shorter for children with RV-A (HR 3.9 [95% CI1.7, 9.0], p = 0.009) and RV-C (HR 3.9 [95% CI 1.9, 7.9], p = 0.001) induced wheezing episode than for children with non-RV induced wheezing. Also, children with RV-A (HR 4.5 [95% CI 1.4, 14], p = 0.001) or an RV-C (HR 5.0 [95% CI 1.9, 13], p < 0.001) infection had a greater risk for a new RV-induced relapse than those with a non-RV infection. Furthermore, the time to the initiation of regular controller medication was shorter among children who experienced either RV-A (HR 4.7 [95% CI 1.6, 14], p = 0.004) or RV-C (HR 3.2 [95% 1.2, 8.5], p = 0.017) induced first wheezing episode than among children with a non-RV induced first wheezing episode. During the 2-month follow-up, children with RV-C infections visited doctors because of an expiratory breathing difficulty more often in comparison to children with RV-A and non-RV (65 %, 24% and 12%, respectively ; p = 0.001).

At the first wheezing episode, any sensitization was a risk factor for the initiation of regular controller medication (HR 2.7 [95% CI 1.4, 5.1], p = 0.003). No other significant risk factors were found either for the occurrence of a new recurrent wheezing episode or a new RV-induced wheezing episode. When adjusted to any sensitization, children with RV-A (HR 3.9 [95% CI 0.3, 11], p = 0.012) and RV-C (HR 2.8 [95% CI 1.0, 7.3], p = 0.041) induced first time wheezing had a greater risk for the initiation of regular controller medication in shorter time than children with non-RV induced wheezing. The risk was not increased for primary outcomes between children with RV-C induced first wheezing episode and RV-A induced first wheezing episode (all p > 0.3) during the 12-months follow-up.

5.7. The efficacy of prednisolone after RV-induced first wheezing episode

Overall, there was no difference between prednisolone and placebo groups for the 3 primary outcomes: occurrence of a new physician-confirmed wheezing episode within 2 months (HR 0.7, 95% CI 0.3 – 1.4, p = 0.30), the number of physician confirmed wheezing episodes within 12 months (RR 0.9 [95% CI 0.6, 1.2], p = 0.43) and the initiation of regular controller medication for asthma symptoms within 12 months (HR 0.8 [95% CI 0.4, 1.7], p = 0.63) (Figure 6). There was neither significant difference between prednisolone and placebo groups and children with RV-A or RV-C associated wheezing episode (p = 0.46). RV-A or RV-C induced first wheezing episode did not modify the effect of randomly assigned prednisolone treatment on the primary outcomes. Children with RV-C infection

(HR 0.66 [95% CI 0.16, 2.7], p = 0.57) did not experience a new physician confirmed wheezing episode in longer time when compared to children with RV-A infection between prednisolone and placebo groups. No association was either found with the occurrence of a new RV-induced wheezing episode (HR 0.39 [95% CI 0.072, 2.1], p = 0.28) in children with RV-C compared to children with RV-A induced wheezing episode. Between prednisolone vs placebo, children with RV-C induced first wheezing episode were not associated with the initiation of regular controller medication in shorter time (HR 1.2 [95% CI 0.26, 6.0], p = 0.79) when compared to children with RV-A induced wheezing episode.

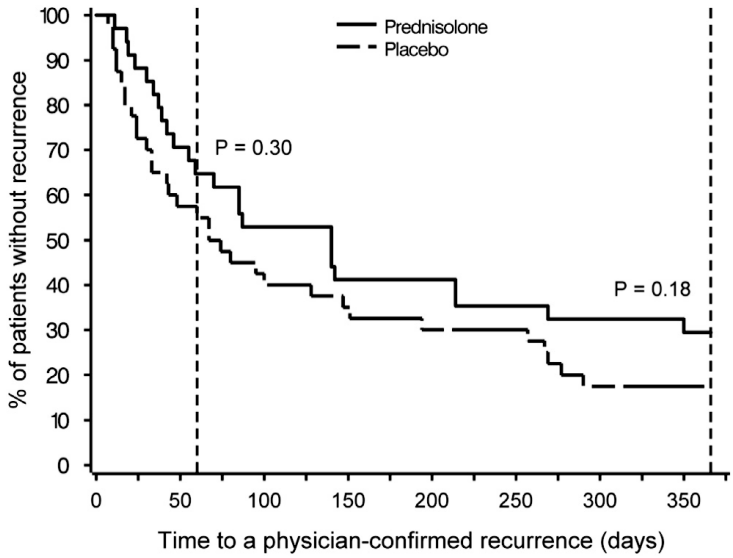

Figure 6. Time to a new physician-confirmed wheezing episode in children with their first rhinovirus-induced wheezing episode receiving either prednisolone or placebo through randomization. The 2-month time point has been marked. No difference was found at the 2-month or 12-month time points. This figure is printed with the permission of copyright holders (Jartti et al. 2015, Study IV).

RV load modified the response of randomly assigned interventions on the occurrence of a physician-confirmed new wheezing episode during the 2-month follow-up. Children with a RV load of greater than 7000 copies/mL were associated with lower occurrence of a new physician-confirmed wheezing episode in prednisolone group when compared to placebo group (HR 0.2 [95% CI 0.1, 0.7], p = 0.01) (Figure 7). Moreover, the number of physician-confirmed new wheezing episodes during the 12-months follow-up was lower in the prednisolone group when compared to placebo group (RR 0.6 [95% CI 0.4-0.98], p = 0.04). Further, children with a rhinovirus load more than 7000 copies/mL had less physician-confirmed new wheezing episodes during the 12-month follow-up in comparison of the prednisolone group to placebo group (HR 0.2 [95% CI 0.1-0.6], p = 0.004). There was no difference between the two treatment groups on the initiation of regular controller medication for asthma symptoms.

The duration of cough, noisy breathing, and rhinitis was shorter in the prednisolone group than in the placebo group 2 weeks after discharge. In the prednisolone there were 2 (6%) children that had severe expiratory breathing difficulties in prednisolone group during the 2-week period after discharge, whereas in the placebo group that number was 9 9 (23%) children in placebo group (p = 0.045). Furthermore, in the prednisolone group 5 (15%) children had 2 or more awakenings for breathing difficulties, whereas in the placebo group the same applied to 17 (43%) children who had 2 2 or more awakenings for breathing difficulties. No other differences were found in secondary outcomes.

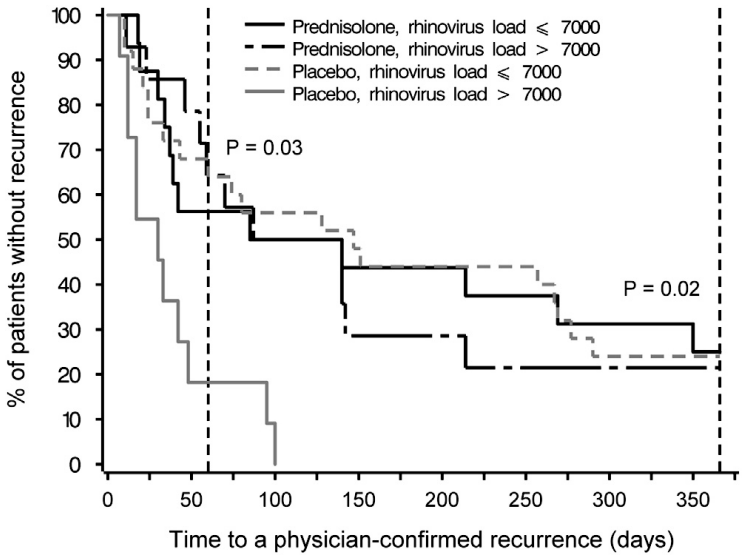

Figure 7. According to rhinovirus load, time to a new physician-confirmed wheezing episode in children in children with their first rhinovirus-induced wheezing episode receiving either prednisolone or placebo through randomization. This figure is printed with the permission of copyright holders (Jartti et al. 2015, Study IV).

6. Discussion

6.1. Virus etiology of the first wheezing episode

In this study, RV was identified as the most common pathogen in the first wheezing episode in small children. It was detected in 76% of the children, thus, having a clearly higher detection rate than shown in previous studies (Jartti et al. 2009, Nascimento et al. 2010, Table 1). Such high detection rates have previously been found in children with recurrent illnesses with high-risk for asthma, and in early wheezing children (Heymann et al. 2004, Jartti et al. 2009). A more sensitive real-time PCR than a conventional PCR has improved the RV diagnostics, whicht is the most likely explanation for high-prevalence of the virus (Jansen et al. 2011). This has also emphasized the importance of RV as a common pathogen in lower respiratory infections and early wheezing (Jartti et al. 2004, Lee et al. 2007, Palmenberg et al. 2009). Moreover, since RV infection is closely linked to wheezing status, atopy and increasing age, a cohort including only wheezing children, high predominance of atopic characteristics, the relatively high mean age of the study patients (12 months) and the fact that children under 3 months of age were excluded from the study, may also explain the high detection rate of the RV, (Lemanske et al. 2005, Kusel et al. 2007, Lukkarinen et al. 2013, Jartti et al. 2009, Midulla et al. 2010, Table 1, Table 2).

Previously, RSV has been detected in up to 80% of wheezing children aged under 24 months (Rakes et al 1999, Bosis et al. 2008, Helminen et al. 2008, Table 1, Table 2). Even though major RSV epidemic occurred during the study period, the detection rate of RSV was still only 28%. Traditional RSV-induced bronchiolitis with non-specific noisy-breathing is a typicaöl illness in infants, whereas wheezing commonly occurs in older children and is linked more closely to RV (Jartti et al. 2009, Nascimento et al. 2010, Midulla et al. 2012, Helminen et al. 2008). HBoV was the third most common causative agent of the first wheezing episode. This supports the earlier findings according to which HBoV is detected in 16 – 23% of lower respiratory tract infections and wheezing among young children (Jartti et al. 2009, Nascimento et al. 2010, Meriluoto et al. 2012, Ricart et al. 2013, Table 1, Table 2). The development of HBoV antibody assays has improved the diagnostics of acute HBoV infections, since the HBoV PCR diagnoses nowadays also asymptomatic infections. The detection rate of HBoV has been up to 27% in respiratory samples from wheezing children aged less than 24 months (Söderlund-Venermo et al. 2009, Jartti et al. 2012). Prolonged HBoV infection has been identified in up to 44% of children with respiratory illnesses which might explain the high prevalence of HBoV infections during the study period (Meriluoto et al. 2012, Jartti et al. 2012, Longtin et al. 2008, Blessing et al. 2009, Martin et al. 2010). However, the persistence of HBoV was not studied by serological analysis during the follow-up which would have ensured acute infections.

Virus coinfections were present in 38% of the wheezing children at study entry, which is in line with the earlier detection rates among children with the first wheezing episode (Jartti et al. 2009, Nascimento et al. 2010). However, the detection rate of coinfections among

wheezing children younger than 24 months has been found to fluctuate between 3 % - 51 % (Miron et al. 2010, Bizzintino et al. 2011, Cox et al. 2013, Fawkner-Corbett et al. 2016). The high detections rates of virus coinfections in small children might be caused by slow viral clearance due to an immature immune system (van der Zalm et al. 2009, Huijskens et al. 2012, Cebey-Lopez et al. 2015). The clinical significance of virus coinfections, whether it indicates antecedent or present infection, is still unknown and warrants further studies.

RV-C was detected in 58% of the children with first wheezing episode. This is in agreement with previous studies on the prevalence of infections caused by the RV species in children with acute wheezing, asthma or lower respiratory tract infection (26% - 68%) (Linsuwanon et al. 2009, Miller et al. 2009, Bizzintino et al. 2011, Cox et al. 2013, Lauinger et al. 2013). The RV-A detection rate has varied from 22% to 60% in lower airway illnesses, however, in this study RV-A was found in 20% of the first time wheezing children (Miller et al. 2009, Martin et al. 2015). RV-B was found in only one child, which supports the earlier findings that RV-B is less common in respiratory tract infections and acute wheezing (Lee et al. 2012, Nakagome et al. 2014). RV species detected simultaneously with other viruses were more common in RV-C cases than in RV-A which is in contrast to previous studies (Miller et al. 2009, Fawkner-Corbett et al. 2016)

The results emphazise the role of RV in respiratory infections as they can be detected in 29% - 59% of children with respiratory symptoms after the first wheezing episode during one-year follow-up. RV starts to dominate in respiratory infections after 9-12 months of age which is in concordance with earlier studies (Jartti et al. 2009, Midulla et al. 2010). RV occurred in 40% of asymptomatic infections which is in agreement in previous studies in which the prevalence of RV has been 8% - 40% (Rakes et al. 1999, Jartti et al. 2004, Fry et al. 2011, Loeffenholz et al. 2014, Principi et al. 2015). This could be explained by the immature immune system of young children, which results into a less effective viral clearance or the sensitivity of the modern PCR tests (Kumar et al. 2008, Jansen et al. 2011, van der Zalm et al. 2011). In the recurrent wheezing episodes, RV clearly dominated representing mainly RV-C which supports the earlier finding that RV-C infected children are in an increased risk for subsequent hospital admissions because of wheezing (Cox et al. 2013).

Previous studies have concluded that in early wheezing repetative RV detections are due to infections by new virus strains, and not by virus persistence with the same strain in early wheezing (Winther et al. 2006, Wright et al. 2007, van der Zalm et al. 2011, Engelmann et al. 2013, Loeffenholz et al. 2014). This is also supported by findings of this study according to which RV infections were mostly caused by different RV strains indicating reinfections. In line with this is also the finding that recurrent wheezing episodes were due to different virus strains when compared to the first wheezing episode (Jartti et al. 2008). RVs seemed to clear out in 2 weeks regardless of the RV type. Already at the 2-week follow-up visit RV represented a different genotype than at the acute phase. This is in line with earlier reports (Jartti et al. 2004, Loeffenholz et al. 2014). Longer RV

persistence has been shown to associate with immunocomprised patients (Kaiser et al. 2006, Kainulainen et al. 2010).

6.2. Associations between the virus etiology and patient characteristics

In this study, the first RV-induced wheezing episode was closely associated with atopic characteristics, especially with the first two manifestations of atopic manifestations, eczema and increased blood eosinophil count. These results are in line with previous findings (Jartti et al. 2009, Nascimento et al. 2010, Midulla et al. 2012, Ricart et al. 2013). In a long-term sequel, the first wheezing episode caused by an RV infection has been associated with an increased risk of a recurrent wheezing episode. This risk might be greater if the patient has atopic characteristics or a family history of asthma (Jartti et al. 2009, Midulla et al. 2012, Lukkarinen et al. 2013). Many earlier studies have linked in the cohort of marginally older children the RV-induced wheezing to atopic characteristics (Jartti et al. 2010, Jackson et al. 2012, Soto-Quiros et al. 2012, Carroll et al. 2012). These atopic characteristics have been specifically IgE sensitization, increased eosinophil count, atopic eczema, and maternal atopic asthma. These results add to the previous findings by indicating that there is an association between parental smoking and an RV caused first wheezing episode. It has been shown before that maternal smoking during pregnancy is an independent risk factor for RV-induced wheezing (van der Zalm et al. 2011). One explanation for the mechanism behind this link might be the bronchial inflammation and poor lung function caused by tobacco smoke, which in turn increase the risk for an RV-induced lower airway illness and wheezing (Kalliola et al. 2013). Interestingly, within in this study RSV etiology did not seem to be linked to atopic characteristics and HBoV became more prevalent with increasing age, which support the earlier findings (Kusel et al. 2007, Söderlund-Venermo et al. 2009, Jartti et al. 2010, Jartti et al. 2012).

Atopy was especially shown to be very common in children with an RV-A or RV-C induced first wheezing episode when compared to children with non-RV induced wheezing as an addition to previous findings (Soto-Quiros et al. 2012, Lukkarinen et al. 2013, Cox et al. 2013). Moreover, it has been earlier shown that children with an RV-A or RV-C infection have a similar risk for wheezing when being sensitized to dust mite (Soto-Quiros et al. 2012). Atopic sensitization might also increase the risk for hospitalization in children with RV-C induced lower airway illnesses (Soto-Quiros et al. 2012, Cox et al. 2013). This result may also indicate that sensitization to many environmental factors predisposes children to more to the RV-A or RV-C infection than to infections of other respiratory viruses. In addition to the earlier findings, the increased blood eosinophil count is notably associated with RV-A and RV-C induced early wheezing (Midulla et al. 2010).

One explanation for the association between an RV infection and allergic sensitization is that allergic sensitization enhaces the Th2 immune responses as well as increases the release of cytokines (Huber et al. 2010, Pritchard et al. 2012). This upregulates the expression of ICAM-I on the bronchial epithelial cell surfaces which accelerates the

proliferation and replication of those species RV-A which are known to utilize ICAM-I as a receptor (Rossi et al. 2013). At the same time, RV-induced wheezing is linked to decreased interferon responses which could explain the connection between an RV infection and allergic sensitization (Wark et al. 2005, Contoli et al. 2006, Jakiela et al. 2008, Sykes et al. 2012). This worsens RV infection pointing out children who are in risk for recurrent wheezing.

6.3. Associations between the virus etiology and illness severity

This study was the first to show an association between the first RV-induced wheezing episodes and prolonged cough. Children with RV-induced wheezing may already have a chronic, partly atopy-related inflammation in the lower respiratory tract which manifestates as cough and is exacerbated by an RV infection. This finding may also explain by the positive response to oral corticosteroid. Earlier studies have also reported decreased interferon responses toward an RV infection (Papadopoulos et al. 2002, Contoli et al. 2006, Sykes et al. 2012). RSV-induced first wheezing episode was linked to hospitalization, which is in line with earlier studies (Marguet et al. 2009, Mansbach et al. 2012). Virus coinfections associated with prolonged wheezing independent of the virus etiology which is in line with some but not with all earlier studies. Earlier studies have shown that children with bronchiolitis caused by the coinfection of RV and RSV was associated with a longer duration of hospitalization and a more severe illness than bronchiolitis caused by an RV infection alone or an RV infection with another virus than RSV (Mansbach et al. 2012, Papadopoulos et al. 2002). In contrast to this, HBoV has been found to interfere RV-induced immune responses and affect the outcome of wheezing (Lukkarinen et al 2014). However, there are also studies that have not found a link between wheezing illnesses and infections with multiple viruses (Marguet et al. 2009, Brand et al. 2012, Debiaggi et al. 2012).

Previous reaserch has shown that in general, RV causes more aggressive illness than RSV, and that RV-A and RV-C cause a higher number of clinically severe lower airway illnesses than RV-B (Arden et al. 2010, Bizzintino et al. 2011, Cox et al. 2013, Martin et al. 2015, Fawkner-Corbett et al. 2016). The result of *Study II* adds to the earlier finding that a rapid onset of an illness is especially dominant in children with an RV-A- and RV-C-induced first wheezing episode (Jartti et al. 2014). The link between a more severe illness and children with an RV-A and RV-C infection is supported by the significant association between the use of bronchodilatators in RV-A and RV-C than non-RV infected children. However, there was no significant difference in the illness severity when comparing RV-A and RV-C infected children only (Müller et al. 2015, Fawkner-Corbett et al. 2016). RV-A and RV-C infections have been linked to impaired antiviral responses and reduced epithelial barrier function that might explain the association between severe wheezing and RV-A and RV-C infections (Jakiela et al. 2008, Baraldo et al. 2012, Contoli et al. 2015). Furthermore, RV has been shown to be a strong risk factor for recurrent wheezing irrespective of whether being detected alone or together with other viruses (Hasegawa et al. 2014, Jackson et al.

2008, Bergroth et al. 2016). Interestingly however, RV-B has been found to be associated with a slower replication and a lower cytokine and/or chemokine production which in turn may contribute to less severe illnesses. This supports the ability of RV-A and RV-C to cause a more severe illness (Nakagome et al. 2014). Supporting the ability of RV-C to cause more severe illness, a gene, CDHR3, has been indicated to increase RV-C binding and replication, and furthermore, be a susceptible gene for wheezing illnesses and hospitalizations in children with asthma (Bonnelykke et al. 2014, Bochkov et al. 2015).

6.4. One-year outcome of the first wheezing episode

RV has been shown to be a strong predictor of recurrent wheezing and together with allergic sensitization the risk for developing asthma is further increased (Jackson et al. 2008, Jackson et al. 2012, Lukkarinen et al. 2013). The results of *Study III* add to the previous findings that both RV-A and RV-C infections at the first wheezing episode are major risk markers of recurrent wheezing when compared to non-RV infections. Only Linsuwanon et al have shown that RV-A or RV-C in codetection with RSV are associated with recurrent wheezing (Linsuwanon et al. 2009). However, the risk of recurrent wheezing was not increased when atopic characteristics were present which further emphasizes the RV's role as a risk factor (Jackson et al. 2012, Lukkarinen et al. 2015). Moreover, this study was the first to show that children with a RV-A or a RV-C induced first wheezing episode also had an increased risk for an RV-induced recurrent wheezing. Of the recurrent wheezing episode, RV was detected in 69% of the children in the *Study III*. This might be explained by deficient innate immune responses, genetics, and atopic inflammation which have all been shown to be linked to RV infected children (Jakiela et al. 2008, Baraldo et al. 2012, Jackson et al. 2012, Caliskan et al. 2013, Contoli et al. 2015).

6.5. Efficacy of systemic corticosteroid in the first wheezing episode

Vinku2 study was the first randomized double-blind, placebo-controlled trial to investigate the effect of prednisolone in the first acute RV-induced wheezing episode. In the prespecified interaction analysis showed that the efficacy of prednisolone was linked to an increased RV load. The hypothesis was confirmed because previous studies have demonstrated that a wheezing episode caused by an RV is an important risk factor for recurrent wheezing and doctor-diagnosed asthma in children (Kusel et al. 2007, Kotaniemi-Syrjänen et al. 2008, Jackson et al. 2008, Midulla et al. 2012). Also, in a *post hoc* analysis oral corticosteroid was found to reduce the recurrent wheezing episodes induced by RV (Lehtinen et al. 2007, Lukkarinen et al. 2013). In this study, the short-term benefits of the prednisolone (for example less respiratory symptoms and relapses resulting in a clinical visit during the 2-week follow-up) were shown to be in line with the earlier study (Jartti et al. 2006). This study further emphasizes the role of RV infection in early wheezing, since there are some studies that have shown the positive effect of oral corticosteroid in wheezing illness in hich the virus etiology was not, however, studied (Plint et al. 2009, Alansari et al. 2013). On the contrary, some studies have shown no

positive effect of oral corticosteroid in viral wheezing, however, the virus etiology was not specified in these studies (Oommen et al. 2003, Panickar et al. 2009). Bisgaard et al found no effect of intermittent inhaled corticosteroid on the progression of wheezing or short-term symptoms independent from the virus etiology (Bisgaard et al. 2006, Busse et al. 2011). Furthermore, systemic corticosteroids combined with nebulized epinephrine have been shown to have a borderline significance in reducing the need for hospitalization in infants with bronchiolitis. This effect was not affected by a positive RSV status (RV was not examined), a personal or family history of atopy, the early presentation in the course of illness or the severity of the illness (Plint et al.2009).

The link between long term-outcomes and high RV load is supported by previous *post hoc* trials showing a long-term effect of prednisolone in RV-positive children with first-time wheezing (Lehtinen et al. 2007, Lukkarinen et al. 2013). During the time between aforementioned trials the improvement of the PCR methods has enhanced the detection of RVs (Jartti et al. 2013). One potential explanation for the efficacy of prednisolone in children with high RV load is that the increased RV replication is associated with more severe airway inflammation (Rakes et al. 1999, Jartti et al. 2006, Contoli et al. 2006, Kusel et al. 2007, Jartti et al. 2010, Jackson et al. 2012, Baraldo et al. 2012, Sykes et al. 2012, Carroll et al. 2012). Allergic airway inflammation is typically associated with low IFN responses, leading to decreased viral clearance, increased virus replication, and eventually to a more severe airway inflammation. An *in vitro* study has shown that broken epithelial barrier is more prone to RV replication than an intact cell surface (Jakiela et al. 2008). Although, this study has shown the long-term effect of prednisolone in the first wheezing wheezing with high viral load of RV, there was no difference in the efficacy of prednisolone between patients with RV-A or RV-C associated first wheezing.

6.6. Strengths and limitations

The strengths of this study are that it focused on purely on the first wheezing episode, recruited patients consecutively, recorded clinical data prospectively, and used RCT design with power calculation, sensitive virus diagnostics and comprehensive clinical laboratory analyses were used. Of the 111 children enrolled, 83% completed the 12-month follow-up. The subjects of the randomized, double-blind, placebo-controlled study design with 12-month follow-up were carefully characterized. The first wheezing episode was confirmed by interviewing parents by using a standard questionnaire and by checking the medical records. For RV typing specific primers from both partial 5' NCR and VP4/VP2 region of the viral genome were used. Typing for VP4/VP2 is known to be more definitive for RV species and type differentiation. It is generally known that 5' NCR primers are more sensitive for RV PCR amplification.

As for limitations, children under the age of 3 months were excluded due to the intervention. However, RV typically triggers wheezing in children older than 3 months which was the main interest (Jartti et al. 2009, Korppi et al. 2015). The results might

not be generalized to outpatients since 80% of the study patients were hospitalized. The sample was relatively small, and it was too small to permit meaningful analysis of inpatient versus outpatient interactions. There was a delay (45 hours' vs 0 hours) in the initiation of the study drug in the current trial compared to the previous trial because of completion of the RV PCR. However, no association between the delay and the primary and secondary outcomes were found. There was only one RV-B and it was excluded from analyses so the differences between RV-A, RV-B and RV-C could not be demonstrated. Moreover, 30% of RV positive samples were typed.

7. Summary and conclusions

The purpose of this study was (I) to investigate the virus etiology of the first wheezing episode in children aged 3 – 23 months and how it is associated with clinical characteristics and illness severity, (II) to analyze RV species and clinical characteristics of children in the first wheezing episode (III) to examine the clinical and virus surveillance after the first wheezing episode with special focus on RV-A and RV-C species and in order to determine the short- and long-term efficacy of oral corticosteroid, prednisolone, after the first RV-induced wheezing episode (IV).

In *Study I*, it was found that acute wheezing in young children is exclusively caused by a virus infection. RV dominated as a causative agent already at the first wheezing episode and was closely associated with age and atopic characteristics of child as well as parental smoking. RV infections were also more closely linked to wheezing than RSV infections. RV was also linked to prolonged cough suggesting a more chronic type of illness in children susceptible to RV-induced wheezing. The second most common pathogen was RSV and the third HBoV. The results add to previous studies to call attention to RV-induced wheezing and the differences between these three major viruses (RV, RSV and HBoV).

The *Study II* showed that RV-C was the most common pathogen causing the first wheezing episode. It was followed by RV-A. RV-B seems to remain rare. Children with RV-A and RV-C-induced first wheezing episode were more often atopic, as well as had a more rapid onset of illness and greater illness severity than those with wheezing induced by other viruses. Further studies are warranted on the pathogenesis and preventive strategies for RV-induced early wheezing. It is clinically important to understand the association between atopic status and wheezing induced by species A, B and C RVs when analyzing subsequent risk for asthma.

Study III showed that in small children, during a 12-months follow-up after the first wheezing episode, RV causes repetitive infections with varying virus strains. Children with RV-A and RV-C induced first wheezing episode had an increased risk to have a new wheezing episode or a new RV-induced wheezing episode in shorter time than non-RV induced first wheezing episode. No difference was found in the efficacy of prednisolone between patients with RV-A or RV-C associated first wheezing. These results call attention to the prevention strategies targeted at infections induced by these two RV species.

In *Study IV*, although prednisolone had no effect on the long-term primary outcomes, the primary interaction analysis showed that prednisolone was effective in children with RV-induced wheezing when high viral loads were measured. Furthermore, prednisolone seemed to have a short-term effect on secondary outcomes in children with RV infection regardless of virus genome load. As a conclusion, the first time wheezing children affected by RV, especially those with high virus genome load, may benefit from short- and long-term use of oral corticosteroid. These results warrant adequately powered trial on the efficacy of prednisolone in RV-affected first-time wheezing children that takes into account virus genome load and atopy status of child.

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References

Aberle JH, Aberle SW, Pracher E, Hutter HP, Kundi M, Popow-Kraupp T. Single versus dual respiratory virus infections in hospitalized infants: impact on clinical course of disease and interferon-gamma response. Pediatr Infect Dis J. 2005; 24: 605-10.

Aberle JH, Aberle SW, Rebhandl W, Pracher E, Kundi M, Popow-Kraupp T. Decreased interferon-gamma response in respiratory syncytial virus compared to other respiratory viral infections in infants. Clin Exp Immunol. 2004; 137: 146-50.

Aguilar JC, Pérez-Breña MP, García ML, Cruz N, Erdman DD, Echevarría JE. Detection and identification of human parainfluenza viruses 1, 2, 3, and 4 in clinical samples of pediatric patients by multiplex reverse transcription-PCR. J Clin Microbiol. 2000; 38: 1191-5.

Alam MM, Khurshid A, Shaukat S, Sharif S, Suleman RM, Angez M, Nisar N, Aamir UB, Naeem M, Zaidi SS. 'Human bocavirus in Pakistani children with gastroenteritis'. J Med Virol. 2015; 87: 656-63.

Alansari K, Sakran M, Davidson BL, Ibrahim K, Alrefai M, Zakaria I. Oral dexamethasone for bronchiolitis: a randomized trial. Pediatrics 2013 ;132: e810-6.

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, Ruuskanen O. Human bocavirus and acute wheezing in children. Clin Infect Dis 2007: 44: 904–10.

Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A. 2005; 102: 12891-6.

Anders KL, Nguyen HL, Nguyen NM, Van Thuy NT, Hong Van NT, Hieu NT, Hong Tham NT, Thanh Ha PT, Lien le B, Vinh Chau NV, Ty Hang VT, van Doorn HR, Simmons CP. Epidemiology and virology of acute respiratory infections during the first year of life: a birth cohort study in Vietnam. Pediatr Infect Dis J. 2015; 34: 361-70.

Andréoletti L, Lesay M, Deschildre A, Lambert V, Dewilde A, Wattré 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: 341-6.

Andrewes CH, Chaproniere DM, Gompels AE, Pereira HG, Roden AT. Propagation of common-cold virus in tissue cultures. Lancet. 1953; 265: 546-7.

Andries K, Dewindt B, Snoeks J, Wouters L, Moereels H, Lewi PJ, Janssen PA. Two groups of rhinoviruses revealed by a panel of antiviral compounds present sequence divergence and differential pathogenicity. J Virol. 1990; 64: 1117-23.

Antunes H, Rodrigues H, Silva N, Ferreira C, Carvalho F, Ramalho H, Gonçalves A, Branca F. Etiology of bronchiolitis in a hospitalized pediatric population: prospective multicenter study. J Clin Virol. 2010; 48: 134-6.

Arden KE, Chang AB, Lambert SB, Nissen MD, Sloots TP, Mackay IM. Newly identified respiratory viruses in children with asthma exacerbation not requiring admission to hospital. J Med Virol. 2010; 82: 1458–61.

Arruda E, Boyle TR, Winther B, Pevear DC, Gwaltney JM Jr, Hayden FG. Localization of human rhinovirus replication in the upper respiratory tract by in situ hybridization. J Infect Dis. 1995; 171: 1329-33.

Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff RM. A novel bocavirus associated with acute gastroenteritis in Australian children. PLoS Pathog. 2009; 5: e1000391.

Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, Williams H; ISAAC Phase Three Study Group. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet. 2006; 368: 733-43.

Ashraf S, Brockman-Schneider R, Bochkov YA, Pasic TR, Gern JE. Biological characteristics and propagation of human rhinovirus-C in differentiated sinus epithelial cells. Virology. 2013; 436: 143-9.

Asthma (online). Current care guidelines. Working group set up by the Finnish Medical Society Duodecim, the Finnish Respiratory Society, the Finnish Paediatric Society and the Finnish Society of Clinical Physiology. Helsinki: The Finnish Medical Society Duodecim, 2012. Available online at: www.kaypahoito.fi.

Baraldo S, Contoli M, Bazzan E, Turato G, Padovani A, Marku B, Calabrese F, Caramori G, Ballarin A, Snijders D, Barbato A, Saetta M, Papi A. Deficient antiviral immune responses in childhood: distinct roles of atopy and asthma. J Allergy Clin Immunol 2012: 130: 1307–14.

Bardin PG. Vaccination for asthma exacerbations. Intern Med J. 2004; 34: 358-60.

Barral PM, Sarkar D, Fisher PB, Racaniello VR. RIG-I is cleaved during picornavirus infection. Virology. 2009; 391: 171-6.

Bastien N, Brandt K, Dust K, Ward D, Li Y. Human Bocavirus infection, Canada. Emerg Infect Dis. 2006; 12: 848-50.

Bendig JW, Franklin OM, Hebden AK, Backhouse PJ, Clewley JP, Goldman AP, Piggott N. Coxsackievirus B3 sequences in the blood of a neonate with congenital myocarditis, plus serological evidence of maternal infection. J Med Virol. 2003; 70: 606-9.

Benschop K, Molenkamp R, van der Ham A, Wolthers K, Beld M. Rapid detection of human parechoviruses in clinical samples by real-time PCR. J Clin Virol. 2008; 41: 69-74.

Bergroth E, Aakula M, Korppi M, Remes S, Kivistö JE, Piedra PA, Camargo CA Jr, Jartti T. Post-bronchiolitis Use of Asthma Medication: A Prospective 1-year Follow-up Study. Pediatr Infect Dis J. 2016; 35: 363-8.

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

Bisgaard H, Gillies J, Groenewald M, Maden C, an International Study Group, The effect of inhaled fluticasone propionate in the treatment of young asthmatic children. A dose comparison study. Am J Respir Crit Care Med 1999; 160: 126–131.

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

Bisgaard H, Szefler S. Prevalence of asthma-like symptoms in young children. Pediatr Pulmonol 2007; 42: 723–728.

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

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, Le Souëf PN. Association between human rhinovirus C and severity of acute asthma in children. Eur Respir J. 2011; 37: 1037-42.

Blessing K, Neske F, Herre U, Kreth HW, Weissbrich B. Prolonged detection of human bocavirus DNA in nasopharyngeal aspirates of children with respiratory tract disease. Pediatr Infect Dis J. 2009; 28: 1018–9.

Blomqvist S, Roivainen M, Puhakka T, Kleemola M, Hovi T. Virological and serological analysis of rhinovirus infections during the first two years of life in a cohort of children. J Med Virol. 2002; 66: 263-8.

Bochkov YA, Palmenberg AC, Lee WM, Rathe JA, Amineva SP, Sun X, Pasic TR, Jarjour NN, Liggett SB, Gern JE. Molecular modeling, organ culture and reverse genetics for a newly identified human rhinovirus C. Nat Med. 2011; 17: 627-32.

Bochkov YA, Gern JE. Clinical and molecular features of human rhinovirus C. Microbes Infect. 2012; 14: 485-94.

Bochkov YA, Grindle K, Vang F, Evans MD, Gern JE. Improved molecular typing assay for rhinovirus species A, B, and C. J Clin Microbiol. 2014; 52: 2461-71.

Bochkov YA, Watters K, Ashraf S, Griggs TF, Devries MK, Jackson DJ, Palmenberg AC, Gern JE. Cadherin-related family member 3, a childhood asthma susceptibility gene product, mediates rhinovirus C binding and replication. Proc Natl Acad Sci U S A. 2015; 112: 5485-90.

Boivin G, De Serres G, Côté S, Gilca R, Abed Y, Rochette L, Bergeron MG, Déry P. Human metapneumovirus infections in hospitalized children. Emerg Infect Dis. 2003; 9: 634-40.

Bønnelykke K, Sleiman P, Nielsen K, Kreiner-Møller E, Mercader JM, Belgrave D, den Dekker HT, Husby A, Sevelsted A, Faura-Tellez G, Mortensen LJ, Paternoster L, Flaaten R, Mølgaard A, Smart DE, Thomsen PF, Rasmussen MA, Bonàs-Guarch S, Holst C, Nohr EA, Yadav R, March ME, Blicher T, Lackie PM, Jaddoe VW, Simpson A, Holloway JW, Duijts L, Custovic A, Davies DE, Torrents D, Gupta R, Hollegaard MV, Hougaard DM, Hakonarson, Bisgaard H. A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. Nat Genet. 2014; 46: 51-5.

Bønnelykke K, Vissing NH, Sevelsted A, Johnston SL, Bisgaard H. Association between respiratory infections in early life and later asthma is independent of virus type. J Allergy Clin Immunol. 2015; 136: 81-86.e4.

Bosis S, Esposito S, Niesters HG, Zuccotti GV, Marseglia G, Lanari M, Zuin G, Pelucchi C, Osterhaus AD, Principi N. Role of respiratory pathogens in infants hospitalized for a first episode of wheezing and their impact on recurrences. Clin Microbiol Infect 2008: 14: 677–84.

Bossios A, Psarras S, Gourgiotis D, Skevaki CL, Constantopoulos AG, Saxoni-Papageorgiou P, Papadopoulos NG. Rhinovirus infection induces cytotoxicity and delays wound healing in bronchial epithelial cells. Respir Res. 2005; 6: 114.

Bouscambert-Duchamp M, Lina B, Trompette A, Moret H, Motte J, Andréoletti L. Detection of human metapneumovirus RNA sequences in nasopharyngeal aspirates of young French children with acute bronchiolitis by real-time reverse transcriptase PCR and phylogenetic analysis. J Clin Microbiol. 2005; 43: 1411-4.

Bouzigon E, Corda E, Aschard H, Dizier MH, Boland A, Bousquet J, Chateigner N, Gormand F, Just J, Le Moual N, Scheinmann P, Siroux V, Vervloet D, Zelenika D, Pin I, Kauffmann F, Lathrop M, Demenais F. Effect of 17q21 variants and smoking exposure in early-onset asthma. N Engl J Med. 2008; 359: 1985-94.

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 Souëf 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, Bush A. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur Respir J. 2008; 32: 1096-110.

Brand HK, de Groot R, Galama JM, Brouwer ML, Teuwen K, Hermans PW, Melchers WJ, Warris A. Infection with multiple viruses is not associated with increased disease severity in children with bronchiolitis. Pediatr Pulmonol. 2012; 47: 393-400.

Brieu N, Guyon G, Rodière M, Segondy M, Foulongne V. Human bocavirus infection in children with respiratory tract disease. Pediatr Infect Dis J. 2008; 27: 969-73.

British Thoracic Society, Scottish Intercollegiate Guideline Network. British guideline on the management of asthma 2014. www.brit-thoracic.org.uk

Brussee JE, Smit HA, Koopman LP, Wijga AH, Kerkhof M, Corver K, Vos AP, Gerritsen J, Grobbee DE, Brunekreef B, Merkus PJ, de Jongste JC. Interrupter resistance and wheezing phenotypes at 4 years of age. Am J Respir Crit Care Med 2004; 169: 209–13.

Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. Lancet. 2010; 376: 826-34.

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, Sorkness CA. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. N Engl J Med. 2011; 364: 1005-15.

Byington CL, Taggart EW, Carroll KC, Hillyard DR. A polymerase chain reaction-based epidemiologic investigation of the incidence of nonpolio enteroviral infections in febrile and afebrile infants 90 days and younger. Pediatrics. 1999; 103: E27.

Caliskan M, Bochkov YA, Kreiner-Møller E, Bønnelykke K, Stein MM, Du G, Du G, Bisgaard H, Jackson DJ, Gern JE, Lemanske RF Jr, Nicolae DL, Ober C. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. N Engl J Med 2013; 368: 1398-407.

Calvo C, García-García ML, Sanchez-Dehesa R, Román C, Tabares A, Pozo F, Casas I. Eight Year Prospective Study of Adenoviruses Infections in Hospitalized Children. Comparison with Other Respiratory Viruses. PLoS One. 2015; 10: e0132162.

Calvo C, Pozo F, Garcıa-Garcıa ML, Sanchez M, Lopez-Valero M, Pérez-Breña P, Casas I. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. Acta Paediatr 2010: 99: 883–7.

Camara AA, Silva JM, Ferriani VP, Tobias KR, Macedo IS, Padovani MA, Harsi CM, Cardoso MR, Chapman MD, Arruda E, Platts-Mills TA, Arruda LK. Risk factors for wheezing in a subtropical environment: role of respiratory viruses and allergen sensitization. J Allergy Clin Immunol. 2004; 113: 551-7.

Camps M, Ricart S, Dimova V, Rovira N, Muñoz-Almagro C, Garcia JJ, Pons-Odena M, Marcos MA, Pumarola T. Prevalence of human metapneumovirus among hospitalized children younger than 1 year in Catalonia, Spain. J Med Virol. 2008; 80: 1452-60.

Canchola J, Vargosko AJ, Kim HW, Parrott RH, Christmas E, Jeffries B, Chanock RM. Antigenic variation among newly isolated strains of parainfluenza type 4 virus. Am J Hyg. 1964; 79: 357-64.

Carroll KN, Wu P, Gebretsadik T, Griffin MR, Dupont WD, Mitchel EF, Hartert TV. Season of infant bronchiolitis and estimates of subsequent risk and burden of early childhood asthma. J Allergy Clin Immunol. 2009; 123: 964-6.

Carroll KN, Gebretsadik T, Minton P, Woodward K, Liu Z, Miller EK, Williams JV, Dupont WD, Hartert TV. Influence of maternal asthma on the cause and severity of infant acute respiratory tract infections. J Allergy Clin Immunol 2012: 129: 1236–42.

Castro-Rodríguez JA, Rodrigo GJ. Efficacy of inhaled corticosteroids in infants and preschoolers with recurrent wheezing and asthma: a systematic review with meta-analysis. Pediatrics 2009; 123: e519–25.

Cebey-López M, Herberg J, Pardo-Seco J, Gómez-Carballa A, Martinón-Torres N, Salas A, Martinón-Sánchez JM, Gormley S, Sumner E, Fink C, Martinón-Torres F; GENDRES network. Viral Co-Infections in Pediatric Patients Hospitalized with Lower Tract Acute Respiratory Infections. PLoS One. 2015; 10: e0136526.

Chan PC, Wang CY, Wu PS, Chang PY, Yang TT, Chiang YP, Kao CL, Chang LY, Lu CY, Lee PI, Chen JM, Shao PL, Huang FY, Lee CY, Huang LM. Detection of human metapneumovirus in hospitalized children with acute respiratory tract infection using real-time RT-PCR in a hospital in northern Taiwan. J Formos Med Assoc. 2007; 106: 16-24.

Chang TS, Lemanske RF Jr, Guilbert TW, Gern JE, Coen MH, Evans MD, Gangnon RE, David Page C, Jackson DJ. Evaluation of the modified asthma predictive index in high-risk preschool children. J Allergy Clin Immunol. 2013; 1: 152-6.

Chano F, Rousseau C, Laferrière C, Couillard M, Charest H. Epidemiological survey of human metapneumovirus infection in a large pediatric tertiary care center. Pract. 2013; 1: 152-6.

Charles CH, Luo GX, Kohlstaedt LA, Morantte IG, Gorfain E, Cao L, Williams JH, Fang F. Prevention of human rhinovirus infection by multivalent fab molecules directed against ICAM-1. Antimicrob Agents Chemother. 2003; 47: 1503-8.

Chiu CC, Shi YF, Yang JJ, Hsiao YC, Tzang BS, Hsu TC. Effects of human Parvovirus B19 and Bocavirus VP1 unique region on tight junction of human airway epithelial A549 cells. PLoS One. 2014; 9: e107970.

Chiu SS, Chan KH, Chu KW, Kwan SW, Guan Y, Poon LL, Peiris JS. Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China. Clin Infect Dis. 2005; 40: 1721-9.

Choi EH, Lee HJ, Kim SJ, Eun BW, Kim NH, Lee JA, Lee JH, Song EK, Kim SH, Park JY, Sung JY. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000-2005. Clin Infect Dis. 2006; 43: 585-92.

Chung JY, Han TH, Kim JS, Kim SW, Park CG, Hwang ES. Th1 and Th2 cytokine levels in nasopharyngeal aspirates from children with human bocavirus bronchiolitis. J Clin Virol. 2008; 43: 223-5.

Cilla G, Oñate E, Perez-Yarza EG, Montes M, Vicente D, Perez-Trallero E. Viruses in community-acquired pneumonia in children aged less than 3 years old: High rate of viral coinfection. J Med Virol. 2008; 80: 1843-9.

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, Johnston SL. Role of deficient type III interferon-lambda production in asthma exacerbations. Nat Med 2006; 12: 1023-6.

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, Papi A. Th2 cytokines impair innate immune responses to rhinovirus in respiratory epithelial cells. Allergy. 2015; 70: 910–20.

Cook DG, Strachan DP, Carey IM. Health effects of passive smoking. 9. Parental smoking and spirometric indices in children. Thorax. 1998; 53: 884-93.

Copenhaver CC, Gern JE, Li Z, Shult PA, Rosenthal LA, Mikus LD, Kirk CJ, Roberg KA, Anderson EL, Tisler CJ, DaSilva DF, Hiemke HJ, Gentile K, Gangnon RE, Lemanske RF Jr. Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. Am J Respir Crit Care Med. 2004; 170: 175-80.

Cox DW, Bizzintino J, Ferrari G, Khoo SK, Zhang G, Whelan S, Lee WM, Bochkov YA, Geelhoed GC, Goldblatt J, Gern JE, Laing IA, Le Souëf PN. Human rhinovirus species C infection in young children with acute wheeze is associated with increased acute respiratory hospital admissions. Am J Respir Crit Care Med. 2013; 188: 1358-64.

Cox NJ, Subbarao K. Influenza. Lancet. 1999; 354: 1277-1282

Csonka P, Kaila M, Laippala P, Iso-Mustajärvi M, Vesikari T, Ashorn P. Oral prednisolone in the acute management of children age 6 to 35 months with viral respiratory infection-induced lower airway disease: a randomized, placebo-controlled trial. J Pediatr. 2003; 143: 725-30.

de Benedictis FM1, Bush A. Corticosteroids in respiratory diseases in children. Am J Respir Crit Care Med. 2012; 185: 12-23.

de Crom SC, van Furth MA, Peeters MF, Rossen JW, Obihara CC. Characteristics of pediatric patients with enterovirus meningitis and no cerebral fluid pleocytosis. Eur J Pediatr. 2012; 171: 795-800.

Debiaggi M, Canducci F, Ceresola ER, Clementi M. The role of infections and coinfections with newly identified and emerging respiratory viruses in children. Virol J. 2012; 9: 247.

Delgado MF, Coviello S, Monsalvo AC, Melendi GA, Hernandez JZ, Batalle JP, Diaz L, Trento A, Chang HY, Mitzner W, Ravetch J, Melero JA, Irusta PM, Polack FP. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. Nat Med. 2009; 15: 34-41.

Deng Y, Gu X, Zhao X, Luo J, Luo Z, Wang L, Fu Z, Yang X, Liu E. High viral load of human bocavirus correlates with duration of wheezing in children with severe lower respiratory tract infection. PLoS One. 2012; 7: e34353.

Deng ZH, Hao YX, Yao LH, Xie ZP, Gao HC, Xie LY, Zhong LL, Zhang B, Cao YD, Duan ZJ. Immunogenicity of recombinant human bocavirus-1,2 VP2 gene virus-like particles in mice. Immunology. 2014; 142: 58-66.

Don M, Söderlund-Venermo M, Valent F, Lahtinen A, Hedman L, Canciani M, Hedman K, Korppi M. Serologically verified human bocavirus pneumonia in children. Pediatr Pulmonol. 2010; 45: 120-6.

Drahos J, Racaniello VR. Cleavage of IPS-1 in cells infected with human rhinovirus. J Virol. 2009; 83: 11581-7.

Ducharme FM, Tse SM, Chauhan B. Diagnosis, management, and prognosis of preschool wheeze. Lancet. 2014; 383: 1593-604.

Durrani SR, Montville DJ, Pratt AS, Sahu S, DeVries MK, Rajamanickam V, Gangnon RE, Gill MA, Gern JE, Lemanske RF Jr, 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: 489-95.

Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrländer C, Heederik D, Piarroux R, von Mutius E; GABRIELA Transregio 22 Study Group. Exposure to environmental microorganisms and childhood asthma. N Engl J Med. 2011; 364: 701-9.

Engelmann I, Mordacq C, Gosset P, Tillie-Leblond I, Dewilde A, Thumerelle C, Pouessel G, Deschildre A. Rhinovirus and asthma: reinfection, not persistence. Am J Respir Crit Care Med. 2013 Nov 1;188:1165-7.

Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Evidence of a novel human coronavirus that is associated with respiratory tract disease in infants and young children. J Infect Dis. 2005; 191: 492-8.

Esposito S, Bosis S, Niesters HG, Tremolati E, Begliatti E, Rognoni A, Tagliabue C, Principi N, Osterhaus AD. Impact of human coronavirus infections in otherwise healthy children who attended an emergency department. J Med Virol. 2006; 78: 1609-15.

Esposito S, Bosis S, Niesters HG, Tremolati E, Sabatini C, Porta A, Fossali E, Osterhaus AD, Principi N. Impact of human bocavirus on children and their families. J Clin Microbiol. 2008; 46: 1337-42.

Fang F, Yu M. Viral receptor blockage by multivalent recombinant antibody fusion proteins: inhibiting human rhinovirus (HRV) infection with CFY196. J Antimicrob Chemother. 2004; 53: 23-5.

Fawkner-Corbett DW, Khoo SK, Duarte MC, Bezerra P, Bochkov YA, Gern JE, Le Souef PN, McNamara PS, Rose K, Fonceca AM, Hopkins M, Britto M, Cuevas LE, Correia J. Rhinovirus-C detection in children presenting with acute respiratory infection to hospital in Brazil. J Med Virol. 2016; 88: 58-63.

Feuillet F, Lina B, Rosa-Calatrava M, Boivin G. Ten years of human metapneumovirus research. J Clin Virol. 2012; 53: 97-105.

Fitzgerald DA, Kilham HA. Bronchiolitis: assessment and evidence-based management. Med J Aust. 2004; 180: 399-404.

Flores P, Guimarães J, Videira Amaral JM. Th1 and th2 cytokine expression in nasopharyngeal secretions during acute bronchiolitis in children younger than two years old. 124 Allergol Immunopathol. 2011; 39: 3-9.

Forno E, Celedón JC. Predicting asthma exacerbations in children. Curr Opin Pulm Med. 2012; 18: 63-9.

Fouchier RA, Hartwig NG, Bestebroer TM, Niemeyer B, de Jong JC, Simon JH, Osterhaus AD. A previously undescribed coronavirus associated with respiratory disease in humans. Proc Natl Acad Sci U S A. 2004; 101: 6212-6.

Freymouth F, Vabret A, Legrand L, Eterradossi N, Lafay-Delaire F, Brouard J, Guillois B. Presence of the new human metapneumovirus in French children with bronchiolitis. Pediatr Infect Dis J. 2003; 22: 92-4.

Fry AM, Lu X, Olsen SJ, Chittaganpitch M, Sawatwong P, Chantra S, Baggett HC, Erdman D. Human rhinovirus infections in rural Thailand: epidemiological evidence for rhinovirus as both pathogen and bystander. PLoS One. 2011; 6: e17780.

Fujitsuka A, Tsukagoshi H, Arakawa M, Goto-Sugai K, Ryo A, Okayama Y, Mizuta K, Nishina A, Yoshizumi M, Kaburagi Y, Noda M, Tashiro M, Okabe N, Mori M, Yokota S, Kimura H. A molecular epidemiological study of respiratory viruses detected in Japanese children with acute wheezing illness. BMC Infect Dis. 2011; 11: 168.

Gama RE, Horsnell PR, Hughes PJ, North C, Bruce CB, al-Nakib W, Stanway G. Amplification of rhinovirus specific nucleic acids from clinical samples using the polymerase chain reaction. J Med Virol. 1989; 28: 73-7.

Gama RE, Hughes PJ, Bruce CB, Stanway G. Polymerase chain reaction amplification of rhinovirus nucleic acids from clinical material. Nucleic Acids Res. 1988; 16: 9346.

García-García ML, Calvo C, Pérez-Breña P, De Cea JM, Acosta B, Casas I. Prevalence and clinical characteristics of human metapneumovirus infections in hospitalized infants in Spain. Pediatr Pulmonol. 2006; 41: 863-71.

Gern JE, Calhoun W, Swenson C, Shen G, Busse WW. Rhinovirus infection preferentially increases lower airway responsiveness in allergic subjects. Am J Respir Crit Care Med. 1997; 155: 1872-6.

Gern JE, Brockman-Schneider R, Bhattacharya S, Malter JS, Busse WW. Serum and low-density lipoprotein enhance interleukin-8 secretion by airway epithelial cells. Am J Respir Cell Mol Biol. 2003; 29: 483-9.

Gern JE, Rosenthal LA, Sorkness RL, Lemanske RF Jr. Effects of viral respiratory infections on lung development and childhood asthma. J Allergy Clin Immunol. 2005; 115: 668-74.

Gern JE, Brooks GD, Meyer P, Chang A, Shen K, Evans MD, Tisler C, Dasilva D, Roberg KA, Mikus LD, Rosenthal LA, Kirk CJ, Shult PA, Bhattacharya A, Li Z, Gangnon R, Lemanske RF Jr. Bidirectional interactions between viral respiratory illnesses and cytokine responses in the first year of life. J Allergy Clin Immunol. 2006; 117: 72-8.

Gern JE. Rhinovirus and the initiation of asthma. Curr Opin Allergy Clin Immunol. 2009; 9: 73-8.

Gern JE. The ABCs of rhinoviruses, wheezing, and asthma. J Virol. 2010; 84: 7418-26

Gern JE. How rhinovirus infections cause exacerbations of asthma. Clin Exp Allergy. 2015; 45: 32-42.

Gerna G, Campanini G, Rovida F, Percivalle E, Sarasini A, Marchi A, Baldanti F. Genetic variability of human coronavirus OC43-, 229E-, and NL63-like strains and their association with lower respiratory tract infections of hospitalized infants and immunocompromised patients. J Med Virol. 2006; 78: 938-49.

Gerna G, Piralla A, Rovida F, Rognoni V, Marchi A, Locatelli F, Meloni F. Correlation of rhinovirus load in the respiratory tract and clinical symptoms in hospitalized immunocompetent and immunocompromised patients. J Med Virol 2009; 81: 1498-507.

Gill MA, Bajwa G, George TA, Dong CC, Dougherty II, Jiang N, Gan VN, Gruchalla RS. Counterregulation between the FcepsilonRI pathway and antiviral responses in human plasmacytoid dendritic cells. J Immunol. 2010; 184: 5999-6006.

Global Asthma Network Steering Group. The Global Asthma Network Website: The Global Asthma Network; 2012. Available from: www.globalasthmanetwork.org.

Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention. Updated 2015. www.ginasthma.org

Guilbert TW, Singh AM, Danov Z, Evans MD, Jackson DJ, Burton R, Roberg KA, Anderson EL, Pappas TE, Gangnon R, Gern JE, Lemanske RF Jr. Decreased lung function after preschool wheezing rhinovirus illnesses in children at risk to develop asthma. J Allergy Clin Immunol 2011; 128: 532-8.

Habibi MS, Openshaw PJ. Benefit and harm from immunity to respiratory syncytial virus: implications for treatment. Curr Opin Infect Dis. 2012; 25: 687-94..

Hall CB, Weinberg GA, Blumkin AK, Edwards KM, Staat MA, Schultz AF, Poehling KA, Szilagyi PG, Griffin MR, Williams JV, Zhu Y, Grijalva CG, Prill MM, Iwane MK. Respiratory syncytial virus-associated hospitalizations among children less than 24 months of age. Pediatrics. 2013; 132: e341-8.

Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, Auinger P, Griffin MR, Poehling KA, Erdman D, Grijalva CG, Zhu Y, Szilagyi P. The burden of respiratory syncytial virus infection in young children. N Engl J Med. 2009; 360: 588-98.

Hall CB. Respiratory syncytial virus and parainfluenza virus. N Engl J Med. 2001; 344: 1917-28.

Hamparian VV, Colonno RJ, Cooney MK, Dick EC, Gwaltney JM Jr, Hughes JH, Jordan WS Jr, Kapikian AZ, Mogabgab WJ, Monto A, et al. A collaborative report: rhinoviruses--extension of the numbering system from 89 to 100. Virology. 1987; 159: 191-2.

Han MK, Huang YJ, Lipuma JJ, Boushey HA, Boucher RC, Cookson WO, Curtis JL, Erb-Downward J, Lynch SV, Sethi S, Toews GB, Young VB, Wolfgang MC, Huffnagle GB, Martinez FJ. Significance of the microbiome in obstructive lung disease. Thorax. 2012; 67: 456-63.

Hansbro NG, Horvat JC, Wark PA, Hansbro PM. Understanding the mechanisms of viral induced asthma: new therapeutic directions. Pharmacol Ther. 2008; 117: 313-53.

Hao W, Bernard K, Patel N, Ulbrandt N, Feng H, Svabek C, Wilson S, Stracener C, Wang K, Suzich J, Blair W, Zhu Q. Infection and propagation of human rhinovirus C in human airway epithelial cells. J Virol. 2012; 86: 13524-32.

Harrach B, Benkő M, Both GW, Brown M, Davison AJ, Echavarría M, Hess M, Jones MS, Kajon A, Lehmkuhl HD, Mautner V, Mittal SK, Wadell G (2011) Family Adenoviridae. King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (eds) Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier, New York. 125–141.

Harris JM Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 1996

Hasegawa K, Mansbach JM, Teach SJ, Fisher ES, Hershey D, Koh JY, Clark S, Piedra PA, Sullivan AF, Camargo CA Jr. 2014. Multicenter study of viral etiology and relapse in hospitalized children with bronchiolitis. Pediatr Infect Dis J. 2014; 33: 809-13.

Hayden FG, Herrington DT, Coats TL, Kim K, Cooper EC, Villano SA, Liu S, Hudson S, Pevear DC, Collett M, McKinlay M; Pleconaril Respiratory Infection Study Group. Efficacy and safety of oral pleconaril for treatment of colds due to picornaviruses in adults: results of 2 double-blind, randomized, placebo-controlled trials. Clin Infect Dis. 2003; 36: 1523-32.

Hedman L, Söderlund-Venermo M, Jartti T, Ruuskanen O, Hedman K. Dating of human bocavirus infection with protein-denaturing IgG-avidity assays-Secondary immune activations are ubiquitous in immunocompetent adults. J Clin Virol. 2010; 48: 44-8.

Heikkinen T, Silvennoinen H, Heinonen S, Vuorinen T. Clinical and socioeconomic impact of moderate-to-severe versus mild influenza in children. Eur J Clin Microbiol Infect Dis. 2016; 35: 1107-13.

Heinonen S, Peltola V, Silvennoinen H, Vahlberg T, Heikkinen T. Signs and symptoms predicting influenza in children: a matched case-control analysis of prospectively collected clinical data. Eur J Clin Microbiol Infect Dis. 2012; 31: 1569-74.

Helminen M, Nuolivirta K, Virta M, Halkosalo A, Korppi M, Vesikari T, Hurme M. IL-10 gene polymorphism at -1082 A/G is associated with severe rhinovirus bronchiolitis in infants. Pediatr Pulmonol. 2008; 43: 391-5.

Henderson J, Hilliard TN, Sherriff A, Stalker D, Al Shammari N, 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: 386-92.

Henrickson KJ, Hoover S, Kehl KS, Hua W. National disease burden of respiratory viruses detected in children by polymerase chain reaction. Pediatr Infect Dis J. 2004; 23(1 Suppl): S11-8.

Hewson CA, Jardine A, Edwards MR, Laza-Stanca V, Johnston SL. Toll-like receptor 3 is induced by and mediates antiviral activity against rhinovirus infection of human bronchial epithelial cells. J Virol. 2005; 79: 12273-9.

Heymann PW, Carper HT, Murphy DD, Platts-Mills TA, Patrie J, McLaughlin AP, Erwin EA, Shaker MS, Hellems M, Peerzada J, Hayden FG, Hatley TK, Chamberlain R. Viral infections in relation to age, atopy and season of admission among children hospitalized for wheezing. J Allergy Clin Immunol 2004: 114: 239–47.

Holgate ST. The sentinel role of the airway epithelium in asthma pathogenesis. Immunol Rev. 2011; 242: 205-19.

Holm-Hansen CC, Midgley SE, Fischer TK. Global emergence of enterovirus D68: a systematic review. Lancet Infect Dis. 2016 Feb 23. [Epub ahead of print]

Holt PG, 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: 1165-71.

Holt PG, Sly PD. Viral infections and atopy in asthma pathogenesis: new rationales for asthma prevention and treatment. Nat Med. 2012; 18: 726-35.

Horowitz L, Zafrir O, Gilboa S, Berger I, Wolach B. Acute asthma. Single dose oral steroids in paediatric community clinics. Eur J Pediatr. 1994; 153: 526-30.

Horsnell C, Gama RE, Hughes PJ, Stanway G. Molecular relationships between 21 human rhinovirus serotypes. J Gen Virol. 1995; 76: 2549-55.

Huber JP, Ramos HJ, Gill MA, Farrar JD. Cutting edge: Type I IFN reverses human Th2 commitment and stability by suppressing GATA3. J Immunol. 2010; 185: 813-7.

Hugg TT, Jaakkola MS, Ruotsalainen R, Pushkarev V, Jaakkola JJ. Exposure to animals and the risk of allergic asthma: a population-based cross-sectional study in Finnish and Russian children. Environ Health. 2008; 7: 28.

Huijskens EG, Biesmans RC, Buiting AG, Obihara CC, Rossen JW. Diagnostic value of respiratory virus detection in symptomatic children using real-time PCR. Virol J. 2012; 9: 276.

Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen TM, Korhonen K, Korppi MO. Teenage asthma after severe early childhood wheezing: an 11-year prospective follow-up. Pediatr Pulmonol. 2005; 40: 316-23.

Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen TM, Piippo-Savolainen E, Korppi M. Eosinophil activity in infants hospitalized for wheezing and risk of persistent childhood asthma. Pediatr Allergy Immunol. 2010; 21: 96-103.

Hyypiä T, Auvinen P, Maaronen M. Polymerase chain reaction for human picornaviruses. J Gen Virol. 1989; 70: 3261-8.

Illi S, von Mutius E, Lau S, Niggemann B, Grüber C, Wahn U; Multicentre Allergy Study (MAS) group. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. Lancet. 2006; 368: 763-70.

Iwane MK, Prill MM, Lu X, Miller EK, Edwards KM, Hall CB, Griffin MR, Staat MA, Anderson LJ, Williams JV, Weinberg GA, Ali A, Szilagyi PG, Zhu Y, Erdman DD. Human rhinovirus species associated with hospitalizations for acute respiratory illness in young US children. J Infect Dis. 2011; 204: 1702-10.

Jaakola S, Lyytikäinen O, Huusko S, Salmenlinna S, Pirhonen J, Savolainen-Kopra C, Liitsola K, Jalava J, Toropainen M, Nnohynek H, Virtanen M, Löflund JE, Kuusi M, Salminen M. Infectious Diseases in Finland 2014. Report 14/2015. National Institute for Health and Welfare.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, Lemanske RF Jr. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med. 2008; 178: 667-72.

Jackson DJ, Virnig CM, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Burton RM, Salazar LP, DaSilva DF, Shanovich KM, Tisler CJ, Gern JE, Lemanske RF Jr. Fractional exhaled nitric oxide measurements are most closely associated with allergic sensitization in school-age children. J Allergy Clin Immunol. 2009; 124: 949-53.

Jackson DJ, Lemanske RF Jr. The role of respiratory virus infections in childhood asthma inception. Immunol Allergy Clin North Am. 2010; 30: 513-22.

Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Pappas TE, Lee WM, Gern JE, Lemanske RF Jr. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. Am J Respir Cri Care Med 2012: 185: 281–5.

Jackson DJ, Gern JE, Lemanske RF Jr. The contributions of allergic sensitization and respiratory pathogens to asthma inception. J Allergy Clin Immunol. 2016; 137: 659-65.

Jacobs SE, Lamson DM, St George K, Walsh TJ. Human rhinoviruses. Clin Microbiol Rev. 2013; 26: 135-62.

Jacques J, Moret H, Minette D, Lévêque N, Jovenin N, Deslée G, Lebargy F, Motte J, Andréoletti L. Epidemiological, molecular, and clinical features of enterovirus respiratory infections in French children between 1999 and 2005. J Clin Microbiol. 2008; 46: 206-13..

Jacques J, Moret H, Minette D, Lévêque N, Jovenin N, Deslée G, Lebargy F, Motte J, Andréoletti L. Epidemiological, molecular, and clinical features of enterovirus respiratory infections in French children between 1999 and 2005. J Clin Microbiol. 2008; 46: 206-13.

Jakiela B, Brockman-Schneider R, Amineva S, Lee WM, Gern JE. Basal cells of differentiated bronchial epithelium are more susceptible to rhinovirus infection. Am J Respir Cell Mol Biol 2008; 38: 517-23.

Jang YJ, Lee SH, Kwon HJ, Chung YS, Lee BJ. Development of rhinovirus study model using organ culture of turbinate mucosa. J Virol Methods. 2005; 125: 41-7.

Jansen RR, Schinkel J, Dek I, Koekkoek SM, Visser CE, de Jong MD, Molenkamp R, Pajkrt D. Quantitation of respiratory viruses in relation to clinical course in children with acute respiratory tract infections. Pediatr Infect Dis J. 2010; 29: 82-4.

Jansen RR, Schinkel J, Koekkoek S, Pajkrt D, Beld M, de Jong MD, Molenkamp R. Development and evaluation of a four-tube real time multiplex PCR assay covering fourteen respiratory viruses, and comparison to its corresponding single target counterparts. J Clin Virol 2011: 51: 179–85.

Jansen RR, Wieringa J, Koekkoek SM, Visser CE, Pajkrt D, Molenkamp R, de Jong MD, Schinkel J. Frequent detection of respiratory viruses without symptoms: toward defining clinically relevant cutoff values. J Clin Microbiol. 2011; 49: 2631-6.

Jartti T, van den Hoogen B, Garofalo RP, Osterhaus AD, Ruuskanen O. Metapneumovirus and acute wheezing in children. Lancet. 2002; 360: 1393-4.

Jartti T, Lehtinen P, Vuorinen T,

Osterback R, van den Hoogen B, Osterhaus AD, Ruuskanen O. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. Emerg Infect Dis 2004: 10: 1095–101.

Jartti T, Lehtinen P, Vuorinen T, Koskenvuo M, Ruuskanen O. Persistence of rhinovirus and enterovirus RNA after acute respiratory illness in children. J Med Virol. 2004; 72: 695-9.

Jartti T, Lehtinen P, Vanto T, Hartiala J, Vuorinen T, Mäkelä MJ, Ruuskanen O. Evaluation of the efficacy of prednisolone in early wheezing induced by rhinovirus or respiratory syncytial virus. Pediatr Infect Dis J 2006: 25: 482–8.

Jartti T, Lehtinen P, Vanto T, Vuorinen T, Hartiala J, Hiekkanen H, Malmberg P, Mäkelä M, Ruuskanen O. Efficacy of prednisolone in children hospitalized for recurrent wheezing. Pediatr Allergy Immunol. 2007; 18: 326-34.

Jartti T, Lee WM, Pappas T, Evans M, Lemanske RF Jr, Gern JE. Serial viral infection in infants with recurrent respiratory illnesses. Eur Respir J 2008: 32: 314–20.

Jartti T, Lehtinen P, Vuorinen T, Ruuskanen O. Bronchiolitis: age and previous wheezing episodes are linked to viral etiology and atopic characteristics. Pediatr Infect Dis J 2009: 28: 311–7.

Jartti T, Kuusipalo H, Vuorinen T, Söderlund-Venermo M, Allander T, Waris M, Hartiala J, Ruuskanen O. Allergic sensitization is associated with rhinovirus-, but no other virus-, induced wheezing in children. Pediatr Allergy Immunol 2010: 21: 1008–14.

Jartti T, Hedman K, Jartti L, Ruuskanen O, Allander T, Söderlund-Venermo M. Human bocavirus-the first 5 years. Rev Med Virol 2012: 22: 46–64.

Jartti T, Söderlund-Venermo M, Hedman K, Ruuskanen O, Mäkelä MJ. New molecular virus detection methods and their clinical value in lower respiratory tract infections in children. Paediatr Respir Rev 2013: 14: 38–45.

Jartti T, Aakula M, Mansbach JM, Piedra PA, Bergroth E, Koponen P, Kivistö JE, Sullivan AF, Espinola JA, Remes S, Korppi M, Camargo CA Jr. Hospital length-of-stay is associated with rhinovirus etiology of bronchiolitis. Pediatr Infect Dis J. 2014. 33:829–34.

Jin Y, Yuan XH, Xie ZP, Gao HC, Song JR, Zhang RF, Xu ZQ, Zheng LS, Hou YD, Duan ZJ. Prevalence and clinical characterization of a newly identified human rhinovirus C species in children with acute respiratory tract infections. J Clin Microbiol. 2009; 47: 2895-900.

Johnston SL. Innate immunity in the pathogenesis of virus-induced asthma exacerbations. Proc Am Thorac Soc. 2007; 4: 267-70.

Juntti H, Osterlund P, Kokkonen J, Dunder T, Renko M, Pokka T, Julkunen I, Uhari M. Cytokine responses in cord blood predict the severity of later respiratory syncytial virus infection. J Allergy Clin Immunol. 2009; 124: 52-58.e1-2.

Kahn JS. Epidemiology of human metapneumovirus. Clin Microbiol Rev. 2006; 19: 546-57.

Kainulainen L, Vuorinen T, Rantakokko-Jalava, K, Osterback R, Ruuskanen O. Recurrent and persistent respiratory tract viral infections in patients with primary hypogammaglobulinemia. J Allergy Clin Immunol. 2010; 126: 120–126

Kaiser L, Fritz RS, Straus SE, Gubareva L, Hayden FG. Symptom pathogenesis during acute influenza: interleukin-6 and other cytokine responses. J Med Virol. 2001; 64: 262-8.

Kaiser L, Aubert JD, Pache JC, et al. Chronic rhinoviral infection in lung transplant recipients. Am J Respir Crit Care Med. 2006; 174: 1392–1399

Kalliola S, Pelkonen AS, Malmberg LP, Sarna S, Hämäläinen M, Mononen I, Mäkelä MJ. Maternal smoking affects lung function and airway inflammation in young children with multiple-trigger wheeze. J Allergy Clin Immunol 2013: 131: 730–5.

Kantola K, Hedman L, Allander T, Jartti T, Lehtinen P, Ruuskanen O, Hedman K, Söderlund-Venermo M. Serodiagnosis of human bocavirus infection. Clin Infect Dis. 2008; 46: 540-6.

Kantola K, Hedman L, Arthur J, Alibeto A, Delwart E, Jartti T, Ruuskanen O, Hedman K, Söderlund-Venermo M. Seroepidemiology of human bocaviruses 1-4. J Infect Dis. 2011; 204: 1403-12.

Kapoor A, Slikas E, Simmonds P, Chieochansin T, Naeem A, Shaukat S, Alam MM, Sharif S, Angez M, Zaidi S, Delwart E. A newly identified bocavirus species in human stool. J Infect Dis. 2009; 199: 196-200.

Kares S, Lönnrot M, Vuorinen P, Oikarinen S, Taurianen S, Hyöty H. Real-time PCR for rapid diagnosis of entero- and rhinovirus infections using LightCycler. J Clin Virol. 2004; 29: 99-104.

Kato A, Favoreto S Jr, Avila PC, Schleimer RP. TLR3- and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. J Immunol. 2007; 179: 1080-7.

Katpally U, Fu TM, Freed DC, Casimiro DR, Smith TJ. Antibodies to the buried N terminus of rhinovirus VP4 exhibit cross-serotypic neutralization. J Virol. 2009; 83: 7040-8

Khoshdel A, Parvin N, Doosti A, Famouri F. Prevalence of Nosocomial Diarrhea Due to Adenoviruses 40 and 41 in a Paediatric Ward in Iran. J Clin Diagn Res. 2015; 9: SC15-7.

Kieninger E, Regamey N. Rhinoviruses: markers of, or causative for, recurrent wheeze and asthma? Eur Respir J. 2012; 39: 238-9.

Kim TK, Bheda-Malge A, Lin Y, Sreekrishna K, Adams R, Robinson MK, Bascom CC, Tiesman JP8, Isfort RJ, Gelinas R. A systems approach to understanding human rhinovirus and influenza virus infection. Virology. 2015; 486: 146-57.

Kling S, Donninger H, Williams Z, Vermeulen J, Weinberg E, Latiff K, Ghildyal R, Bardin P. Persistence of rhinovirus RNA after asthma exacerbation in children. Clin Exp Allergy. 2005; 35: 672-8.

Kloepfer KM, Gern JE. Virus/allergen interactions and exacerbations of asthma. Immunol Allergy Clin North Am. 2010; 30: 553-63.

Knowles, N.J., Hovi, T, Hyypiä, T., King, A.M.Q., Lindberg, A.M., Pallansch, M.A., Palmenberg, A.C., Simmonds, P., Skern, T., Stanway, G., Yamashita, T. and Zell, R. (2012). Picornaviridae. In: Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses. Ed: King, A.M.Q., Adams, M.J., Carstens, E.B. and Lefkowitz, E.J. San Diego: Elsevier, pp 855-880.

Konstantinidou A, Anninos H, Spanakis N, Kotsiakis X, Syridou G, Tsakris A, Patsouris E. Transplacental infection of Coxsackievirus B3 pathological findings in the fetus. J Med Virol. 2007; 79: 754-7.

Korpi-Steiner NL, Bates ME, Lee WM, Hall DJ, Bertics PJ. Human rhinovirus induces robust IP-10 release by monocytic cells, which is independent of viral replication but linked to type I interferon receptor ligation and STAT1 activation. J Leukoc Biol. 2006; 80: 1364-74.

Korppi M, Kotaniemi-Syrjänen A, Waris M, Vainionpää R, Reijonen TM. Rhinovirus-associated wheezing in infancy: comparison with respiratory syncytial virus bronchiolitis. Pediatr Infect Dis J. 2004; 23: 995-9.

Korppi M. Virus-induced wheezing in infants aged 12-24 months and bronchiolitis in infants under 6 months are different clinical entities. Acta Paediatr. 2015; 104: e539.

Kotaniemi-Syrjänen A, Reijonen TM, Korhonen K, Waris M, Vainionpää R, Korppi M. Wheezing due to rhinovirus infection in infancy: Bronchial hyperresponsiveness at school age. Pediatr Int. 2008; 50: 506-10.

Kotaniemi-Syrjänen A, Reijonen TM, Romppanen J, Korhonen K, Savolainen K, Korppi M. Allergen-specific immunoglobulin E antibodies in wheezing infants: the risk for asthma in later childhood. Pediatrics. 2003; 111: e255-61.

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

Kouni S, Karakitsos P, Chranioti A, Theodoridou M, Chrousos G, Michos A. Evaluation of viral co-infections in hospitalized and non-hospitalized children with respiratory infections using microarrays. Clin Microbiol Infect. 2013; 19: 772-7.

Krawiec ME, Westcott JY, Chu HW, Balzar S, Trudeau JB, Schwartz LB, Wenzel SE. Persistent wheezing in very young children is associated with lower respiratory inflammation. Am J Respir Crit Care Med. 2001; 163: 1338-43.

Kuiken T, Taubenberger JK. Pathology of human influenza revisited. Vaccine. 2008;26: D59-D66

Kumar S, Wang L, Fan J, Kraft A, Bose ME, Tiwari S, Van Dyke M, Haigis R, Luo T, Ghosh M, Tang H, Haghnia M, Mather EL, Weisburg WG, Henrickson KJ. Detection of 11 common viral and bacterial pathogens causing community-acquired pneumonia or sepsis in asymptomatic patients by using a multiplex reverse transcription-PCR assay with manual (enzyme hybridization) or automated (electronic microarray) detection. J Clin Microbiol. 2008; 46: 3063-72.

Kurukulaaratchy RJ, Fenn MH, Waterhouse LM, Matthews SM, Holgate ST, Arshad SH. Characterization of wheezing phenotypes in the first 10 years of life. Clin Exp Allergy 2003; 33: 573–578.

Kurukulaaratchy RJ, Matthews S, Holgate ST, Arshad SH. Predicting persistent disease among children who wheeze during early life. Predicting persistent disease among children who wheeze during early life. Eur Respir J. 2003; 22: 767-71.

Kusel MM, de Klerk NH, Kebadze T, Vohma V, Holt PG, Johnston SL, Sly PD. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. J Allergy Clin Immunol 2007: 119: 1105–10.

Kusel MM, Kebadze T, Johnston SL, Holt PG, Sly PD. Febrile respiratory illnesses in infancy and atopy are risk factors for persistent asthma and wheeze. Eur Respir J. 2012; 39: 876-82.

Kuypers J, Wright N, Corey L, Morrow R. Detection and quantification of human metapneumovirus in pediatric specimens by real-time RT-PCR. J Clin Virol. 2005; 33: 299-305.

Kuypers J, Wright N, Ferrenberg J, Huang ML, Cent A, Corey L, Morrow R. Comparison of real-time PCR assays with fluorescent-antibody assays for diagnosis of respiratory virus infections in children. J Clin Microbiol. 2006; 44: 2382-8.

Lachowicz-Scroggins ME1, Boushey HA, Finkbeiner WE, Widdicombe JH. Interleukin-13-induced mucous metaplasia increases susceptibility of human airway epithelium to rhinovirus infection. Am J Respir Cell Mol Biol. 2010; 43: 652-61.

Lai CK, Beasley R, Crane J, Foliaki S, Shah J, Weiland S; International Study of Asthma and Allergies in Childhood Phase Three Study Group. 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: 476-83.

Lakhanpaul M, MacFaul R, Werneke U, Armon K, Hemingway P, Stephenson T. An evidence-based guideline for children presenting with acute breathing difficulty. Emerg Med J. 2009; 26: 850-3.

Lanari M, Giovannini M, Giuffré L, Marini A, Rondini G, Rossi GA, Merolla R, Zuccotti GV, Salvioli GP; Investigators R.A.DA.R. Study Group. Prevalence of respiratory syncytial virus infection in Italian infants hospitalized for acute lower respiratory tract infections, and association between respiratory syncytial virus infection risk factors and disease severity. Pediatr Pulmonol. 2002; 33: 458-65.

Landry M. 2011. Rhinoviruses, p 1400–1409. In Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, WarnockDW(ed), Manual of clinical microbiology, 10th ed. ASM Press, Washington, DC.

Lau S, Illi S, Sommerfeld C, Niggemann B, Völkel K, Madloch C, Grüber C, Nickel R, Forster J, Wahn U; Multicentre Allergy Study Group. Transient early wheeze is not associated with impaired lung function in 7-yr-old children. Eur Respir J 2003; 21: 834–841.

Lau SK, Yip CC, Tsoi HW, Lee RA, So LY, Lau YL, Chan KH, Woo PC, Yuen KY. Clinical features and complete genome characterization of a distinct human rhinovirus (HRV) genetic cluster, probably representing a previously undetected HRV species, HRV-C, associated with acute respiratory illness in children. J Clin Microbiol. 2007; 45: 3655-64.

Lau C, Wang X, Song L, North M, Wiehler S, Proud D, Chow CW. Syk associates with clathrin and mediates phosphatidylinositol 3-kinase activation during human rhinovirus internalization. J Immunol. 2008; 180: 870-80.

Lau SK, Yip CC, Lin AW, Lee RA, So LY, Lau YL, Chan KH, Woo PC, Yuen KY. Clinical and molecular epidemiology of human rhinovirus C in children and adults in Hong Kong reveals a possible distinct human rhinovirus C subgroup. J Infect Dis. 2009; 200: 1096-103.

Lauinger IL, Bible JM, Halligan EP, Bangalore H, Tosas O, Aarons EJ, MacMahon E, Tong CY. Patient characteristics and severity of human rhinovirus infections in children. J Clin Virol. 2013; 58: 216-20.

Le Souëf PN. Gene-environmental interaction in the development of atopic asthma: new developments. Curr Opin Allergy Clin Immunol. 2009; 9: 123-7.

Ledford RM, Patel NR, Demenczuk TM, Watanyar A, Herbertz T, Collett MS, Pevear DC. VP1 sequencing of all human rhinovirus serotypes: insights into genus phylogeny and susceptibility to antiviral capsid-binding compounds. J Virol. 2004; 78: 3663-74.

Lee BE, Chawla R, Langley JM, Forgie SE, Al-Hosni M, Baerg K, Husain E, Strong J, Robinson JL, Allen U, Law BJ, Dobson S, Davies HD. Paediatric Investigators Collaborative Network on Infections in Canada (PICNIC) study of aseptic meningitis. BMC Infect Dis. 2006; 6: 68.

Lee WM, Kiesner C, Pappas T, Lee I, Grindle K, Jartti T, Jakiela B, Lemanske RF Jr, Shult PA, Gern JE. A diverse group of previously unrecognized human rhinoviruses are common causes of respiratory illnesses in infants. PLoS One. 2007; 2: e966.

Lee WM, Lemanske RF Jr, Evans MD, Vang F, Pappas T, Gangnon R, Jackson DJ, Gern JE. Human rhinovirus species and season of infection determine illness severity. Am J Respir Crit Care Med. 2012; 186: 886-91.

Lehtinen P, Ruohola A, Vuorinen T, Vanto T, Ruuskanen O, Jartti T. Prednisolone reduces recurrent wheezing after a first wheezing episode associated with rhinovirus infection or eczema. J Allergy Clin Immunol 2007; 119: 570-5.

Leigh R, Oyelusi W, Wiehler S, Koetzler R, Zaheer RS, Newton R, Proud D. Human rhinovirus infection enhances airway epithelial cell production of growth factors involved in airway remodeling. J Allergy Clin Immunol. 2008; 121: 1238-1245.e4.

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, Gern JE. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. J Allergy Clin Immunol. 2005; 116: 571-7.

Lin YC, Lu PL, Lin KH, Chu PY, Wang CF, Lin JH, Liu HF. Molecular Epidemiology and Phylogenetic Analysis of Human Adenovirus Caused an Outbreak in Taiwan during 2011. PLoS One. 2015; 10: e0127377.

Linder JE, Kraft DC, Mohamed Y, Lu Z, Heil L, Tollefson S, Saville BR, Wright PF, Williams JV, Miller EK. Human rhinovirus C: Age, season, and lower respiratory illness over the past 3 decades. J Allergy Clin Immunol. 2013; 131: 69-77.e1-6.

Linsuwanon P, Payungporn S, Samransamruajkit R, Posuwan N, Makkoch J, Theanboonlers A, Poovorawan Y. High prevalence of human rhinovirus C infection in Thai children with acute lower respiratory tract disease. J Infect. 2009; 59: 115-21.

Linsuwanon P, Payungporn S, Samransamruajkit R, Theamboonlers A, Poovorawan Y. Recurrent human rhinovirus infections in infants with refractory wheezing. Emerg Infect Dis. 2009 Jun;15(6):978-80.

Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev. 2014; 27: 441-62.

Loeffelholz MJ, Trujillo R, Pyles RB3, Miller AL, Alvarez-Fernandez P, Pong DL, Chonmaitree T. Duration of rhinovirus shedding in the upper respiratory tract in the first year of life. Pediatrics. 2014; 134: 1144-50.

Longtin J, Bastien M, Gilca R, Leblanc E, de Serres G, Bergeron MG, Boivin G. Human bocavirus infections in hospitalized children and adults. Emerg Infect Dis. 2008; 14: 217-21.

Lopez-Souza N, Dolganov G, Dubin R, Sachs LA, Sassina L, Sporer H, Yagi S, Schnurr D, Boushey HA, Widdicombe JH. Resistance of differentiated human airway epithelium to infection by rhinovirus. Am J Physiol Lung Cell Mol Physiol. 2004; 286:L 373-81.

Lowe LA, Simpson A, Woodcock A, Morris J, Murray CS, Custovic A, and the NAC Manchester Asthma and Allergy Study Group. Wheeze phenotypes and lung function in preschool children. Am J Respir Crit Care Med 2005; 171: 231–37.

Lukashev AN, Ivanova OE, Eremeeva TP, Iggo RD. Evidence of frequent recombination among human adenoviruses. J Gen Virol. 2008; 89(: 380-8.

Lukkarinen H, Söderlund-Venermo M, Vuorinen T, Allander T, Hedman K, Simell O, Ruuskanen O, Jartti T. Human bocavirus 1 may suppress rhinovirus-associated immune response in wheezing children. J Allergy Clin Immunol 2014: 133: 256–8.

Lukkarinen M, Lukkarinen H, Lehtinen P, Vuorinen T, Ruuskanen O, Jartti T. Prednisolone reduces recurrent wheezing after first rhinovirus wheeze: a 7-year follow-up. Pediatr Allergy Immunol 2013: 24: 237–43.

Lukkarinen M, Vuorinen T, Lehtinen P, Ruuskanen O, Jartti T. Sensitization at the first wheezing episode increases risk for long-term asthma therapy. Pediatr Allergy Immunol. 2015; 26: 687-91.

Luo H, Zhang Z, Zheng Z, Ke X, Zhang X, Li Q, Liu Y, Bai B, Mao P, Hu Q, Wang H. Human bocavirus VP2 upregulates IFN-β pathway by inhibiting ring finger protein 125-mediated ubiquitination of retinoic acid-inducible gene-I. J Immunol. 2013; 191: 660-9.

Lynch JP, Mazzone SB, Rogers MJ, Arikkatt JJ, Loh Z, Pritchard AL, Upham JW, Phipps S. The plasmacytoid dendritic cell: at the cross-roads in asthma. Eur Respir J. 2014; 43: 264-75.

Lönnrot M, Sjöroos M, Salminen K, Maaronen M, Hyypiä T, Hyöty H. Diagnosis of enterovirus and rhinovirus infections by RT-PCR and time-resolved fluorometry with lanthanide chelate labeled probes. J Med Virol. 1999; 59: 378-84.

Maertzdorf J, Wang CK, Brown JB, Quinto JD, Chu M, de Graaf M, van den Hoogen BG, Spaete R, Osterhaus AD, Fouchier RA. Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. J Clin Microbiol. 2004; 42: 981-6.

Mak RK, Tse LY, Lam WY, Wong GW, Chan PK, Leung TF. Clinical spectrum of human rhinovirus infections in hospitalized Hong Kong children. Pediatr Infect Dis J. 2011; 30: 749-53.

Malcolm E, Arruda E, Hayden FG, Kaiser L. Clinical features of patients with acute respiratory illness and rhinovirus in their bronchoalveolar lavages. J Clin Virol. 2001; 21: 9-16.

Malhotra R, Ward M, Bright H, Priest R, Foster MR, Hurle M, Blair E, Bird M. Isolation and characterisation of potential respiratory syncytial virus receptor(s) on epithelial cells. Microbes Infect. 2003; 5: 123-33.

Mallol J, Crane J, von Mutius E, Odhiambo J, Keil U, Stewart A; ISAAC Phase Three Study Group. The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three: a global synthesis. Allergol Immunopathol (Madr). 2013; 41: 73-85.

Malmström K, Pitkäranta A, Carpen O, Pelkonen A, Malmberg LP, Turpeinen M, Kajosaari M, Sarna S, Lindahl H, Haahtela T, Mäkelä MJ. Human rhinovirus in bronchial epithelium of infants with recurrent respiratory symptoms. J Allergy Clin Immunol. 2006; 118: 591-6.

Mansbach JM, McAdam AJ, Clark S, Hain PD, Flood RG, Acholonu U, Camargo CA Jr. Prospective multicenter study of the viral etiology of bronchiolitis in the emergency department. Acad Emerg Med. 2008; 15: 111-8.

Mansbach JM, Piedra PA, Teach SJ, Sullivan AF, Forgey T, Clark S, Espinola JA, Camargo CA Jr; MARC-30 Investigators. Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. Arch Pediat Adolesc Med 2012: 166: 700–6.

Marcone DN, Culasso A, Carballal G, Campos R, Echavarría M. Genetic diversity and clinical impact of human rhinoviruses in hospitalized and outpatient children with acute respiratory infection, Argentina. J Clin Virol. 2014; 61: 558-64.

Marcone DN, Ellis A, Videla C, Ekstrom J, Ricarte C, Carballal G, Vidaurreta SM, Echavarría M. Viral etiology of acute respiratory infections in hospitalized and outpatient children in Buenos Aires, Argentina. Pediatr Infect Dis J. 2013; 32: e105-10.

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

Martin ET, Fairchok MP, Kuypers J, Magaret A, Zerr DM, Wald A, Englund JA. Frequent and prolonged shedding of bocavirus in young children attending daycare. J Infect Dis. 2010; 201: 1625-32.

Martin EK, Kuypers J, Chu HY, Lacombe K, Qin X, Strelitz B, Bradford M, Jones C, Klein EJ, Englund JA. Molecular epidemiology of human rhinovirus infections in the pediatric emergency department. J Clin Virol. 2015; 62: 25-31.

Martinez F, Wright A, Taussig L, Holberg C, Halonen M, Morgan W. Asthma and wheezing in the first six years of life. N Engl J Med. 1995; 332:133–8.

Matricardi PM, Illi S, Grüber C, Keil T, Nickel R, Wahn U, Lau S. Wheezing in childhood: incidence, longitudinal patterns and factors predicting persistence. Eur Respir J. 2008; 32: 585-92.

Matricardi PM, Illi S, Keil T, Wagner P, Wahn U, Lau S. Predicting persistence of wheezing: one algorithm does not fit all. Eur Respir J. 2010; 35: 701-3.

Matsuzaki Y, Katsushima N, Nagai Y, Shoji M, Itagaki T, Sakamoto M, Kitaoka S, Mizuta K, Nishimura H. Clinical features of influenza C virus infection in children. J Infect Dis. 2006;193: 1229-35.

McIntyre, C.L., Knowles, N.J. and Simmonds, P. Proposals for the classification of human rhinovirus species A, B and C into genotypically assigned types. J. Gen. Virol. 2013; 94: 1791-1806.

McErlean P, Shackelton LA, Lambert SB, Nissen MD, Sloots TP, Mackay IM. Characterisation of a newly identified human rhinovirus, HRV-QPM, discovered in infants with bronchiolitis. J Clin Virol. 2007; 39: 67-75.

McLean GR, Walton RP, Shetty S, Peel TJ, Paktiawal N, Kebadze T, Gogsadze L, Niespodziana K, Valenta R, Bartlett NW, Johnston SL. Rhinovirus infections and immunisation induce cross-serotype reactive antibodies to VP1. Antiviral Res. 2012; 95: 193-201.

Medici MC, Arcangeletti MC, Rossi GA, Lanari M, Merolla R, Paparatti UD, Chezzi C; Osservatorio VRS Study Group. Four year incidence of respiratory syncytial virus infection in infants and young children referred to emergency departments for lower respiratory tract diseases in Italy: the "Osservatorio VRS" Study (2000-2004). New Microbiol. 2006; 29: 35-43.

Mehta AK, Duan W, Doerner AM, Traves SL, Broide DH, Proud D, Zuraw BL, Croft M. Rhinovirus infection interferes with induction of tolerance to aeroantigens through OX40 ligand, thymic stromal lymphopoietin, and IL-33. J Allergy Clin Immunol. 2016; 137: 278-88. e6.

Meissner HC. Viral Bronchiolitis in Children. N Engl J Med. 2016; 374: 62-72.

Meriluoto M, Hedman L, Tanner L, Simell V, Mäkinen M, Simell S, Mykkänen J, Korpelainen J, Ruuskanen O, Ilonen J, Knip M, Simell O, Hedman K, Söderlund-Venermo M. Association of human bocavirus 1 infection with respiratory disease in childhood followup study, Finland. Emerg Infect Dis 2012: 18: 264–71.

Message SD, Laza-Stanca V, Mallia P, Parker HL, Zhu J, Kebadze T, Contoli M, Sanderson G, Kon OM, Papi A, Jeffery PK, Stanciu LA, Johnston SL. Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. Proc Natl Acad Sci U S A. 2008; 105: 13562-7.

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

Midulla F, Pierangeli A, Cangiano G, Bonci E, Salvadei S, Scagnolari C, Moretti C, Antonelli G, Ferro V, Papoff P. Rhinovirus bronchiolitis and recurrent wheezing: 1-year follow-up. Eur Respir J 2012: 39: 396–402.

Midulla F, Nicolai A, Ferrara M, Gentile F, Pierangeli A, Bonci E, Scagnolari C, Moretti C, Antonelli G, Papoff P. Recurrent wheezing 36 months after bronchiolitis is associated with rhinovirus infections and blood eosinophilia. Acta Paediatr. 2014; 103: 1094-9.

Miller EK, Lu X, Erdman DD, Poehling KA, Zhu Y, Griffin MR, Hartert TV, Anderson LJ, Weinberg GA, Hall CB, Iwane MK, Edwards KM; New Vaccine Surveillance Network. Rhinovirus-associated hospitalizations in young children. J Infect Dis. 2007; 195: 773-81.

Miller EK, Edwards KM, Weinberg GA, Iwane MK, Griffin MR, Hall CB, Zhu Y, Szilagyi PG, Morin LL, Heil LH, Lu X, Williams JV; New Vaccine Surveillance Network. A novel group of rhinoviruses is associated with asthma hospitalizations. J Allergy Clin Immunol. 2009; 123: 98-104.e1.

Miller EK, Khuri-Bulos N, Williams JV, Shehabi AA, Faouri S, Al Jundi I, Chen Q, Heil L, Mohamed Y, Morin LL, Ali A, Halasa NB. Human rhinovirus C associated with wheezing in hospitalised children in the Middle East. J Clin Virol. 2009; 46: 85-9.

Miller EK, Williams JV, Gebretsadik T, Carroll KN, Dupont WD, Mohamed YA, Morin LL, Heil L, Minton PA, Woodward K, Liu Z, Hartert TV. Host and viral factors associated with severity of human rhinovirus-associated infant respiratory tract illness. J Allergy Clin Immunol. 2011; 127: 883-91.

Miller EK, Mackay IM. From sneeze to wheeze: what we know about rhinovirus Cs. J Clin Virol. 2013; 57: 291-9. Miron D, Srugo I, Kra-Oz Z, Keness Y, Wolf D, Amirav I, Kassis I. Sole pathogen in acute bronchiolitis: is there a role for other organisms apart from respiratory syncytial virus? Pediatr Infect Dis J 2010: 29: e7–10.

Morfin F, Dupuis-Girod S, Frobert E, Mundweiler S, Carrington D, Sedlacek P, Bierings M, Cetkovsky P, Kroes AC, van Tol MJ, Thouvenot D. Differential susceptibility of adenovirus clinical isolates to cidofovir and ribavirin is not related to species alone. Antivir Ther. 2009 ;14: 55-61.

Morgan WJ, Stern DA, Sherrill DL, Guerra S, Holberg CJ, Guilbert TW, Taussig LM, Wright AL, 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: 1253-8.

Mosser AG, Brockman-Schneider R, Amineva S, Burchell L, Sedgwick JB, Busse WW, Gern JE. Similar frequency of rhinovirus-infectible cells in upper and lower airway epithelium. J Infect Dis. 2002; 185: 734-43.

Mosser AG, Vrtis R, Burchell L, Lee WM, Dick CR, Weisshaar E, Bock D, Swenson CA, Cornwell RD, Meyer KC, Jarjour NN, Busse WW, Gern JE. Quantitative and qualitative analysis of rhinovirus infection in bronchial tissues. Am J Respir Crit Care Med. 2005; 171: 645-51.

Mullins JA, Erdman DD, Weinberg GA, Edwards K, Hall CB, Walker FJ, Iwane M, Anderson LJ. Human metapneumovirus infection among children hospitalized with acute respiratory illness. Emerg Infect Dis. 2004; 10: 700-5.

Müller L, Mack I, Tapparel C, Kaiser L, Alves MP, Kieninger E, Frey U, Regamey N, Latzin P. Human Rhinovirus Types and Association with Respiratory Symptoms During the First Year of Life. Pediatr Infect Dis J. 2015; 34: 907-9.

Nakagome K, Bochkov YA, Ashraf S, Brockman-Schneider RA, Evans MD, Pasic TR, Gern JE. Effects of rhinovirus species on viral replication and cytokine production. J Allergy Clin Immunol. 2014; 134: 332-41.

Nascimento MS, Souza AV, Ferreira AV, Rodrigues JC, Abramovici S, Silva Filho LV. High rate of viral identification and coinfections in infants with acute bronchiolitis. Clinics (Sao Paulo) 2010: 65: 1133–7.

National Heart, Lung and Blood Institute (NAEPP). Expert Panel Report 3 (EPR3): Guidelines for the diagnosis and management of asthma. Full Report 2007. http://www.nhlbi.nih.gov/files/docs/guidelines/asthgdln.pdf

Niespodziana K, Cabauatan CR, Jackson DJ, Gallerano D, Trujillo-Torralbo B, Del Rosario A, Mallia P, Valenta R, Johnston SL. Rhinovirus-induced VP1-specific Antibodies are Group-specific and Associated With Severity of Respiratory Symptoms. EBioMedicine. 2014; 2: 64-70.

Nissen SP, Kjaer HF, Høst A, Nielsen J, Halken S. The natural course of sensitization and allergic diseases from childhood to adulthood. Pediatr Allergy Immunol. 2013; 24: 549-55.

Nokso-Koivisto J, Kinnari TJ, Lindahl P, Hovi T, Pitkäranta A. Human picornavirus and coronavirus RNA in nasopharynx of children without concurrent respiratory symptoms. J Med Virol. 2002; 66: 417-20.

Nurani G, Lindqvist B, Casasnovas JM. Receptor priming of major group human rhinoviruses for uncoating and entry at mild low-pH environments. J Virol. 2003; 77: 11985-91.

Oikarinen M, Tauriainen S, Oikarinen S, Honkanen T, Collin P, Rantala I, Mäki M, Kaukinen K, Hyöty H. Type 1 diabetes is associated with enterovirus infection in gut mucosa. Diabetes. 2012; 61: 687-91.

Oommen A, Lambert PC, Grigg J. Efficacy of a short course of parent-initiated oral prednisolone for viral wheeze in children aged 1-5 years: randomised controlled trial Lancet 2003;362:1433-8.

Osterback R, Tevaluoto T, Ylinen T, Peltola V, Susi P, Hyypia T, Waris M. Simultaneous detection and differentiation of human rhino- and enteroviruses in clinical specimens by real-time PCR with locked nucleic acid probes. J Clin Microbiol 2013; 51: 3960-7.

Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. JAMA. 2002; 288: 963-72.

Palmenberg AC, Spiro D, Kuzmickas R, Wang S, Djikeng A, Rathe JA, Fraser-Liggett CM, Liggett SB. Sequencing and analyses of all known human rhinovirus genomes reveal structure and evolution. Science. 2009; 324: 55-9.

Panickar J, Lakhanpaul M, Lambert PC, Kenia P, Stephenson T, Smyth A, et al. Oral prednisolone for preschool children with acute virus-induced wheezing. N Engl J Med 2009; 360: 329-38.

Papadopoulos NG, Bates PJ, Bardin PG, Papi A, Leir SH, Fraenkel DJ, Meyer J, Lackie PM, Sanderson G, Holgate ST, Johnston SL. Rhinoviruses infect the lower airways. J Infect Dis. 2000; 181: 1875-84.

Papadopoulos NG, Stanciu LA, Papi A, Holgate ST, Johnston SL. A defective type 1 response to rhinovirus in atopic asthma. Thorax. 2002; 57: 328-32.

Papadopoulos NG, Moustaki M, Tsolia M, Bossios A, Astra E, Prezerakou A, Gourgiotis D, Kafetzis D. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. Am J Respir Crit Care Med. 2002; 165: 1285-9.

Papadopoulos NG, Papi A, Psarras S, Johnston SL. Mechanisms of rhinovirus-induced asthma. Paediatr Respir Rev. 2004; 5: 255-60.

Papi A, 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: 9707-20.

Papi A, Papadopoulos NG, Degitz K, Holgate ST, 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: 318-26.

Peltola V, Heikkinen T, Ruuskanen O. Clinical courses of croup caused by influenza and parainfluenza viruses. Pediatr Infect Dis J. 2002; 21: 76-8.

Peltola V, Waris M, Österback R, Susi P, Ruuskanen O, Hyypiä T. Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. J Infect Dis 2008; 197: 382-9.

Pientong C, Ekalaksananan T, Teeratakulpisarn J, Tanuwattanachai S, Kongyingyoes B, 120 Limwattananon C. Atypical bacterial pathogen infection in children with acute bronchiolitis in 121 northeast Thailand. J Microbiol Immunol Infect. 2011; 44: 95-100.

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

Piralla A, Rovida F, Campanini G, Rognoni V, Marchi A, Locatelli F, Gerna G. Clinical severity and molecular typing of human rhinovirus C strains during a fall outbreak affecting hospitalized patients. J Clin Virol. 2009; 45: 311-7.

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, Klassen TP; Pediatric Emergency Research Canada (PERC). Epinephrine and dexamethasone in children with bronchiolitis. N Engl J Med 2009; 360: 2079-89.

Principi N, Zampiero A, Gambino M, Scala A, Senatore L, Lelii M, Ascolese B, Pelucchi C, Esposito S. Prospective evaluation of rhinovirus infection in healthy young children. J Clin Virol. 2015; 66: 83-9.

Pritchard AL, Carroll ML, Burel JG, White OJ, Phipps S, Upham JW. Innate IFNs and plasmacytoid dendritic cells constrain Th2 cytokine responses to rhinovirus: a regulatory mechanism with relevance to asthma. J Immunol. 2012; 188: 5898-905.

Proud D, Hudy MH, Wiehler S, Zaheer RS, Amin MA, Pelikan JB, Tacon CE, Tonsaker TO, Walker BL, Kooi C, Traves SL, Leigh R. Cigarette smoke modulates expression of human rhinovirus-induced airway epithelial host defense genes. PLoS One. 2012; 7: e40762.

Proud D, Turner RB, Winther B, Wiehler S, Tiesman JP, Reichling TD, Juhlin KD, Fulmer AW, Ho BY, Walanski AA, Poore CL, Mizoguchi H, Jump L, Moore ML, Zukowski CK, Clymer JW. Gene expression profiles during in vivo human rhinovirus infection: insights into the host response. Am J Respir Crit Care Med. 2008; 178: 962-8.

Pruikkonen H, Uhari M, Dunder T, Pokka T, Renko M. Initial oxygen saturation values can predict the need to hospitalise children with mild wheezing. Acta Paediatr. 2014; 103: 951-6.

Qouy Molecular Biology and Evolution 2010

Rakes GP, Arruda E, Ingram JM, Hoover GE, Zambrano JC, Hayden FG, Platts-Mills TA, Heymann PW. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. Am J Respir Crit Care Med 1999; 159:785-90.

Rakshi K, Couriel JM. Management of acute bronchiolitis. Arch Dis Child. 1994; 71: 463-9.

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; American Academy of Pediatrics. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. Pediatrics. 2014; 134: e1474-502.

Remes ST, Koskela HO, Iivanainen K, Pekkanen J. Allergen-specific sensitization in asthma and allergic diseases in children: the study on farmers′ and non-farmers′ children. Clin Exp Allergy. 2005 ; 35: 160-6.

Rhodes HL, Sporik R, Thomas P, Holgate ST, Cogswell JJ. Early life risk factors for adult asthma: a birth cohort study of subjects at risk. J Allergy Clin Immunol. 2001; 108: 720-5.

Ricart S, Marcos MA, Sarda M, Anton A, Muñoz-Almagro C, Pumarola T, Pons M, Garcia-Garcia JJ. Clinical risk factors are more relevant than respiratory viruses in predicting bronchiolitis severity. Pediatr Pulmonol 2013: 48: 456–63.

Rittichier KR, Bryan PA, Bassett KE, Taggart EW, Enriquez FR, Hillyard DR, Byington CL. Diagnosis and outcomes of enterovirus infections in young infants. Pediatr Infect Dis J. 2005; 24: 546-50.

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

Rona RJ, Duran-Tauleria E, Chinn S. Family size, atopic disorders in parents, asthma in children, and ethnicity. J Allergy Clin Immunol. 1997 Apr;99(4):454-60.

Rosenthal LA, Avila PC, Heymann PW, Martin RJ, Miller EK, Papadopoulos NG, Peebles RS Jr, Gern JE; Infections and Asthma Committee, Environmental and Occupational Respiratory Diseases Interest Section, American Academy of Allergy, Asthma & Immunology. Viral respiratory tract infections and asthma: the course ahead. J Allergy Clin Immunol. 2010; 125: 1212-7.

Rossi GA, Medici MC, Arcangeletti MC, Lanari M, Merolla R, Paparatti UD, Silvestri M, Pistorio A, Chezzi C; Osservatorio RSV Study Group. Risk factors for severe RSV-induced lower respiratory tract infection over four consecutive epidemics. Eur J Pediatr. 2007; 166: 1267-72.

Royston L, Tapparel C. Rhinoviruses and Respiratory Enteroviruses: Not as Simple as ABC. Viruses. 2016; 8. pii: E16. doi: 10.3390/v8010016.

Rubner FJ, Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Pappas TE, Gern JE, Lemanske RF Jr. Early life rhinovirus wheezing, allergic sensitization, and asthma risk at adolescence. J Allergy Clin Immunol. 2016. pii: S0091-6749(16)30276-7. [Epub ahead of print]

Rusconi F, Galassi C, Corbo GM, Forastiere F, Biggeri A, Ciccone G, Renzoni E. Risk factors for early, persistent, and late-onset wheezing in young children. SIDRIA Collaborative Group. Am J Respir Crit Care Med. 1999; 160: 1617-22.

Ruuskanen O, Lahti E, Jennings LC, Murdoch DR. Viral pneumonia. Lancet. 2011; 377: 1264-75.

Saglani S, Payne DN, Zhu J, Wang Z, Nicholson AG, Bush A, Jeffery PK. Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. Am J Respir Crit Care Med. 2007; 176: 858-64.

Savenije OE, Mahachie John JM, Granell R, Kerkhof M, Dijk FN, de Jongste JC, Smit HA, Brunekreef B, Postma DS, Van Steen K, Henderson J, Koppelman GH. Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood. J Allergy Clin Immunol. 2014; 134: 170-7.

Savolainen C, Blomqvist S, Mulders MN, Hovi T. Genetic clustering of all 102 human rhinovirus prototype strains: serotype 87 is close to human enterovirus 70. J Gen Virol. 2002; 83: 333-40.

Savolainen C, Mulders MN, Hovi T. Phylogenetic analysis of rhinovirus isolates collected during successive epidemic seasons. Virus Res. 2002; 85: 41-6.

Savolainen-Kopra C, Blomqvist S, Kaijalainen S, Jounio U, Juvonen R, Peitso A, Saukkoriipi A, Vainio O, Hovi T, Roivainen M. All known human rhinovirus species are present in sputum specimens of military recruits during respiratory infection. Viruses. 2009; 1: 1178-89.

Savolainen-Kopra C, Blomqvist S, Smura T, Roivainen M, Hovi T, Kiang D, Kalra I, Yagi S, Louie JK, Boushey H, Boothby J, Schnurr DP. 5′ noncoding region alone does not unequivocally determine genetic type of human rhinovirus strains. J Clin Microbiol. 2009; 47:

Sawyer MH. Enterovirus infections: diagnosis and treatment. Semin Pediatr Infect Dis. 2002; 13: 40-7.

Schildgen O, Müller A, Allander T, Mackay IM, Völz S, Kupfer B, Simon A. Human bocavirus: passenger or pathogen in acute respiratory tract infections? Clin Microbiol Rev. 2008; 21: 291-304.

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

Sears MR, Herbison GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. Clin Exp Allergy. 1989; 19: 419-24.

Sears MR, Johnston NW. Understanding the September asthma epidemic. J Allergy Clin Immunol. 2007; 120: 526-9. Epub 2007 Jul 20.

Sethi SK, Bianco A, Allen JT, Knight RA, Spiteri MA. Interferon-gamma (IFN-gamma) down-regulates the rhinovirus-induced expression of intercellular adhesion molecule-1 (ICAM-1) on human airway epithelial cells. Clin Exp Immunol. 1997; 110: 362-9.

Sigurs N, Aljassim F, Kjellman B, Robinson PD, Sigurbergsson F, Bjarnason R, Gustafsson PM. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. Thorax. 2010; 65: 1045-52.

Sigurs N, Bjarnason R, Sigurbergsson F, 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: 1501-7.

Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, Kjellman B. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. Am J Respir Crit Care Med. 2005; 171: 137-41.

Silvennoinen H, Peltola V, Lehtinen P, Vainionpää R, Heikkinen T. Clinical presentation of influenza in unselected children treated as outpatients. Pediatr Infect Dis J. 2009; 28: 372-5.

Simmonds, P., McIntyre, C.L., Savolainen-Kopra, C., Tapparel, C., Mackay, I.M. and Hovi, T. Proposals for the classification of human rhinovirus species C into genotypically assigned types. J. Gen. Virol. 2010; 91: 2409–2419.

Simoes EA. Environmental and demographic risk factors for respiratory syncytial virus lower respiratory tract disease. J Pediatr. 2003; 143: S118-26.

Simpson A, Tan VY, Winn J, Svensén M, Bishop CM, Heckerman DE, Buchan I, Custovic A. Beyond atopy: multiple patterns of sensitization in relation to asthma in a birth cohort study. Am J Respir Crit Care Med. 2010; 181: 1200-6.

Singer JI, Maur PR, Riley JP, Smith PB. Management of central nervous system infections during an epidemic of enteroviral aseptic meningitis. J Pediatr. 1980; 96: 559-63.

Singh AM, Moore PE, Gern JE, Lemanske RF Jr, Hartert TV. Bronchiolitis to asthma: a review and call for studies of gene-virus interactions in asthma causation. Am J Respir Crit Care Med. 2006; 175: 108-19.

Singleton RJ, Bulkow LR, Miernyk K, DeByle C, Pruitt L, Hummel KB, Bruden D, Englund JA, Anderson LJ, Lucher L, Holman RC, Hennessy TW. Viral respiratory infections in hospitalized and community control children in Alaska. J Med Virol. 2010; 82: 1282-90.

Slater L, Bartlett NW, Haas JJ, Zhu J, Message SD, Walton RP, Sykes A, Dahdaleh S, Clarke DL, Belvisi MG, Kon OM, Fujita T, Jeffery PK, Johnston SL, Edwards MR. Co-ordinated role of TLR3, RIG-I and MDA5 in the innate response to rhinovirus in bronchial epithelium. PLoS Pathog. 2010; 6: e1001178.

Sloots TP, McErlean P, Speicher DJ, Arden KE, Nissen MD, Mackay IM. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. J Clin Virol. 2006; 35: 99-102.

Sly PD, Boner AL, Björksten B, Bush A, Custovic A, Eigenmann PA, Gern JE, Gerritsen J, Hamelmann E, Helms PJ, Lemanske RF, Martinez F, Pedersen S, Renz H, Sampson H, von Mutius E, Wahn U, Holt PG. Early identification of atopy in the prediction of persistent asthma in children. Lancet. 2008; 372: 1100-6.

Smuts HE, Workman LJ, Zar HJ. Human rhinovirus infection in young African children with acute wheezing. BMC Infect Dis. 2011; 11: 65.

Smyth RL, Openshaw PJ. Bronchiolitis. Lancet. 2006; 368: 312-22.

Soto-Quiros M, Avila L, Platts-Mills TA, Hunt JF, Erdman DD, Carper H, Murphy DD, Odio S, James HR, Patrie JT, Hunt W, O′Rourke AK, Davis MD, Steinke JW, Lu X, Kennedy J, Heymann PW. High titers of IgE antibody to dust mit allergen and risk for wheezing among asthmatic children infected with rhinovirus. Allergy Clin Immunol 2012: 129: 1499–505.

Spanier AJ, Hornung R, Lierl M, Lanphear BP. Environmental exposures and exhaled nitric oxide in children with asthma. J Pediatr. 2006; 149: 220-6.

Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, Wright AL, Martinez FD. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet. 1999; 354: 541-5.

Stellato C. Glucocorticoid actions on airway epithelial responses in immunity: functional outcomes and molecular targets. J Allergy Clin Immunol. 2007; 120: 1247-63.

Stoltz DJ, Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Gern JE, Lemanske RF Jr. Specific patterns of allergic sensitization in early childhood and asthma & rhinitis risk. Clin Exp Allergy. 2013; 43: 233-41.

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

Stott EJ, Killington RA. Rhinoviruses. Annu Rev Microbiol. 1972; 26: 503-24.

Sugai K, Kimura H, Miyaji Y, Tsukagoshi H, Yoshizumi M, Sasaki-Sakamoto T, Matsunaga S, Yamada Y, Kashiwakura J, Noda M, Ikeda M1, Kozawa K, Ryo A, Yoshihara S, Ogata H, Okayama Y. MIP-1α level in nasopharyngeal aspirates at the first wheezing episode predicts recurrent wheezing. J Allergy Clin Immunol. 2016; 137: 774-81.

Sykes A, Edwards MR, Macintyre J, del Rosario A, Bakhsoliani E, Trujillo-Torralbo MB, Kon OM, Mallia P, McHale M, Johnston SL. Rhinovirus 16-induced IFN-a and IFN-b are deficient in bronchoalveolar lavage cells in asthmatic patients. J Allergy Clin Immunol 2012; 129: 1506-14.

Söderlund-Venermo M, Lahtinen A, Hedman L, Hedman L, Kemppainen K, Lehtinen P, Allander T, Ruuskanen O, Hedman K. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. Emerg Infect Dis 2009: 15: 1423–30.

Talbot HK, Crowe JE Jr, Edwards KM, Griffin MR, Zhu Y, Weinberg GA, Szilagyi PG, Hall CB, Podsiad AB, Iwane M, Williams JV; New Vaccine Surveillance Network. Coronavirus infection and hospitalizations for acute respiratory illness in young children. J Med Virol. 2009; 81: 853-6.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, and Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution. 2011; 28:2731–2739.

Tapiainen T, Aittoniemi J, Immonen J, Jylkkä H, Meinander T, Nuolivirta K, Peltola V, Salo E, Seuri R, Walle SM, Korppi M. Finnish guidelines for the treatment of laryngitis, wheezing bronchitis and bronchiolitis in children. Acta Paediatr. 2016; 105: 44-9.

Tapparel C, Cordey S, Van Belle S, Turin L, Lee WM, Regamey N, Meylan P, Mühlemann K, Gobbini F, Kaiser L. New molecular detection tools adapted to emerging rhinoviruses and enteroviruses. J Clin Microbiol. 2009; 47: 1742-9.

Tapparel C, Siegrist F, Petty TJ, Kaiser L. Picornavirus and enterovirus diversity with associated human diseases. Infect Genet Evol. 2013; 14: 282-93.

Tariq SM, Matthews SM, Hakim EA, Stevens M, Arshad SH, Hide DW. The prevalence of and risk factors for atopy in early childhood: a whole population birth cohort study. J Allergy Clin Immunol. 1998 May;101(5):587-93.

Tauriainen S, Oikarinen S, Oikarinen M, Hyöty H. Enteroviruses in the pathogenesis of type 1 diabetes. Semin Immunopathol. 2011; 33: 45-55.

Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD. Tucson Children's Respiratory Study: 1980 to present. J Allergy Clin Immunol. 2003; 111: 661-75; 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, Szefler SJ. Preseasonal treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma exacerbations. J Allergy Clin Immunol. 2015; 136: 1476-85.

Thumerelle C, Deschildre A, Bouquillon C, Santos C, Sardet A, Scalbert M, Delbecque L, Debray P, Dewilde A, Turck D, 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: 75-82.

Toelle BG, Xuan W, Peat JK, Marks GB. Childhood factors that predict asthma in young adulthood. Eur Respir J. 2004; 23: 66-70.

Torgersen H, Skern T, Blaas D. Typing of human rhinoviruses based on sequence variations in the 5′ non-coding region. J Gen Virol. 1989; 70: 3111-6.

Triantafilou K, Vakakis E, Richer EA, Evans GL, Villiers JP, Triantafilou M. Human rhinovirus recognition in non-immune cells is mediated by Toll-like receptors and MDA-5, which trigger a synergetic pro-inflammatory immune response. Virulence. 2011; 2: 22-9.

Tulic MK, Hurrelbrink RJ, Prêle CM, Laing IA, Upham JW, Le Souef P, Sly PD, Holt PG. TLR4 polymorphisms mediate impaired responses to respiratory syncytial virus and lipopolysaccharide. J Immunol. 2007; 179: 132-40.

Turner SW, Young S, Landau LI, Le Souëf PN. Reduced lung function both before bronchiolitis and at 11 years. Arch Dis Child. 2002; 87: 417-20.

Uncapher CR, DeWitt CM, Colonno RJ. The major and minor group receptor families contain all but one human rhinovirus serotype. Virology. 1991; 180: 814-7.

Utokaparch S, Marchant D, Gosselink JV McDonough JE, Thomas EE, Hogg JC, Hegele RG. The relationship between respiratory viral loads and diagnosis in children presenting to a pediatric hospital emergency department. Pediatr Infect Dis J 2011;30:e18-23.

van Benten I, Koopman L, Niesters B, Hop W, van Middelkoop B, de Waal L, van Drunen K, Osterhaus A, Neijens H, Fokkens W. Predominance of rhinovirus in the nose of symptomatic and asymptomatic infants. Pediatr Allergy Immunol. 2003;14: 363-70.

van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, Osterhaus AD. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med. 2001; 7: 719-24.

van den Hoogen BG, van Doornum GJ, Fockens JC, Cornelissen JJ, Beyer WE, de Groot R, Osterhaus AD, Fouchier RA. Prevalence and clinical symptoms of human metapneumovirus infection in hospitalized patients. J Infect Dis. 2003; 188: 1571-7.

van der Gugten AC, van der Zalm MM, Uiterwaal CS, Wilbrink B, Rossen JW, van der Ent CK. Human rhinovirus and wheezing: short and long-term associations in children. Pediatr Infect Dis J. 2013; 32: 827-33.

van der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJ, Wolthers KC, Wertheim-van Dillen PM, Kaandorp J, Spaargaren J, Berkhout B. Identification of a new human coronavirus. Nat Med. 2004; 10: 368-73.

van der Hulst AE, Klip H, Brand PL. Risk of developing asthma in young children with atopic eczema: a systematic review. J Allergy Clin Immunol. 2007; 120: 565-9.

van der Zalm MM, van Ewijk BE, Wilbrink B, Uiterwaal CS, Wolfs TF, van der Ent CK. Respiratory pathogens in children with and without respiratory symptoms. J Pediatr. 2009; 154: 396-400, 400.e1.

van der Zalm MM, Uiterwaal CS, Wilbrink B, Koopman M, Verheij TJ, van der Ent CK The influence of neonatal lung function on rhinovirus-associated wheeze. Am J Respir Crit Care Med 2011: 183: 262–7.

van der Zalm MM, Wilbrink B, van Ewijk BE, Overduin P, Wolfs TF, van der Ent CK. Highly frequent infections with human rhinovirus in healthy young children: a longitudinal cohort study. J Clin Virol. 2011; 52: 317-20.

van Elden LJ, van Loon AM, van Alphen F, Hendriksen KA, Hoepelman AI, van Kraaij MG, Oosterheert JJ, Schipper P, Schuurman R, Nijhuis M. Frequent detection of human coronaviruses in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. J Infect Dis. 2004; 189: 652-7.

Volle R, Nourrisson C, Mirand A, Regagnon C, Chambon M, Henquell C, Bailly JL, Peigue-Lafeuille H, Archimbaud C. Quantitative real-time RT-PCR assay for research studies on enterovirus infections in the central nervous system. J Virol Methods. 2012; 185: 142-8.

von Hertzen L, Mäkelä MJ, Petäys T, Jousilahti P, Kosunen TU, Laatikainen T, Vartiainen E, Haahtela T. Growing disparities in atopy between the Finns and the Russians: a comparison of 2 generations. J Allergy Clin Immunol. 2006; 117: 151-7.

Vuorinen T, Vainionpää R, Hyypiä T. Five years' experience of reverse-transcriptase polymerase chain reaction in daily diagnosis of enterovirus and rhinovirus infections. Clin Infect Dis. 2003; 37: 452-5.

Wang Q, Nagarkar DR, Bowman ER, Schneider D, Gosangi B, Lei J, Zhao Y, McHenry CL, Burgens RV, Miller DJ, Sajjan U, Hershenson MB. Role of double-stranded RNA pattern recognition receptors in rhinovirus-induced airway epithelial cell responses. J Immunol. 2009; 183: 6989-97.

Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, Holgate ST, Davies DE. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. J Exp Med. 2005; 201: 937-47.

Weinberg GA, Erdman DD, Edwards KM, Hall CB, Walker FJ, Griffin MR, Schwartz B; New Vaccine Surveillance Network Study Group. Superiority of reverse-transcription polymerase chain reaction to conventional viral culture in the diagnosis of acute respiratory tract infections in children. J Infect Dis. 2004; 189: 706-10.

Weinberger DM, Simonsen L, Jordan R, Steiner C, Miller M, Viboud C. Impact of the 2009 influenza pandemic on pneumococcal pneumonia hospitalizations in the United States. J Infect Dis. 2012; 205: 458-65.

Williams JV, Harris PA, Tollefson SJ, Halburnt-Rush LL, Pingsterhaus JM, Edwards KM, Wright PF, Crowe JE Jr. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. N Engl J Med. 2004; 350: 443-50.

Williams JV, Wang CK, Yang CF, Tollefson SJ, House FS, Heck JM, Chu M, Brown JB, Lintao LD, Quinto JD, Chu D, Spaete RR, Edwards KM, Wright PF, Crowe JE Jr. The role of human metapneumovirus in upper respiratory tract infections in children: a 20-year experience. J Infect Dis. 2006; 193: 387-95.

Winther B, Block SL, Reisinger K, Dutkowski R. Impact of oseltamivir treatment on the incidence and course of acute otitis media in children with influenza. Int J Pediatr Otorhinolaryngol. 2010; 74: 684-8.

Winther B, Hayden FG, Hendley JO. Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: Association with symptomatic illness and effect of season. J Med Virol. 2006; 78: 644-50.

Wisdom A, Leitch EC, Gaunt E, Harvala H, Simmonds P. Screening respiratory samples for detection of human rhinoviruses (HRVs) and enteroviruses: comprehensive VP4-VP2 typing reveals high incidence and genetic diversity of HRV species C. J Clin Microbiol. 2009; 47: 3958-67.

Wisdom A, Kutkowska AE, McWilliam Leitch EC, Gaunt E, Templeton K, Harvala H, Simmonds P. Genetics, recombination and clinical features of human rhinovirus species C (HRV-C) infections; interactions of HRV-C with other respiratory viruses. PLoS One. 2009; 4: e8518.

Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y, Wong BH, Poon RW, Cai JJ, Luk WK, Poon LL, Wong SS, Guan Y, Peiris JS, Yuen KY. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol. 2005; 79: 884-95.

Wright PF, Deatly AM, Karron RA, Belshe RB, Shi JR, Gruber WC, Zhu Y, Randolph VB. Comparison of results of detection of rhinovirus by PCR and viral culture in human nasal wash specimens from subjects with and without clinical symptoms of respiratory illness. J Clin Microbiol. 2007; 45: 2126-9.

Wu P, Dupont WD, Griffin MR, Carroll KN, Mitchel EF, Gebretsadik T, 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: 1123-9. POIS?

Wu P, Hartert TV. Evidence for a causal relationship between respiratory syncytial virus infection and asthma. Expert Rev Anti Infect Ther. 2011; 9: 731-45.

Xatzipsalti M, Psarros F, Konstantinou G, Gaga M, Gourgiotis D, Saxoni-Papageorgiou P, Papadopoulos NG. Modulation of the epithelial inflammatory response to rhinovirus in an atopic environment. Clin Exp Allergy. 2008; 38: 466-72.

Yamazaki H1, Tsutsumi H, Matsuda K, Nagai K, Ogra PL, Chiba S. Effect of maternal antibody on IgA antibody response in nasopharyngeal secretion in infants and children during primary respiratory syncytial virus infection. Gen Virol. 1994; 75: 2115-9.

Yoshida LM, Suzuki M, Nguyen HA, Le MN, Dinh Vu T, Yoshino H, Schmidt WP, Nguyen TT, Le HT, Morimoto K, Moriuchi H, Dang DA, Ariyoshi K.

Respiratory syncytial virus: co-infection and paediatric lower respiratory tract infections. Eur Respir J. 2013; 42: 461-9.

Young S, O'Keeffe PT, Arnott J, Landau LI. Lung function, airway responsiveness, and respiratory symptoms before and after bronchiolitis. Arch Dis Child. 1995; 72: 16-24.

Yu H, Huang J, Huai Y, Guan X, Klena J, Liu S, Peng Y, Yang H, Luo J, Zheng J, Chen M, Peng Z, Xiang N, Huo X, Xiao L, Jiang H, Chen H, Zhang Y, Xing X, Xu Z, Feng Z, Zhan F, Yang W, Uyeki TM, Wang Y, Varma JK. The substantial hospitalization burden of influenza in central China: surveillance for severe, acute respiratory infection, and influenza viruses, 2010-2012. Influenza Other Respir Viruses. 2014; 8: 53-65.

Appendix 1. Parental questionnaire.*

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 <u>suspected</u> 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 <u>last 12 months</u>:
   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 <u>current</u> 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 <u>episode of breathing difficulties?</u>
   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 2. Clinical score chart at the emergency room or hospital ward.

Name _____ Social security number _____
(After study entry, to be filled in the mornings 2 hours after bronchodilator).

	An example				
Date, time	7.9.2007 8.00				
Heart rate/min	112				
Breathing frequency/min	55				
Oxygen saturation	98				
Body temperature	37.6				
Inspiratory:expiratory time: 0 (2:1), 1 (1:1), 2 (1:2), 3 (1:3)	1:2				
Degree of dyspnea: 0 (normal), 1 (mild), 2 (moderate), 3 (severe)	2				
Auscultatory findings on wheezing: 0 (none), 1 (expiratory), 2 (inspiratory and expiratory), 3 (audible without stethoscope)	2				
Type of breathing: 0 (normal), 1 (use of stomach muscles), 2 (use of intercostal muscles), 3 (nasal flaring)	1				
Pneumonic crackles: 0 (no), 1 (mild), 2 (moderate), 3 (severe)	0				
Otitis media: 0 (no), 1 (yes)	0				
Antibiotic: 0 (no), 1 (yes), what and when initiated	0				
Other symptoms +/++/+++ and their connection to study medication: 1 (no), 2 (maybe), 3 (yes)					
Cough	++/1				
Rhinitis	+/1				
Eye symptoms	-				
Eczema	-				
Any other symptoms/signs (report everything)	Restlesness ++/1				
Other notes, e.g. the cause of symptoms	flu				
Study drug given (tally)	lll				
Mediacation (drug name, dosage) before physical examination (tally)	ER: albuterol 1.2 mg x2, ward: albuterol 1.5 mg x2				
…and before blood draw	+ albuterol 1.5 mg x2				
Oxygen (hour) or i.v. or nasogastric hydration (volume/time)	Oxygen 5 h i.v. fluid 700 ml				
Exact times: Study entry at ER/ward (1), study drug initiation (2), ready for discharge considering breathing difficulties (3), actual discharge (4).	(1) 15.30				

Name _____ **Social security number** _____

Daily symptom and medications until 2-week visit.

Date (fill in one column per day)	An example 1.1.07								
Hospitalization for expiratory breathing difficulty, yes or no	Yes								
Cough, 0 (no) - 3 (severe)	2								
Expiratory breathing difficulty, 0 (no) - 3 (severe)	1								
Noisy breathing, 0 (no) - 3 (loud)	0								
Rhinitis, 0 (no) - 3 (severe)	0								
Night wakening for breathing difficulties, 0 (no), 1 (once), 2 (often), 3 (continuously)	1								
Temperature, exact or on scale 0 (no) - 3 (high)	Fever 37.9, or 1								
Any other symptom (report any deviation from normal)	Fell in stairs, tearful								
Other notes (e.g. cause of symptom)	scute otitis media, playing with a cat								
Study drug taken (tally)	111								
Bronchodilator (name, dose, number of doses; tally)	Ventoline 0.1 mg, puffs 1111								
Other medication	Naprosyn mixt. 5 mg/ml 3 ml/dose, 111 Amorion mixt 80 mg/ml 3.7 ml x2								
Doctor's appointment: where, why, name of the doctor, and treatment	Healthcenter Mäntymäki 1, fever, cough, tearful, Naprosyn and Amorion for acute otitis media								

If any questions, do not hesitate to contact study physician by phone.

Appendix 4. Symptom diary 2.

Name _____ **Social security number** _____

Daily symptoms and medications for 2 months. Check a box if yes.

Month _____ (fill in one column per day)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Rhinitis																															
Cough																															
Expiratory breathing difficulty																															
Noisy breathing																															
Nocturnal symptoms																															
Bronchodilator																															
Oral corticosteroid																															
Inhaled corticosteroid																															
Antibiotic																															
Doctor's visit for expiratory breathing difficulty																															
Hospitalization for expiratory breathing difficulty																															

Month _____ (fill in one column per day)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Rhinitis																															
Cough																															
Expiratory breathing difficulty																															
Noisy breathing																															
Nocturnal symptoms																															
Bronchodilator																															
Oral corticosteroid																															
Inhaled corticosteroid																															
Antibiotic																															
Doctor's visit for expiratory breathing difficulty																															
Hospitalization for expiratory breathing difficulty																															

If any questions, do not hesitate to contact study physician by phone.