Tampere University

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Post-Bronchiolitis Lung Function

Long-term outcome after hospitalisation in early infancy

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ACADEMIC DISSERTATION
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ACADEMIC DISSERTATION
Tampere University, Faculty of Medicine and Health Technology
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To my family

ABSTRACT

Background: Bronchiolitis, a lower respiratory tract infection (LRTI) caused by different viruses, is a common reason for hospitalisation in early childhood. Hospitalisation for bronchiolitis increases the risk for lung function abnormalities at school age, in adolescence and in adulthood. Respiratory syncytial virus (RSV) has been associated especially to irreversible changes in spirometry. However, children who have suffered from severe bronchiolitis in infancy form a heterogeneous group and lung function reduction does not concern all of them. Thus, identifying those who are at high risk would help to focus preventive and therapeutic measures.

Aims: The aim of this thesis was to evaluate baseline and post-bronchodilator (post-bd) lung function at the age of 10-13 years in children hospitalised for bronchiolitis in early infancy. Lung function was compared between former bronchiolitis patients and age- and sex-matched controls. Post-bronchiolitis lung function was investigated in relation to weight status and polymorphisms in Toll-like receptors (TLR) encoding genes. Early-life and preschool-age risk factors were studied in relation to irreversible airway obstruction, assessed by reduced post-bd FEV1 or post-bd FEV1/FVC.

Methods: Originally, healthy, term infants, who were hospitalised for bronchiolitis at the age of less than six months were enrolled. Viral aetiology was studied in nasopharyngeal aspirates, and RSV was the predominant causative virus. At the age of 10-13 years, 89 children (cases) of those 166 invited attended a clinical examination with interview and baseline and post-bd spirometry. The follow-up study at the age of 10-13 years included age- and sex-matched controls, 108 of whom performed spirometry. Lung function was measured previously at the age of 5-7 years with impulse oscillometry (IOS) in 103 cases, and measurements at both ages were available from 62 cases.

Results: Bronchiolitis in early infancy showed an association with decreased forced expiratory volume in one second (FEV1) and FEV1/forced vital capacity (FVC) at the age of 10-13 years. In analyses adjusted for current asthma and maternal smoking

in infancy, bronchiolitis was associated with a 2.4-fold risk of baseline FEV1 reduction and a 4.4-fold risk of post-bd FEV1 reduction, but the significance of association between bronchiolitis and FEV1/FVC reduction was lost. The risk of baseline and post-bd FEV1 reductions remained significant in cases with RSV positive bronchiolitis, but not in those with RSV negative bronchiolitis.

Among the bronchiolitis group, overweight children showed lower baseline and post-bd FEV1/FVC values as continuous and categorised variables compared to normal weight children. Variant genotype of *TLR10* rs4129009 was associated with better baseline and post-bd FEV1/FVC after bronchiolitis, and the result was similar when *TLR10* was analysed either separately or combined with *TLR1* or *TLR2*. Irreversible airway obstruction after bronchiolitis was associated with physician-diagnosed asthma at less than 12 months of age. Worse results in IOS at preschool age predicted irreversible airway obstruction at 10-13 years of age.

Conclusions: The present study demonstrated an association between hospitalisation for bronchiolitis in infancy and lung function reduction in early adolescence. Reductions in post-bd FEV1 and FEV1/FVC suggesting irreversible changes were present as early as at 10-13 years of age. Among the bronchiolitis group, overweight predicted lower FEV1/FVC values; in contrast, the variant *TLR10* rs4129009 genotype was associated with higher FEV1/FVC. Children with asthma diagnosis as early as before the age of 12 months and children with worse results in IOS at preschool age seem to be at greater risk to have irreversible changes in lung function after bronchiolitis.

TIIVISTELMÄ

Tausta: Bronkioliitti eli ilmatiehyttulehdus on viruksen aiheuttama alahengitystieinfektio lapsilla, mikä voi johtaa sairaalahoitoon erityisesti pienten lasten kohdalla. Sairaalahoitoisen bronkioliitin jälkeen keuhkojen toimintahäiriöitä on kuvattu sekä lapsilla, että aikuisilla. Respiratory syncytial viruksen eli RS-viruksen aiheuttama bronkioliitti näyttäisi liittyvän erityisesti palautumattomaan keuhkoputkien toiminnan häiriöön eli sellaiseen häiriöön, mikä ei palaudu normaaliksi bronkodilataattorilla (keuhkoputkia avaavalla lääkkeellä). Keuhkojen toiminta ei kuitenkaan ole poikkeava kaikilla bronkioliitin sairastaneista lapsista. Riskiryhmien tunnistaminen on tärkeää, jotta keuhkojen toiminnan heikentymistä voitaisiin ehkäistä ja lääkehoito sekä seuranta kohdistaa oikein.

Tavoitteet: Tämän väitöskirjatutkimuksen tavoite oli tutkia lasten keuhkojen toimintaa 10-13 vuoden iässä varhaisessa imeväisiässä sairastetun bronkioliitin jälkeen. Tavoitteena oli vertailla tuloksia bronkioliittiryhmän ja iän sekä sukupuolen suhteen vakioidun kontrolliryhmän välillä, sekä arvioida ylipainon ja Tollin kaltaisia reseptoreita (TLR) koodaavien geenien yksittäisten emästen muutosten merkitystä keuhkojen toimintaan bronkioliitin sairastaneilla lapsilla. Tarkoituksena oli lisäksi tutkia riskitekijöitä palautumattoman keuhkojen toiminnan häiriön esiintymiseen bronkioliitin jälkeen.

Menetelmät: Alun perin tutkimukseen otettiin mukaan terveitä, täysiaikaisina syntyneitä lapsia, jotka joutuivat bronkioliitin vuoksi sairaalahoitoon alle kuuden kuukauden iässä. Bronkioliitin virusetiologia määritettiin nenänielun imulimanäytteistä ja RSV oli positiivinen suurimmalla osasta lapsia. Tutkimuskäynti 10-13 vuoden iässä sisälsi haastattelun sekä spirometriatutkimuksen ennen ja jälkeen bronkodilataation, ja tämän tutkimuksen suoritti 89 lasta 166 kutsutusta. Tutkimuskäynti oli kontrolloitu eli 108 iän ja sukupuolen perusteella vakioitua kontrollipotilasta kävi läpi saman tutkimusprotokollan. Aikaisemmin seurantatutkimuksessamme 103 bronkioliitin sairastaneen lapsen keuhkofunktio tutkittiin impulssioskillometrialla (IOS) 5-7 vuoden iässä. Sekä IOS-, että spirometriamittaukset olivat 62 lapselta.

Tulokset: Varhaislapsuudessa sairastettu bronkioliitti lisäsi uloshengityksen sekuntikapasiteetin (FEV1) sekä sekuntikapasiteetin ja nopean vitaalikapasiteetin suhteen (FEV1/FVC) poikkeavuuksia 10-13 vuoden iässä. Bronkioliitin sairastaneilla lapsilla oli 2.4-kertainen riski saada poikkeava FEV1 tulos ennen bronkodilataatiota ja 4.4-kertainen riski saada poikkeava FEV1 tulos bronkodilataation jälkeen kontrollipotilaisiin verrattuna; tulos ei ollut riippuvainen tämänhetkisestä astmasta tai altistumisesta varhaislapsuudessa äidin tupakoinnille. Sitä vastoin FEV1/FVC ei eronnut ryhmien välillä, kun samat sekoittavat tekijät huomioitiin. FEV1 poikkeavuuksia esiintyi erityisesti RSV positiivisen bronkioliitin jälkeen.

Bronkioliittiryhmässä FEV1/FVC arvot olivat matalampia ylipainoisilla kuin normaalipainoisilla lapsilla, ja tulos oli samanlainen luokiteltuina ja jatkuvina muuttujina. *TLR10* rs4129009 geenivariaatio liittyi parempiin FEV1/FVC arvoihin ennen ja jälkeen bronkodilataation. Löydös pysyi samana *TLR10/TLR1* ja *TLR10/TLR2* kombinaatioissa. Palautumaton keuhkojen toiminnan häiriö oli yleisempi niillä lapsilla, jotka olivat saaneet astmadiagnoosin jo ennen yhden vuoden ikää. Esikouluiässä mitatun IOS tutkimuksen huonompi tulos ennusti palautumattoman keuhkojen toiminnan häiriön esiintymistä 10-13 vuoden iässä.

Yhteenveto: Varhaisessa imeväisiässä sairastettu sairaalahoitoinen bronkioliitti lisäsi riskiä keuhkojen toiminnan häiriöille varhaisessa nuoruusiässä. Palautumattomia keuhkofunktion toiminnan häiriöitä esiintyi bronkioliitin sairastaneilla lapsilla jo 10-13 vuoden iässä. FEV1/FVC oli matalampi ylipainoisilla kuin normaalipainoisilla bronkioliitin sairastaneilla lapsilla. Sitä vastoin *TLR10* geenivariaatio liittyi parempiin bronkioliitin jälkeisiin FEV1/FVC arvoihin. Lapsilla, jotka olivat saaneet astmadiagnoosin jo ensimmäisenä elinvuotenaan, oli kohonnut riski palautumattomille keuhkofunktiomuutoksille. Esikouluiässä mitatun IOS tutkimuksen tulos ennusti palautumattoman keuhkojen toiminnan häiriön esiintymistä 10-13-vuotiaana.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following five original publications. In the text, the original publications are referred by numerals I-V.

I Riikonen R, Lauhkonen E, Törmänen S, Backman K, Koponen P, Helminen M, Nuolivirta K, Korppi M. Prospective study confirms that bronchiolitis in early infancy increases the risk of reduced lung function at 10-13 years of age. Acta Paediatr 2019; 108: 124-130.

II Riikonen R, Törmänen S, Saari A, Koponen P, Helminen M, Nuolivirta K, Korppi M, Lauhkonen E. Preliminary communication suggests overweight was associated with reduced lung function in adolescence after infant bronchiolitis. Acta Paediatr 2019; 108: 1729-1730.

III Riikonen R, Korppi M, Törmänen S, Nuolivirta K, Helminen M, He Q, Lauhkonen E. *Toll-like receptor 10* rs4129009 gene polymorphism is associated with post-bronchiolitis lung function in adolescence. Acta Paediatr 2020; 109: 1634-1641.

IV Riikonen R, Korppi, M, Törmänen S, Nuolivirta K, Helminen M, He Q, Lauhkonen E. Genetic variations in Toll-like receptors 4 or 7 were not linked to post-bronchiolitis lung function in adolescence. Acta Paediatr 2021; 110: 959-960.

V Riikonen R, Korppi M, Törmänen S, Koponen P, Nuolivirta K, Helminen M, He Q, Lauhkonen E. Risk factors for irreversible airway obstruction after infant bronchiolitis. Respir Med 2021; 187: 106545.

The thesis contains data not included in the original articles (Unpublished data).

AUTHOR'S CONTRIBUTIONS

The author of this dissertation contributed to all five original publications as the main author. None of the original publications have been a part of another academic dissertation. The original study protocol of this long-term follow-up was designed by Professor Matti Korppi. Data collection at age of 10-13 years was performed by Eero Lauhkonen, MD, PhD and Sari Törmänen, MD, PhD. The coauthors collaborated in the planning and writing the manuscripts and gave their expertise during processing the studies.

The additional contributions of the authors in the original publications were as follows:

I	The author of this dissertation contributed to planning the study, designing the methodology, classifying the previously collected data, performing the statistical analyses, writing the manuscript and being responsible for the publication process.
II	The author of this dissertation contributed to planning the study, designing the methodology, classifying the previously collected data, performing the statistical analyses, writing the manuscript and being responsible for the publication process. Classifying data on weight status was performed by Docent Antti Saari.
III, IV	The author of this dissertation contributed to planning the study, designing the methodology, classifying the previously collected data, performing the statistical analyses, writing the manuscript and being responsible for the publication process. TLR genotyping was performed by Professor Qiushui He.
V	The author of this dissertation contributed to planning the study, designing the methodology, classifying the previously collected data, performing the statistical analyses, writing the manuscript and being responsible for the publication process.

ABBREVIATIONS

ALSPAC	Avon Longitudinal Study of Parents and Children
ANCOVA	Analysis of covariance
ATS	American Thoracic Society
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
DNA	Deoxyribonucleic acid
ECA	Environment and Childhood Asthma Study
EPP	Equal pressure point
ERS	European Respiratory Society
FEV1	Forced expiratory volume in one second
FRC	Functional residual capacity
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
GOLD	Global Initiative for Chronic Obstructive Lung Disease
ICS	Inhaled corticosteroids
IOS	Impulse oscillometry
LRTI	Lower respiratory tract infection
MAAS	Manchester Asthma and Allergy Study
MEF25-75	Maximal expiratory flow at 25-75% of FVC
MEF50	Maximal expiratory flow at 50% of FVC
OR	Odds ratio
PCR	Polymerase chain reaction
PIAF	Perth Infant Asthma Follow-up
Post-bd	Post-bronchodilator
RBM	Reticular basement membrane
Rrs	Respiratory system resistance
Rrs5	Respiratory system resistance at 5 Hz
SD	Standard deviation
RSV	Respiratory syncytial virus
SWS	Southampton Women's Survey

TCRS	Tucson Children's Respiratory Study
TLR	Toll-like receptor (receptor)
TLR	Toll-like receptor (gene)
VmaxFRC	Maximum flow at FRC
Xrs	Respiratory system reactance
Xrs5	Respiratory system reactance at 5 Hz
zBMI	Body mass index z-score
95%CI	95% confidence interval

SYMBOLS

Q	Airflow
ΔP	Pressure difference
R	Resistance
η	Gas viscosity
l	Airway tube length
r	Airway tube radius

1 INTRODUCTION

Viral bronchiolitis is a common cause of hospitalisations worldwide. Diagnosis of bronchiolitis covers a heterogenous group of lower respiratory tract infections (LRTI) with different clinical pictures (1), and the outcome after bronchiolitis is dependent, at least, on hospitalisation age (2, 3) and viral aetiology (4, 5). The most common cause for bronchiolitis during the first year of life is respiratory syncytial virus (RSV) with a hospitalisation rate of 3.5 per 1,000 children (6). The risk of severe RSV infection is increased in infants who are less than six months of age (7, 8).

Development of lung function is a continuous process that begins prenatally, is genetically regulated and modified by environmental factors throughout life. Lung function growth in childhood determines the maximal level of lung function in early adulthood and factors that influence such growth play a role in the later development of lung function abnormalities. The growth of lung function includes growth of the lung tissue and growth of the airways, which are regulated by different genes (9) and further modified by different factors.

Eight previous post-bronchiolitis follow-ups have studied lung function outcome and the evidence of reduced lung function is indisputable (10-17). Six previous post-bronchiolitis follow-ups have evaluated post-bronchodilator (post-bd) lung function reflecting irreversible changes (10-12, 14, 15, 17); two studies measured post-bd lung function at more than one age (15, 17), but none repeated post-bd measurements both before and at school age. Previously in the present follow-up (18), lung function was studied with impulse oscillometry (IOS) at the age of 5-7 years; 20% of former bronchiolitis patients showed abnormal IOS, but only one had post-bd abnormality.

Hospitalisation for bronchiolitis increases the risk of pulmonary asthma (19, 20) and is associated with lung function reduction in asthmatic subjects (14, 15, 21). Reversible airway obstruction is one basis of an asthma diagnosis (22), and persistent asthma leads to irreversible changes in a small group of patients (23-25), probably due to airway remodelling (26-28). It has been suggested that long-term impacts of severe bronchiolitis could be induced by altered maturation of the immune system in infancy, or alternatively, bronchiolitis may be the first sign of a susceptibility to asthma (4, 29).

The oldest post-bronchiolitis follow-ups that have continued for over 18 years have reported irreversible changes in lung function also in non-asthmatic subjects (10, 21), especially after RSV bronchiolitis (10, 21). According to the findings observed in birth cohort studies, children with reduced expiratory flows in infancy are more vulnerable to early-childhood LRTIs (30-32), transient wheezing symptoms (33) and persistently low lung function (34, 35), suggesting premorbid origin. In contrast, experimental studies have suggested that RSV infection may inhibit growth of lung function via direct changes in airway structure (36-39). Longitudinal lung function data after severe bronchiolitis in infancy requiring hospital admission is scarce and based mainly on baseline measurements.

Overweight is thought to have a dual role in childhood asthma: it is associated with development of wheezing symptoms and asthma, and it increases the severity of symptoms in asthmatic patients (40). Overweight may accelerate lung growth in childhood that leads to discrepancy in growths of the airways and lungs, and such pattern is associated with more severe symptoms in asthmatic patients (41). In relation to asthma, overweight is probably a more significant predictor of post-bd spirometry (42). Only one previous cohort study has evaluated overweight in relation to post-bronchiolitis lung function, and there was an association between overweight and baseline lung function in school-aged children; however, post-bd measurements were not done (43). Previously in the present follow-up, obesity (overweight excluded) was associated with baseline and post-bd lung function measured with IOS at the age of 5-7 years (44).

Toll-like receptors (TLR) recognise pathogens and trigger immune responses against them. Normal TLR functions regulate the balance between Th1- and Th2-oriented immunity, and alterations in TLR functions may disturb such balance (29). Supporting this, genetic variations in the encoding genes of TLRs have been associated with development of asthma (45). However, studies evaluating associations between *TLR* polymorphisms and lung function are rare. Previously in the present cohort, polymorphisms in encoding genes of TLR4, TLR6 and TLR7 showed associations with IOS at the age of 5-7 years, and polymorphisms in encoding genes of TLR1 and TLR10 with post-bronchiolitis asthma.

This thesis aimed to study lung function outcome before and after bronchodilation at the age of 10-13 years after bronchiolitis in early infancy. Aim was to compare lung function between former bronchiolitis patients and age- and sex-matched controls. In addition, aim was to evaluate possible factors that impact on post-bronchiolitis lung function: the present studies focus on effects of overweight, *TLR* polymorphisms and early-life and preschool-age risk factors.

2 REVIEW OF THE LITERATURE

2.1 Infant bronchiolitis

2.1.1 Description, diagnosis and definition

Bronchiolitis is an inflammation in the smallest airways, bronchioles, which is mainly caused by viral infection. Bronchioles comprise membranous bronchioles that are the most distal part of conducting airways with a thin fibromuscular wall, and respiratory bronchioles in the respiratory zone of the lungs with a direct connection to alveolar ducts. The diameter of bronchioles is less than 1 mm, and the cartilage that surrounds the larger conducting airways is absent (46).

The diagnosis of viral bronchiolitis is clinical and based on typical signs and symptoms such as rhinitis, cough and wheezing or crackles on auscultation. Tachypnoea and thoracic retractions suggest increased respiratory effort, and in young infants, apnoea may be the first sign of bronchiolitis (47). Bronchiolitis is commonly recognised as a lower respiratory tract infection with wheezing; however, the sounds identified in auscultation may be variable, especially in RSV bronchiolitis (48, 49). The term "wheezing" refers expiratory breathing difficulty, and it has been estimated that approximately 30% of children have at least one episode of wheezing during the first three years of life (35, 50). Viral induced wheezing differs in terms of aetiology, clinical symptoms and outcomes between young infants and older children (2, 4, 51).

The guidelines published in different countries for bronchiolitis are highly variable in terms of highlighting differential diagnosis or defining upper age limits for bronchiolitis (52). In the United Stated, the term bronchiolitis covers wheezing in children under 24 months of age and in most European countries, bronchiolitis means the first wheezing episode in children under 12 months of age (51-53). The terminology used to describe lower respiratory tract symptoms in children is not concordant in long-term birth-cohort or hospital-cohort studies. These studies have used various terms including "bronchiolitis", "wheezing", "viral-induced wheezing", "wheezing bronchitis" and "lower-respiratory tract infection" to define the disease

and applied upper age limits ranging from six months to three years of age, suggesting high variability of mechanisms behind wheezing.

2.1.2 Epidemiology and aetiology

Bronchiolitis causes the majority of infection-related hospitalisations in infants (54). Approximately 2-3% of infants are hospitalised for bronchiolitis every year (47, 52). Due to high prevalence, bronchiolitis is a leading cause of respiratory failure in infants (55); however, only a small proportion of hospitalised children needs treatment in the paediatric intensive care unit (56).

In severe bronchiolitis, viral aetiology could be confirmed in nearly 100% of infections after implementation of polymerase chain reaction (PCR) to study respiratory samples. The main causative agent of bronchiolitis during the first year of life is the highly contagious RSV, which causes up to 80% of all hospitalisations (57), while the second most common viral aetiology for bronchiolitis is rhinovirus (4, 58), which dominates the viral aetiology of infection-induced wheezing in children older than 12 months (3).

RSV bronchiolitis is characterised by destruction of airway epithelial cells due to direct cytopathological effect of RSV, which leads to increased production of mucus and debris and causes a mechanical block in the airways (47, 57). Rhinovirus bronchiolitis is less invasive but disrupts epithelial barrier function (59) and may cause wheezing with similar mechanisms seen in atopic asthma (4). The other viral aetiologies of bronchiolitis consist of at least parainfluenza viruses, bocavirus, metapneumovirus, adenoviruses, coronaviruses, enterovirus and influenza viruses, and coinfections are common (49). In comparison with other aetiologies, RSV bronchiolitis has been associated with a more severe clinical course of the disease (60-62).

2.1.3 Risk factors for severe bronchiolitis

Prematurity, low birth weight, congenital heart disease, chronic lung disease and immunodeficiency are known risk factors for development of severe bronchiolitis (63, 64). However, most children who are hospitalised for bronchiolitis are otherwise healthy and born at full term. Several physiological factors predispose all infants to more severe manifestation of respiratory infections than is seen in older children and adults. First, small children have small airways that are more vulnerable to changes

in diameter. In addition, bronchi in young children are highly compliant, which may lead to increased airway narrowing compared to adults, and developing respiratory muscles presents with limited contractile ability (65). The ribs of infants are located more horizontally, and the thoracic cage of infants mainly consists of cartilage, making it highly compliant. This leads to decreased outward pull of the chest and less efficient mechanics of breathing (66). Functional residual capacity is lower in infants, which increases vulnerability to impairment of gas exchange (65).

Young age increases the risk for severe bronchiolitis (67), probably due to immaturity of the immune system or physiological reasons. In addition, severe bronchiolitis is more common in boys than girls (68-70). This may partly be explained by the differences in immune responses between genders (71, 72) and partly by the sex-related differences in airway properties and lung growth in young children (73). Tobacco smoke exposure, being born in winter months (i.e. at the beginning of RSV epidemic season), being born by Caesarean section, malnutrition, lack of breastfeeding, low socio-economic status, living in crowded conditions, day-care attendance, having older siblings and multiple gestation have been associated with severe course of bronchiolitis (68-70, 74, 75).

2.2 Respiratory syncytial virus bronchiolitis

2.2.1 Description of respiratory syncytial virus bronchiolitis

Respiratory syncytial virus (RSV) is an enveloped, single-stranded RNA virus surrounded by a lipid bilayer. Its two main membrane proteins are the fusion protein F and the glycoprotein G, which are suggested to play a crucial role as antigens. RSV serotypes, type A and type B, differ for G proteins (7).

RSV affects the superficial epithelial cells of the upper airways, epithelial cells in the small bronchioles and pneumocytes in the alveoli (7, 76). Infected cells, and further immune responses, activate pro-inflammatory cytokines and inflammatory cells, mainly neutrophils (77, 78). Tissue oedema, necrosis of the epithelial cells and increased mucus production lead to obstruction of small airways, air trapping in the alveoli, and localised atelectasis after the trapped air is absorbed (47). These changes may induce impaired gas-exchange and lead to hypoxemia and increased work of breathing.

RSV causes worldwide epidemics every year, and nearly all children are infected with RSV before two years of age (79). In Finland, large RSV epidemics typically alternate with smaller epidemics every other year, with peaks during the winter-to-spring months (80). RSV A and B circulate every year or every two years (7, 57), and the severity of RSV bronchiolitis seems to be relatively similar in the case of different serotypes (81). Immunity against RSV is not long-term, but severe infections mainly occur during early infancy (54), suggesting an accomplished yet incomplete adaptive immunity to RSV.

2.2.2 Immunology of respiratory syncytial virus infection

Neutrophils, monocytes, macrophages and dendritic cells, although matured prenatally, function weakly in neonates, resulting in impaired innate immunity and reduced interaction with adaptive immune system. Mature single CD4 and CD8 positive T cells are present at birth, but they still reflect the fetal life, and exposure to environmental antigens other than maternal alloantigens is restricted (82).

Pattern recognition receptors, such as TLRs, are expressed by epithelial cells, fibroblasts and antigen presenting cells in the respiratory tract, and they mediate innate immunity against different pathogens, RSV included (83). The mechanisms of an increased risk of severe infection during infancy have been hypothesised relate to genetic variations of *TLR* genes, leading to altered TLR signalling (29, 84, 85), but the evidence is inconsistent (86).

Both Th1-oriented and Th2-oriented pathways are identified in RSV infection (87). With simplification, the theory on the balance between Th1 and Th2 hypothesises that Th1 cells activate cell-mediated responses against intracellular pathogens such as viruses, whereas Th2 cells are responsible for protection against extracellular pathogens such as parasites, and for humoral immunity by regulating antibody production (88). Th1 pathway is assumed to be able to generate organ-specific autoimmune diseases when over-activated, whereas Th2 pathway is assumed to lead to the development of allergies and asthma (88). It is speculated that activation of neonatal T cells or altered TLR functions may lead to skewed balance of Th1/Th2 pathways in adaptive immunity (29, 82). Currently, immune regulation is considered to be more complex and to comprise more than two major pathways (89).

2.3 Flow-volume spirometry

2.3.1 Mechanics of forced expiratory flows

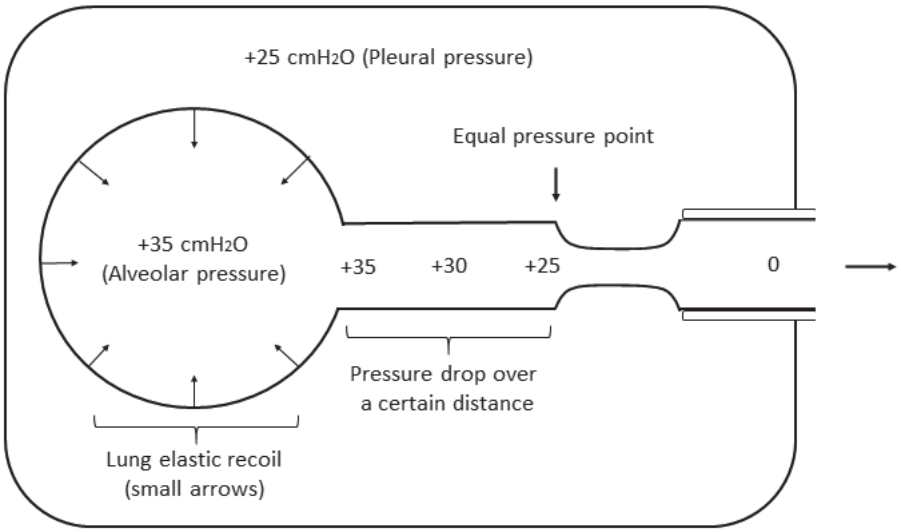
Expiration is normally a passive process. Airflow out of alveoli requires an increase in intra-alveolar pressure over atmospheric pressure. This is resulted from a change in pleural pressure to less negative after inspiration due to combination of chest contraction, inward recoil of the lungs and relaxation of the diaphragm and inspiratory intercostal muscles. Since small airways collapse at the end of expiration, air is trapped in the alveoli, which allows constant gas exchange. This resting volume of the respiratory system is called functional residual capacity (FRC). In normal breathing, pleural pressure is always negative in relation to atmospheric pressure. In forced expiration, activation of the expiratory muscles increases the pleural pressure above atmospheric pressure. Due the elastic recoil pressure of the alveolar wall, intra-alveolar pressure remains always above pleural pressure, causing a large pressure difference down the airways during forced expiration (90).

Certain physiological theories help in understanding forced expiratory flows. According to application of Ohm's law, airflow in the lungs ($\dot{V}$) is directly proportional to the pressure difference between the alveoli and the airway opening (transairway pressure, ΔP) and inversely proportional to airway resistance (R).

$$\dot{V} = \frac{\Delta P\ (Transairway)}{R}$$

Forced expiratory flow cannot increase unlimitedly with increasing effort from respiratory muscles. During a forced expiration, the pressure inside the airway decreases toward airway opening whereas the pleural pressure outside the airway remains relatively similar. The point in the bronchial tree at which pleural pressure is equal to intra-airway pressure is known as the equal pressure point (EPP) and greater alveolar driving pressure does not increase the flow any further. Beyond this point, toward airway opening, the pleural pressure is greater than the pressure in the airway, which leads to dynamic compression of the airway (Figure 1) (90, 91). The EPP is not stationary during a course of forced expiration; it is more central in high lung volume and moves into smaller airways with decreasing lung volume (92).

Figure 1. Schematic illustration of equal pressure point theory of forced expiration. Non-cartilaginous airways are non-barred and cartilaginous airways are barred in the picture. Modified from (90) and (91).



Lung elastic recoil and airflow resistance determine maximal expiratory flow (93) and changes in either have an impact on location of EPP in the airways. In healthy lungs, EPP is reached in large airways that are surrounded by cartilage and do not collapse. In airway obstruction, due to higher resistance of narrowed airways, greater pressure gradient for given flow rate is needed and EPP is reached closer to alveoli. If EPP is reached in the bronchioles without surrounding cartilage, airway collapses that is seen as a typical depression in the flow-volume curve (91). Lung elastic recoil refers lung tendency to deflate and results from elastic characteristics of lung periphery and from surface tension in alveoli (91). The sum of elastic recoil and pleural pressures results alveolar pressure; decrease in elastic recoil pressure means smaller difference between alveolar and pleural pressures and EPP moves towards alveoli. In peripheral airways, loss of elastic support increases their tendency to collapse.

The velocity of the flow accelerates with airway narrowing at EPP. The maximal acceleration of the flow can be determined by wave speed theory, which is based on the properties of the airway wall and inhaled gas. The point at which the flow has reached wave speed by acceleration is called the "choke point" and, beyond this point, further acceleration is not possible (92).

In restrictive diseases, the balance between the inward recoil of the lungs and outward recoil of the chest wall is disturbed. Most commonly, restrictive lung disease

makes lung parenchyma stiffer (or less compliant) and lung ability to inflate is limited that leads to decreased flows in all lung volumes.

2.3.2 Description of flow-volume spirometry

Flow-volume spirometry is a technique for recording volumes of air during forced inspiration and expiration in relation to time, and it is considered the standard method for studying lung function (94). Airway closure was suggested as a potential factor for limiting expiratory flow as early as in the 18th century; however, clinical application of forced expiration manoeuvres was not described until the 1940s (95). Currently, maximal expiratory flow can be registered at airway opening using various types of spirometers, among which pneumotachometers are most commonly used (96). Pneumotachometer consist of a built-in flow-resistive device: pressures downstream and upstream of this device are registered and pressure difference is converted into electrical voltage from which airflow and volumes are calculated (96).

The manoeuvre of flow-volume spirometry includes three separate phases. First, the patient takes a fast, deep breath to reach maximal inspiration. This is followed by maximal flow of expiration with rapid blow. After forced expiration, complete exhalation is continued to the end of the test. Correctly accomplished manoeuvre is critical for reliable results, and the procedure should be demonstrated first by the technician. The manoeuvre needs to be repeated until at least three acceptable, similar and artefact-free curves are seen, and the highest values should be chosen for interpretation (94).

The results of flow-volume spirometry are reported as volume-time or flow-volume loops. Both are important in estimating the quality of blows, but flow-volume loops are the basis of spirometry in clinical use (94). The primary outcome variables of flow-volume spirometry are forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and FEV1/FVC. Peak expiratory flow (PEF), maximal expiratory flow at 50% of FVC (MEF50) and maximal expiratory flow at 25-75% of FVC (MEF25-75) are also used to determine lung function, but their clinical significance is lower (97). MEF50 represents the flow where half of FVC remains to be exhaled (94), and it seems to correlate well with MEF25-75 (98).

2.3.3 Clinical outcome variables

The literature describes four standard lung volumes: tidal volume, inspiratory and expiratory reserve volumes and residual volume. Four standard lung capacities are calculated from volumes. Forced vital capacity (FVC) is the sum of tidal volume and inspiratory and expiratory reserve volumes and represents the maximal volume of air exhaled during maximal forced expiration after maximal inspiration. The other lung capacities are inspiratory capacity, functional residual capacity and total lung capacity (90). In restrictive lung abnormalities, lung ability to inflate is decreased, which leads to reduction of FVC (99). Obstructive lung abnormalities may lead to air trapping that increases lung residual volume and decreases the volume of air that can be expired during forced expiration (FVC) (90).

FEV1 is the maximal volume of air that is exhaled during the first second of forced expiration after maximal inspiration. Airway obstruction is commonly determined as disproportionately reduced FEV1 in relation to FVC, which reflects abnormal airway narrowing during maximal exhalation. In the most peripheral obstruction, FEV1 is usually normal, but in the more severe disease or with more central location of the obstruction, FEV1 decreases more rapidly in relation to FVC (99).

The first sign associated with abnormal spirometric function in obstructive diseases is the depression of the terminal portion of the spirogram, even with an almost normal initial part. This leads to a concave shape of the flow-volume curve, which actually reflects peripheral location of airway obstruction. According to this, one can hypothesise that measuring MEF25-75 or MEF50 values may be used to detect small airway obstruction. However, wide range of normal values, high intra-individual variability and the dependence on lung volumes limits its use in clinical decision-making (99, 100). In recent years, new spirometric indices, mainly based on the concave form of the flow-volume loop, have been suggested to detect peripheral obstruction (100, 101). The lack of a reliable spirometric tool for determining peripheral airway obstruction hampers the diagnosis of early stages of asthma and chronic obstructive pulmonary disease (COPD).

2.3.4 Bronchodilator response and irreversible airway obstruction

Bronchial responsiveness reflects the relaxation of airway smooth muscle (102). The activation of bronchodilator response is complex and at least airway epithelium, nerves and mediators are involved (99). Responses to bronchodilators vary

depending on the administrated medicine, distinctive physical characteristics and changes in baseline FEV1, which together present a challenge to the assessment of significant reversibility (102). According to ATS/ERS (American Thoracic Society/European Respiratory Society) and GINA (Global Initiative for Asthma) recommendations (22, 99), the increase between baseline and post-bd FEV1 in the bronchodilation test should be more than 12% for significant bronchodilator response. In a recent study, bronchodilation test data from three large population-based studies was combined, including a total of over 30,000 subjects aged over 16 years, and FEV1 increase was 12% or more in 17% of asthmatic patients, in 18% of COPD patients and, notably, in 5% of subjects without airway disease (103).

Irreversible airway obstruction, assessed with spirometry, encompasses all mechanisms leading to reduced airway size during forced expiration that is not reversible with bronchodilators. In the research, the definition of irreversible airway obstruction varies; however, reduced post-bd FEV1 or FEV1/FVC, or alternatively, reduced baseline FEV1 or FEV1/FVC without significant reversibility in a bronchodilation test are typically used (104). In a birth cohort study from New Zealand, reduced post-bd FEV1/FVC was associated with accelerated decline in baseline lung function from nine to 26 years of age in 1,037 participants (26), suggesting an association between longitudinal lung function loss and irreversible airway obstruction.

In asthma, irreversible airway obstruction has been suggested to be a proxy of structural changes in the airway wall (27, 28, 105), and it seems to exist only in some asthmatic patients (24, 25, 27, 106). A study from Maryland, USA evaluated irreversible lung function deficits in 121 young adults with history of moderate-to-severe childhood asthma, and prevalence of reduced results ($\leq 5^{th}$ percentile) were 16% for post-bd FEV1 and 34% for post-bd FEV1/FVC (106). A Brazilian follow-up study enrolled 358 children and adolescents with medical treatment for asthma and, after four-year follow-up, post-bd FEV1/FVC ratio was reduced (under lower limit of normal) in 9.5% of participants (25). That study excluded four participants whose FEV1/FVC ratio was observed to be reduced in the first visit, and 25 participants who discontinued asthma treatment during the four-year follow-up (25).

Irreversible airway obstruction is a defining feature in COPD: a post-bd FEV1/FVC ratio <70% or, alternatively, post-bd FEV1/FVC $<5^{th}$ percentile of predicted is considered diagnostic for COPD (107). This definition is widely used for describing COPD in research but has drawn criticism for underestimating other pathways for lung function loss if clinical features is not taken account (108).

2.4 Impulse oscillometry

2.4.1 Airway resistance in relation to flow

Airway resistance refers to the pressure across the airways that is needed to produce a unit flow of gas. The higher the airway resistance, the greater the pressure needed to maintain the air flow. According to Poiseulle's equation, the pressure difference across the airways is directly related to the length of the airway tube (l) and the gas viscosity (η) and inversely related to the fourth power of the airway tube radius (r). (109)

$$\Delta P = \frac{8l\eta Q}{\pi r^4} \qquad R = \frac{8l\eta}{\pi r^4}$$

This means that the changes in airway resistance are mostly affected by the change of diameter of the airway tube. Physiologically, airway resistance increases with decreasing lung volumes in expiration due to narrowing airways. By the same theory, airway obstruction causes a pathological increase in airway resistance.

In human airways, both laminar and turbulent flows are present. In small airways with lower velocities, the flow is mainly laminar, and in larger airways with higher velocities, the flow becomes more turbulent (110). In laminar flow, the Poiseuille equation for pressure difference is as presented above. In turbulent flow, the Poiseuille equation requires modification and airway resistance is inversely related to the fifth power of radius meaning that the impact of airway obstruction on resistance is even higher (65, 109).

Since airways are connected both in series and side-by-side, total airway resistance is calculated reciprocally. As a result, airway resistance declines towards trachea and towards alveolar level, and small airways contribute only approximately 10% of total airway resistance in older children and adults (109). In infancy, small airways contribute more to total airway resistance than in older children and adults (65).

2.4.2 Description of impulse oscillometry

Forced oscillation technique was developed in the 1950s to measure respiratory mechanics non-invasively using sound waves. In this technique, the term "forced"

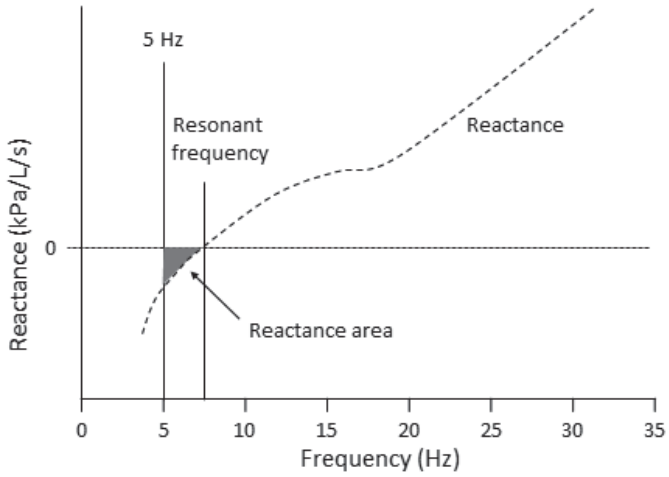
describes sound waves as external, periodic driving forces that generate oscillation in the airways. IOS is a modified application of forced oscillation technique, and it uses multiple frequencies of sound waves at once (111).

IOS registers normal breathing, and it does not require active co-operation such as forced blows. For this reason, it is more convenient than spirometry in young children. During the procedure, the patient is in a sitting position wearing a nose-clip and tries to present as artefact-free breathing as possible. The input impulses of IOS (sound waves) are conducted to airways through a mouthpiece (111). The output of IOS is resulted as respiratory system impedance that represents the sum of forces resisting propagation of pressure waves in oscillation. Respiratory system impedance includes respiratory system resistance (Rrs) and respiratory system reactance (Xrs) (111). The results of IOS are presented as Rrs and Xrs curves as a function of oscillation frequency.

Rrs reflects the resistive forces of the airflow across the respiratory system and depends mainly on airway resistance. In healthy lungs, Rrs is nearly independent on oscillation frequency, and central airway obstruction increases Rrs independent from oscillation frequency. In peripheral obstruction, frequency dependence of Rrs increases; in such case, Rrs is highest at low frequencies and decreases at higher frequencies. Due to this phenomenon, pressure waves at lower frequencies are often described to reach the periphery (112, 113).

Xrs is a visionary part of impedance and reflects the out-of-phase elastic rebound of the expanding airways. Xrs includes the mass-inertive forces of the flowing air column (inertance) and the elastic properties of the lungs to store capacitive energy (capacitance), which are dependent on the frequency of oscillation (113). At lower frequencies, the capacitive properties are dominant and at higher frequencies, the inertive properties are dominant. Therefore, Xrs at lower frequencies primarily reflects the capacitance in the lung periphery while Xrs at higher frequencies primarily reflects the inertance in the larger airways (111, 112). The oscillation frequency at which reactance crosses zero (resonant frequency) is the point where magnitudes of inertance and capacitance are equal (Figure 2). The area from 5 Hz to resonant frequency and between reactance zero and reactance curve (as a function of oscillation frequency) is termed the reactance area (Figure 2) (113). In peripheral lung diseases, Xrs decreases, resonant frequency increases and the reactance area gets larger.

Figure 2. Schematic illustration of respiratory system reactance presented as a function of oscillation frequency. Shaded area is reactance area. Modified from (113)



2.4.3 Correlation between results of impulse oscillometry and spirometry

Compared to spirometry that measures flows and volumes during maximal blows, IOS registers normal breathing at rest. Several previous studies have described modest to strong correlations between Rrs5 or Xrs5 and FEV1 in non-asthmatic and asthmatic subjects (114-117). In contrast, some variables of IOS seem to be more sensitive for detecting small airway abnormalities in asthma compared to variables of spirometry (118, 119). A study from California, USA with 115 children assessed asthma control with measurements of IOS and spirometry, and the best indicators of small airway dysfunction were the reactance area and the difference in Rrs from 5 Hz to 20 Hz (118). In addition, a study from Maryland, USA, compared bronchodilator responses between 66 children with well-controlled asthma and 16 non-asthmatic children with measurements of spirometry and IOS, and the difference in bronchodilator response between the groups was significant for the reactance area, but not for any spirometric or other IOS indices (119).

2.5 Growth of lung function

2.5.1 Lung growth

Development of the airways and lungs begins during the first gestational weeks with the formation of the trachea, bronchi and pleura. All airways and approximately 10% of gas exchange surface area are formed by branching morphogenesis. Each branching produces a new airway generation, finally reaching the average of 21-25 generations. The first 16 generations comprise the conducting airways, and further branching forms respiratory bronchioles and immature shapes of future alveolar ducts and alveolar sacs (120). Respiratory bronchioles are formed until the end of the second trimester. Forward from 24 gestational weeks, the terminal ends of the airways grow forming large airspaces and leading to thinning of the airspace walls that allows gas exchance (120).

The development of the remaining 90% of gas exchange surface area comprises the formation of alveolar septa that subdivide large airspaces into alveoli (120). This process is called septation or alveolarisation, and it begins approximately at 36 gestational weeks (65). The production of surfactant, which lowers surface tension and protects the alveoli from closing at low lung volumes, is sufficient on average at 32 gestational weeks (110). Fully developed alveolar sac consists of alveoli with thin epithelium, internal septae and capillary network surrounding the alveoli (120, 121). Approximately 10-15% of alveoli are fully developed at birth (65, 122).

While the development of conductive airways is complete before birth, the diameter of airways increases throughout childhood. In contrast, alveolarisation mainly occurs postnatally, and lung volume doubles by age six months and triples by age one year. In young children, growth of the lung parenchyma is faster than that of the airways. The predominant hypothesis is that alveolar multiplying continues during first living years and, after that, alveoli increase in volume and surface area. Some authors have suggested that alveolarisation may continue until early adulthood (65). Due to underdeveloped lungs in infancy, it can be hypothesised that environmental factors during childhood, especially during the first years, may impact on lung growth leading to altered lung function. In this regard, increasing evidence has shown that early-life events have a crucial role in the development of significantly reduced lung function later associated with COPD (123-125).

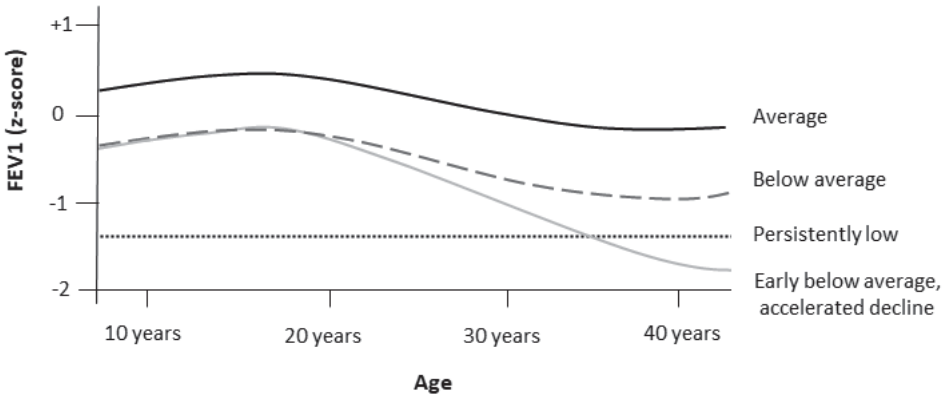
2.5.2 Lung function trajectories

Development of lung function is affected by genetic, prenatal and postnatal factors (122). It has been estimated that genetics explain over 50% of pulmonary function in adults (126). Intrauterine lung growth may be disturbed due to multiple reasons including anatomical abnormalities of the chest wall, maternal hypoxia, maternal use of alcohol or certain drugs, and maternal smoking or nicotine intake (122). Postnatal factors that impact on lung function in childhood include environmental and developmental factors such as respiratory infections (123-125), tobacco smoke exposure (127) and own personal smoking later in childhood (128).

Physiological determinants of lung volumes are body size, age, sex and ethnicity. Standing height is the most dominant factor associated with lung volumes (99), but insufficient alone to predict physiological growth of lung function (129). In normal growth, FEV1 increases linearly until the growth spurt in adolescence, during which there is a shift in growth in relation to height (24, 130). Like in height growth, the onset and magnitudes of the pulmonary function growth spurt vary between sexes and individually within the same sex (130). The peak in FEV1 growth with plateau is reached in early adulthood and followed by a physiological decline with ageing (24).

Theoretical models for height-adjusted FEV1 trajectories have demonstrated different patterns of airway growth after early years (24, 125). A large longitudinal, population-based study demonstrated six baseline FEV1 trajectories as height-, age- and sex-specific z-scores from childhood to the sixth decade of life in 2,438 children enrolled in Tasmanian schools at the age of seven years (125). Half of participants had average or persistently high FEV1 trajectories and 8% of participants had low FEV1 in childhood but reached an average maximal FEV1 in early adulthood and thereafter showed normal decline with ageing. The remaining 42% of participants belonged to three subgroups with lower FEV1 trajectories than average: persistently low FEV1 (6%), below average FEV1 (32%) and below average FEV1 through childhood with accelerated decline with ageing (4%) (Figure 3). These three FEV1 trajectories were associated with reduced post-bd FEV1/FVC at the age of 53, which suggests an increased risk of development of COPD. Early-life risk factors for low FEV1 trajectories included childhood asthma and atopy, along with childhood respiratory infections (125).

Figure 3. Three low childhood FEV1 trajectories and average FEV1 trajectory demonstrated in the Tasmanian longitudinal study. Modified from the reference (125).



2.6 Lung function reduction

2.6.1 Theory of dysanapsis

Physiologically important observations about lung function growth have been made by Green and Mead, who created a theory of dysanapsis to describe the discrepancy between airway growth and lung size (131). This theory was based on wide variations of maximum expiratory flows in healthy adults with similar lung volumes and suggested as physiological differences in airway calibre and geometry. More importantly, it was thought that if the discrepancy was present at birth, it stayed until adulthood (132). Typically, dysanapsis is considered to describe small airway`s anatomy and physiology in relation to lung size, highlighting its prenatal origin. In spirometry, dysanapsis can be defined as low FEV1/FVC ratio with FEV1 and FVC within the normal range (99). In a recent retrospective study of two community-based samples, dysanapsis was quantified using computerised tomography findings for older adults, and lower airway calibre in relation to lung size was associated with greater COPD risk, defined as FEV1/FVC <0.70 and COPD-presumptive symptoms (133). Interestingly, the result was similar in 520 never smokers and 2,726 smoking participants, providing anatomical evidence for the dysanapsis theory.

The theory of dysanapsis has been further supported by the findings of a birth cohort study from Tucson, Arizona (TCRS) that demonstrated persistently low expiratory flows from birth in children with transient wheezing symptoms in early

childhood (134). In the same cohort, infant lung function predicted lower baseline and post-bd FEV1/FVC from school age to adulthood (35) and reduced airway calibre in chest high-resolution computerised tomography at the age of 26 years (135). However, there are no methods for assessing airway reversibility in infancy, although lung function and airway responses to different stimuli can be measured at that age. This means that reduced neonatal flows may reflect either irreversible reduction of airway calibre during maximal expiration or reversible changes in airway tone. In TCRS, children who wheezed in early childhood and continued wheezing at preschool age did not show reduced infant flows (134). However, two thirds of infants with the lowest tercile of lung function were diagnosed with asthma by age 26 years (135).

2.6.2 Lung function reduction in childhood asthma

In a birth cohort study from Copenhagen, Denmark, measurements of airway reactivity and forced expiration were described as trajectories from birth to 13 years of age in 367 children with asthmatic mothers (136). In all, airway reactivity was persistently higher and maximal expiratory flows were persistently lower in children diagnosed with asthma by age 13 years compared to those without asthma diagnoses. In 97 children with asthma, trajectories did not deteriorate due to duration of the disease or asthma exacerbations, and trajectories did not improve when symptoms were in remission (136, 137). The authors concluded that symptomatic disease as well as exacerbations are rather consequences than causes of decreased lung function in typical childhood asthma (136, 137). Similarly, FEV1 or FEV1/FVC reductions in individuals with childhood-onset asthma did not show progression in longitudinal studies continuing until adulthood despite continuing symptoms (138, 139).

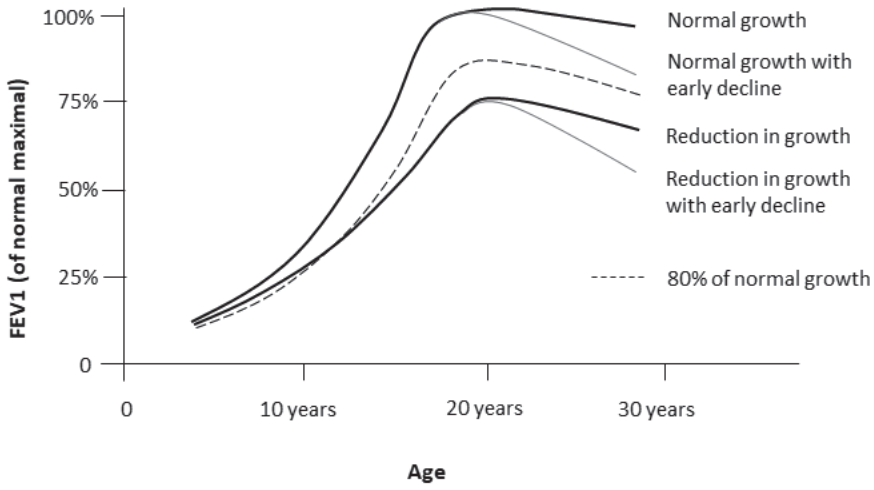
2.6.3 Reduction in growth of lung function

As described, growth of FEV1 during childhood follows normally certain child-specific percentile, meaning an individual trajectory (24). In those with reduced flows in infancy, transition into higher percentiles later in childhood is likely to be relatively common (34). In contrast, normal growth of FEV1 does not cross percentiles downwards (24, 92). At school age, such downwards crossing has been documented in a group of children with persistent, symptomatic asthma (17, 24), possibly as a result of airway remodelling (26, 27). Other potential reasons for reduced growth of

lung function are environmental exposures such as tobacco smoke or air pollution (128, 140) and rare childhood pulmonary or neuromuscular diseases (141, 142).

A British study demonstrated four baseline FEV1 trajectories from preschool age up to early adulthood in 684 individuals with chronic, symptomatic asthma and significant airway reactivity at the enrolment at the age of five to 12 years (Figure 4) (24). The trajectories included normal FEV1 growth with normal decline by ageing (25%), normal FEV1 growth with early decline by ageing after normal attended maximal level (26%), reduced FEV1 growth leading to decreased attended maximal level (23%), and growth with both limitations (26%). Lower baseline FEV1, smaller bronchodilator response, higher airway reactivity and younger age at enrolment and, in addition, male gender were associated with reduced FEV1 growth. Subjects with reduced FEV1 growth were more likely to have reduced post-bd FEV1/FVC (<0.70) at 26 years of age than those with normal FEV1 growth (24).

Figure 4. Possible patterns of FEV1 trajectories from childhood to adulthood in patients with severe childhood-onset asthma. Modified from (24).



2.6.4 Airway remodelling in asthma and COPD

Airway remodelling is a collective term that encompasses the structural changes in the airway wall (105, 130, 143). Previous literature has suggested two types of airway remodelling (144, 145). Physiological remodelling comprises airway maturation and growth during normal lung development and, in addition, those transient responses

to lung injury or inflammation that recovers back to normal. Pathological remodelling comprises structural changes resulted from disturbed lung development and, in addition, those responses to chronic lung injury or inflammation that leads to persistent structural changes of the airway wall. Such pathological remodelling is seen, for example, in obstructive pulmonary diseases (144, 145). Mechanisms of airway remodelling are not fully understood (145).

Asthma is a heterogeneous disease of the airways, characterised by chronic, mainly eosinophilic inflammation with CD4+ helper T cell activation, and it is defined by expiratory airflow obstruction with asthma-presumptive symptoms (22). The main characteristics of airway remodelling in asthma are epithelial injury, increased thickness of the sub-epithelial reticular basement membrane (RBM), higher airway smooth muscle mass and altered goblet cells with mucus hypersecretion (145). Airway remodelling in asthma is thought to be a result from long-term inflammation, based on the findings that treatment with ICS limits airway remodelling (145-148). In contrast, some findings have suggested non-inflammatory mechanisms in the development of structural changes in the asthmatic airway (149).

In studies evaluating endobronchial biopsies, structural changes have been observed in adults with asthma (144), in school-aged children with severe asthma (28, 150, 151) and in preschool-aged children with severe recurrent wheeze (152, 153). Children with non-atopic or atopic asthma presented with similar RBM thickness (152). In severe childhood asthma, RBM thickness reached by school age the same level seen in adult asthma (154). A follow-up study including 53 children from Finland with severe wheeze or cough evaluated endobronchial biopsies at the median age of 12 months, and RBM was similar to that in controls without symptoms (155). However, RBM thickening at one year of age was associated with the need for treatment at three years of age (156). The findings suggest heterogeneous pathogenesis in respiratory disorders with onset in infancy rather than absence of airway remodelling in young asthmatic children.

COPD is a disease of the airways and lung parenchyma characterised by mainly neutrophilic inflammation with CD8+ helper T cell activation (144, 157), and it is defined by an irreversible airway obstruction with persistent respiratory symptoms (107). Pathological mechanisms of COPD include chronic bronchitis with increased mucus secretion, emphysema resulting from alveolar wall destruction and small airway disease characterised by airway remodelling that includes metaplasia of small airways, fibrosis of the airway walls and increased smooth muscle mass (158). Small airway disease may play the most important role in the progression of the disease in the early stages of COPD (158).

2.6.5 Lung function reduction after bronchiolitis

Evidently, hospitalisation for bronchiolitis increases the risk of later lung function abnormalities (10-17). However, it is not known whether bronchiolitis reflects pre-existing variations between individuals or whether bronchiolitis itself triggers lung function changes. In birth cohort studies, abnormalities in infant lung function or airway reactivity have been associated with early-childhood LRTIs (30-32, 159, 160), suggesting pre-existing differences in lung function. In contrast, two thirds of 93 hospitalised RSV bronchiolitis patients showed hyperinflation shortly after bronchiolitis and 17% at the one-year follow-up in a British study (161), indicating at least short-term consequences caused by severe RSV infection.

Infant bronchiolitis is associated with increased risk of asthma (20, 21). It has been thought that bronchiolitis may be the first sign of susceptibility to asthma, or that it may induce long-term impacts via altered maturation of immune system or airway damage (4, 29). In a birth cohort from Copenhagen, Denmark, bronchial hyperreactivity predicted bronchiolitis in high-risk infants with asthmatic mothers, supporting the theory of pre-existing susceptibility to asthma (159). In hospital cohort studies, the risk of asthma was highlighted in those with rhinovirus aetiology of bronchiolitis (162). Rhinovirus infections has been linked to similar airway wall changes as seen in atopic asthma, including changed epithelial barrier function, high cytokine levels and Th2-oriented cell characteristics (4).

Lung function reductions after RSV bronchiolitis have been reported in subjects with and without asthma (17, 21). Interesting results were found in a prospective multi-centre study, which showed a significant decrease in relative risk of preschool-age wheezing in premature non-atopic children who received immune prophylaxis against RSV, but not in those with a history of atopy, suggesting that the mechanism of recurrent wheezing after RSV infections is not dependent on atopy (163). It is suggested that RSV-induced airway hyperreactivity and inflammation are partly related to abnormal neural control of smooth muscle tone, which may lead to persistent structural changes of the airway wall, increasing airway reactivity and decreasing lung function permanently (39). In mouse models, RSV has been capable of causing structural changes in the airways, such as subepithelial fibrosis, increased collagen and thickening of the bronchial basal membrane (36-38).

2.7 Cohort studies: Lung function in relation to bronchiolitis

2.7.1 Birth cohorts: Long-term outcome

Tucson Children Respiratory Study (TCRS)

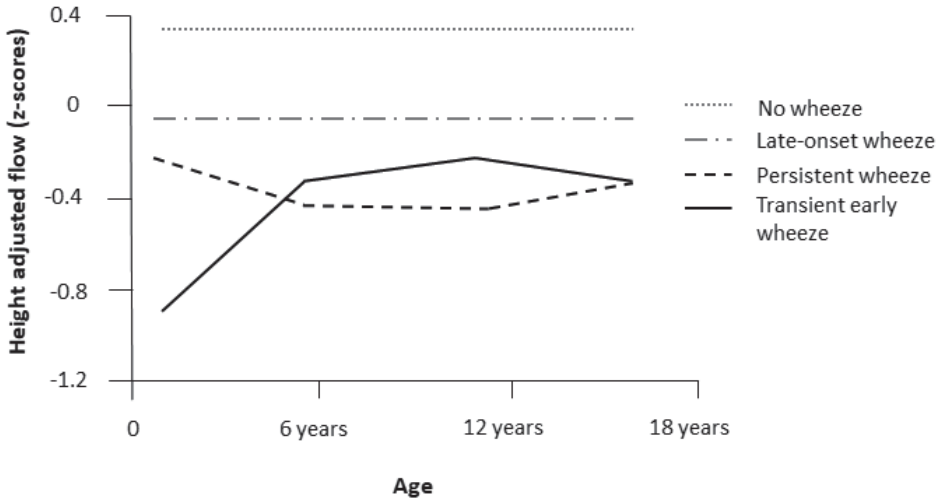
TCRS is a community-based birth cohort study that originally enrolled 1,246 healthy infants (164). First lung function measurements were performed in 125 participants before any LRTIs when the children were less than three months of age (33). At the one-year follow-up, the authors found an increased risk for parent-reported wheeze in infants with decreased respiratory conductance and in girls with decreased levels of FRC (48). By age one year, 36 (29%) children were diagnosed with LRTI: two thirds of children with LRTI had wheezing symptoms and none were hospitalised. No significant differences in infant lung function were found between children with non-wheezing LRTI and children who did not have LRTI by one year of age (48).

Longitudinal findings of TCRS are based on measurements of expiratory flows. In infancy, expiratory flows were tested using chest-compression technique (48). This method requires a fitted inflatable jacket, which is set around abdomen and thorax of the sleeping infant. The jacket is rapidly overblown at the end of the inspiration, which produces forced expiration and creates a partial expiratory flow-volume curve. Since measurements are taken during tidal breathing, FRC is used as the volume landmark for calculations, and results are commonly reported as maximum flows at functional residual capacity (VmaxFRC) (165). In TCRS, VmaxFRC did not show significant associations with emergence of LRTI (48); instead, it was associated with later lung function decline (35).

Similar technique than in infancy was used to study VmaxFRC in 526 children in a follow-up at the age of six years (33). Later lung function measurements were performed using spirometry in 542 children at 11 years of age and in 426 children at 16 years of age (134). For comparisons of lung function outcomes, the authors divided the children into four groups: those who had not wheezed at any age (no wheeze, 53%), those who had at least one physician-diagnosed wheeze before the age of three years but wheezed no more at the age of six years (transient early wheeze, 19%), those who had not wheezed before the age of three years but who wheezed at the age of six years (late-onset wheeze, 15%), and those who had wheezed before the age of three years and still wheezed at the age of six years (persistent wheeze, 13%) (134).

In comparison to never wheezers, transient early wheezers formed the only subgroup of children with lower infant $V_{max}FRC$ (33). In addition, transient early wheezers showed significantly lower $V_{max}FRC$ at six years of age (33) and significantly lower MEFF25-75, FEV1 and FEV1/FVC ratio at 11 and 16 years of ages (134). To describe longitudinal lung function trajectories, the authors converted $V_{max}FRC$ and MEF25-75 values into height-adjusted flows (z-scores). Lung function trajectory of early transient wheezers was characterised by decreased flows in infancy, mild increase in flows by the age of six years, and persistently reduced flows until the age of 16 years (Figure 5) (134). Early transient wheezers were unlikely to have atopy or family history of asthma, but they were more often exposed to smoking during pregnancy in relation to children who had never wheezed, and the majority of them had started to wheeze during the first year of life (33, 164). Those transient early wheezers, who still did not wheeze at school age, were not at increased risk for airway hyperresposiveness measured at 11-year follow-up (166).

Figure 5. Trajectories of height adjusted flows presented as z scores and classified by wheezing phenotypes of TCRS study. Modified from (134).



The other subgroup of children that showed abnormal results were persistent wheezers. In comparison with never wheezers, they did not demonstrate reduced infant $V_{max}FRC$ but had significantly lower $V_{max}FRC$ at six years of age (33) and significantly lower MEF25-75, FEV1 and FEV1/FVC ratio at 11 and 16 years of ages (134). Lung function trajectory of persistent wheezers was characterised by mild decrease in flows from birth to the age of six years and persistently reduced flows until the age of 16 years (Figure 5) (134). Persistent wheezers presented with higher

IgE levels and were more likely to have asthmatic mothers than children without wheeze (33). Children with RSV aetiology of early-life wheezing had a 3-5-fold risk of persistent wheeze at six years of age, but the risk decreased markedly by the age of 13 years (164). Interestingly, those children who developed persistent symptoms after RSV infection were not more likely to be atopic than other study subjects (164).

Southampton Women's Survey (SWS)

SWS from Southampton, UK is prenatally recruited cohort for evaluation of early-life environmental factors on child growth, including assessment of asthma phenotypes and lung function. VmaxFRC was measured at the age of less than four months in 150 infants born at 35 gestational weeks or later using rapid thoracic compression technique. In addition, spirometry was measured in 791 children at the age of six years. Complete follow-up data with lung function measurements in infancy and at six years of age was available in 95 children (167).

The authors compared lung function results between two different classifications of wheezing phenotypes, one from TCRS and the other from the Avon Longitudinal Study of Parents and Children (ALSPAC) (168). ALSPAC is a longitudinal population-based birth cohort study from Bristol, UK with over 10,000 enrolled children but without measurements of infant lung function (169). In ALSPAC, classification of wheezing phenotypes was based on probability of wheezing calculated from estimated wheezing prevalence (168). In this classification, early wheezers were divided into transient early wheezers who grew out of wheeze by the age of 3.5 years and prolonged early wheezers who grew out of wheeze by the age of six years, and persistent wheezers were slightly younger at the onset of wheeze (168).

According to TCRS classification, transient early wheezers had significantly lower maximal flows than never wheezers in infancy and at six years of age; according to ALSPAC classification, such associations were only seen in those with prolonged early wheezing. According to TCRS classification, persistent wheezers showed reduced maximal flows at six years of age but not in infancy; according to ALSPAC classification, the reduction was significant at both ages (167).

Perth Infant Asthma Follow-up (PIAF)

In the PIAF birth cohort study from Perth, Australia, 246 full-term, healthy infants underwent rapid thoracic compression technique to measure VmaxFRC at one month of age before any LRTI (170). Lung function measurements at the ages of one, six and 12 months were evaluated in relation to wheeze during the first two

years of life in 160 children with complete data available (30). Children who had wheezed during the first two years showed significantly lower infant VmaxFRC values than children who had never wheezed. Children who wheezed only during the first year, resolved the difference by the age of 12 months, but in children who wheezed during the second year, the difference persisted up to the age 12 of months (30).

In PIAF, doctor-diagnosed bronchiolitis at the age of less than 24 months was present in 17 children. VmaxFRC was lower throughout the first year of life in children with early-life bronchiolitis than in those without (170), as confirmed in multivariate analyses adjusted for gender, age, length, weight and maternal smoking in pregnancy (171). Early-life bronchiolitis was associated with reduced MEF25-75 at 11 years of age but not with other markers of spirometry (171).

Manchester Asthma and Allergy Study (MAAS)

The MAAS birth cohort study from Mancester, UK evaluated development of asthma and allergies in prenatally recruited full-term children (172). Expiratory flows were measured at the age of one month in a subgroup of 69 children with atopic parents using rapid thoracic compression technique, and lower infant VmaxFRC was associated with parent-reported wheezing and cough during the first year of life (32).

Furthermore, at the ages of three, five, eight, and 11 years, lung function was studied using whole-body plethysmography. Lung function data was interpreted as trajectories of specific airway resistance, and children were assigned to wheezing phenotypes according to TCRS classification (173). Irrespective of wheezing phenotype, specific airway resistance increased significantly with ageing. Such increase was faster in males and in those with persistent wheeze or atopy; in addition, increase in specific airway resistance accelerated with increasing number of asthma exacerbations. Specific airway resistance was higher in children with any phenotype of wheeze than in those without wheeze; however, there was no progression over time in children with transient or late-onset wheeze. Hospital admissions due to wheezing and severe asthma exacerbations during the first three years of life showed an association with consistently higher specific airway resistance (173).

Later, PIAF, ALSPAC and MAAS follow-ups were combined to ascertain FEV1 trajectories from five to 16 years of age, and the authors were able to identify four FEV1 trajectories: persistently high, normal, below average and persistently low trajectories (34). The study produced several important findings. Post-bd FEV1 and FEV1/FVC were in line with baseline measurements, which suggests an irreversible component of lung function decline. The persistently low trajectory was associated

with asthma and, in addition, with early-life factors that included severe, recurrent wheeze, atopic sensitisation and exposure to tobacco smoke. In relation to VmaxFRC, the optimal solution for prediction of FEV1 was classifying it into three trajectories: above average, normal and below average. As expected, lower VmaxFRC was associated with below average FEV1 trajectory; however, only 25% of participants with low VmaxFRC showed constantly low values. This implies that most children with low expiratory flows in infancy transitioned into higher trajectories later in childhood (34).

Environment and Childhood Asthma (ECA) Study

The ECA study is a Norwegian birth cohort study that included 3,754 healthy infants, 802 of whom underwent infant lung function testing during the first five days of life (31). That study evaluated lung function development without classifying children into wheezing phenotypes, and with the primary aims of assessing whether the tracking of lung function was already present in the first years of life (174), and whether LRTIs impact on lung function development at school age (31). Reduction in infant respiratory compliance was associated with presence of LRTIs during the first two years of life (31).

Longitudinal findings of lung function were based on expiratory flows, measured with tidal flow-volume loops in infancy and at two years of age, and in addition, with forced flow-volume loops at ten years of age (31, 174). At the age of two years, recurrent wheezing and atopy were associated with reduced expiratory flows, but after adjustments, infant lung function was the only independent predictor of tidal expiration (174). This suggests that most airflow deficits in early childhood originated before birth or in the very first days of life. The presence of LRTIs during the first two years of life showed no association with change in lung function from birth to 10 years of age. In addition, the presence of LRTIs was not related to lung function at the age of ten years in analyses adjusted for other possible predictors including infant lung function and current asthma (31).

2.7.2 Hospital cohorts: Post-bronchiolitis lung function

Hospital cohort studies evaluate outcomes after severe bronchiolitis treated in hospital, which is an important difference compared to birth cohort studies with small numbers of hospitalised children.

Eight previous post-bronchiolitis studies have reported lung function outcomes (10-17), and all have found an association between bronchiolitis and abnormalities in lung function. In five cohorts, children were hospitalised at the age of less than 12 months and the majority of cases were RSV positive (12, 13, 15-17). Norwegian and Swedish follow-ups measured post-bd spirometry, and both found significant associations between bronchiolitis and irreversible lung function changes with measurements at early school age (12), in early adolescence (15) and in early adulthood (21). In the Swedish study, post-bronchiolitis lung function was studied separately in asthmatic and non-asthmatic subjects at the age of 18 years, and both groups showed irreversible reductions (21). In addition, a study from Missouri, US reported significant decline in post-bd lung function from 5 to 7.6 years of age after RSV bronchiolitis (17).

In three other bronchiolitis cohorts (10, 11, 14), hospitalisation age was less than 24 months, viral aetiology of bronchiolitis was more variable, and lung function outcome was less concordant. RSV was the predominant causative agent only in the Finnish cohort study, which recruited subjects during an RSV epidemic (175). In that study, post-bd lung function was measured at 30 years of age, and the association between bronchiolitis and irreversible changes remained significant after excluding asthmatic patients (10). In contrast, another Finnish study found no significant associations between bronchiolitis and post-bd spirometry at 18 years of age (11), and post-bd spirometry was decreased only in asthmatic patients in a Swedish post-bronchiolitis study at that same age (14).

2.7.3 Hospital cohorts: Outcome at the age of 10-13 years

Five previous post-bronchiolitis cohort studies have reported lung function results including measurements at 10-13 years of age (13, 15-17, 176). In four of them, the subjects had been hospitalised at the age of less than 12 months for bronchiolitis mainly caused by RSV (13, 15-17). In one study, the viral aetiology was more heterogenous and hospitalisation age was less than 24 months.

Post-bronchiolitis study from Nottingham, UK

In total, 101 children who were hospitalised for bronchiolitis at the age of less than 12 months were included in the oldest post-bronchiolitis follow-up from the UK, and RSV comprised 66% of viral aetiology (13). Baseline lung function was studied with spirometry at the age of ten years, and 61 former bronchiolitis patients

had lower baseline FEV1 and MEF50, more asthma diagnoses and more episodes of cough and wheezing when compared to 47 controls (13).

Post-bronchiolitis study from Bergen, Norway

The Norwegian study enrolled 131 children who were hospitalised for bronchiolitis at the age of less than 12 months (16). In that study, 74% of cases were RSV positive, and baseline lung function from 108 former bronchiolitis patients was reported at the age of 11 years with special focus on viral aetiology of bronchiolitis (16). Compared to 89 controls, former bronchiolitis patients showed lower FEV1, FEV1/FVC ratio and MEF25-75; however, after stratified analyses, the associations remained significant only in 25 children with RSV negative bronchiolitis. Only the RSV negative group showed higher prevalence of asthma compared to control group (16).

In the same post-bronchiolitis follow-up, lung function of 53 former bronchiolitis patients and 31 controls was re-evaluated at the age of 18 years, and findings were reported as changes in baseline spirometry from 11 to 18 years of ages (177). Lung function trajectories did not differ between the bronchiolitis group and controls: trajectories of FVC, FEV1, FEV1/FVC ratio and MEF25-75 in former bronchiolitis patients showed no decline or increase compared to those of controls. Instead, former bronchiolitis patients had lower FEV1, FEV1/FVC ratio and FEV25-75 at 11 and at 18 years of ages (177). Findings suggest that the difference between the groups developed at earlier ages.

Post-bronchiolitis study from St. Louis, Missouri, USA

The study from Missouri, US reported longitudinal trajectories of lung function including measurements with spirometry at the ages five, six, 7.6 (mean) and 12.1 (mean) years (17). Originally, this study included 206 children who were hospitalised for RSV bronchiolitis at the age of less than 12 months, 122 of whom performed spirometry on at least two follow-up visits. In this study, baseline FEV1 and FVC trajectories were characterised by a linear increase until the age of 7.6 years followed by a significant decline up to the age of 12.1 years, and baseline FEV1/FVC trajectory decreased significantly between five and 7.6 years of age. Significant reductions in FEV1, FVC and FEV1/FVC trajectories across the timeline were also observed in a subgroup of children with post-bd measurements available, but none of the participants performed post-bd spirometry at the mean age of 12.1 years (17). Findings of that study reflects mainly lung function outcome in post-bronchiolitis asthma, since the majority of patients had asthma diagnosis and nearly two thirds of

them were symptomatic. However, the findings may suggest progressive pattern of lung function decline in asthmatic patients hospitalised for RSV bronchiolitis during the first year of life.

Post-bronchiolitis study from Borås, Sweden

The Swedish study is the only previous post-bronchiolitis follow-up with measurements of post-bd spirometry in early adolescence. This study recruited 47 children with RSV bronchiolitis at the age of less than 12 months, and baseline and post-bd spirometry was first measured at the age of 13 years in 44 participants (15). At that age, former bronchiolitis patients showed lower FEV1/FVC values before and after bronchodilation in comparison to 86 age- and sex-matched controls. Specifically, current asthma (cases and controls combined) was associated with baseline and post-bd FEV1/FVC, and asymptomatic cases showed lower baseline FEV1/FVC than asymptomatic controls (15).

Follow-up of the Swedish cohort continued until 18 years of age (21). At that age, baseline FEV1 and baseline and post-bd FEV1/FVC and MEF25-75 were lower in 46 participants with former RSV bronchiolitis compared to 92 controls, and asthmatic and non-asthmatic cases presented with post-bd reductions (21). The findings suggest irreversible changes of lung function in children hospitalised for RSV bronchiolitis during the first living year. Crucially, the irreversible pattern was already present in adolescence and remained up to early adulthood.

Post-bronchiolitis study from Kuopio, Finland

The Finnish cohort study reported lung function results at the ages of seven, 12 and 18 years (11, 176, 178), only the last of which was controlled and included post-bd measurements (11). The cohort included 100 children who were hospitalised for bronchiolitis at the age of less than 24 months, and RSV and rhinovirus aetiologies both comprised around one third of cases (162, 179). At seven years of age, lung function was measured in 79 children, applying the lower limits of normality <80% for FEV1, <88% for FEV1/FVC and <62% for MEF50 of height-related predicted values. Reduced baseline FEV1, FEV1/FVC and MEF50 were present in 5%, 16% and 18% of participants, respectively (178).

The same limits were used to define abnormal lung function at the age of 12 years; at this time, baseline FEV1, FEV1/FVC and MEF50 reductions were reported in 18%, 16% and 16% of 80 children, respectively (176), reflecting increasing rates of baseline FEV1 reductions between the ages of seven and 11 years. At the age of 12 years, rhinovirus bronchiolitis was associated with lower baseline FEV1/FVC

values, and children with RSV bronchiolitis showed lower FVC values, suggesting restrictive pattern of lung function reduction (176).

The most recent lung function measurements at the age of 18 years showed lower baseline MEF50 in 49 former bronchiolitis patients compared to controls; however, other baseline values, or any post-bd values, did not differ between the groups. By viral aetiology, rhinovirus positivity was associated with lower MEF50 values and higher bronchodilator responses, and RSV positivity with lower FVC values. Interestingly, this study found no associations between early-life wheezing and post-bd spirometry, even though follow-up continued until adulthood (11).

2.7.4 Previous findings in the present follow-up

In the present post-bronchiolitis follow-up, baseline and post-bd lung function were measured previously at the age of 5-7 years with IOS, and acceptable data were available from 103 children (18). The results were reported as z-scores calculated from height-adjusted predicted values, and pathological limits were defined as $\geq$+1.65 for Rrs5 and $\leq$-1.65 for Xrs5. Pathological IOS was reported in 20% of children with accentuation on Xrs5. Post-bd IOS was pathological only in one child, and 11% of children showed a significant ($\geq$35%) decrease in Rrs5 after bronchodilation, implying mainly reversible obstruction at preschool age after early-life bronchiolitis. Baseline or post-bd IOS did not differ between children with or without regular ICS use (18).

In the present cohort, 13% of children had current asthma at the age of 5-7 years, in addition, 14% of children without current asthma had used ICS medication for asthma before 5-7-year follow-up visit, meaning 27% prevalence of preschool-age asthma (180). The risk factors for current asthma at preschool age were early-life atopy, non-RSV aetiology and maternal asthma (180). At the age of 10-13 years, current asthma was present in 13% of former bronchiolitis patients, and risk factors for asthma were maternal asthma and presence of allergic rhinitis at the age of 5-7 years (181). Viral aetiology of bronchiolitis showed no significant associations with asthma at the age of 10-13 years (181).

2.8 Predictive factors of post-bronchiolitis lung function

2.8.1 Exposure to smoking

Exposure to smoking prenatally or in infancy is associated with increased risk of LRTIs and wheezing (127, 182). Findings on associations between tobacco smoke exposure and infant lung function is not concordant (127), but several studies have reported significant associations concerning reductions in expiratory flows (30, 183, 184), in measures of tidal breathing (185), and in respiratory system compliance (186). In a study from Indiana, Canada, 76 healthy infants underwent measurements of expiratory flows and airway reactivity; interestingly, tobacco smoke exposure was associated with reduced lung function but not with increased airway reactivity (187).

In general, results on the association between tobacco smoke exposure and lung function later in childhood varies (127). Findings from birth cohort studies suggest an impact of smoke exposure on lung function especially at preschool years (184, 188, 189), with weakening effect with ageing (189). In follow-ups that have continued until adulthood, the adverse effects of smoke exposure on lung function increased with own personal smoking, suggesting persistent susceptibility to tobacco smoke, or alternatively, cumulative inhibitory consequences (125, 190).

In post-bronchiolitis studies, connections between smoke exposure and lung function have been variable. In the Finnish post-bronchiolitis study, the risk of reduced FEV1 at seven years of age was 12.8-fold in children whose mothers smoked during pregnancy (178). However, the association between maternal smoking and lung function was lost at follow-up until 11 years of age (176). In another Finnish post-bronchiolitis follow-up, even 60% of those who were exposed to maternal smoking in infancy had abnormalities in lung function at the age of 18-20 years (191). In a Swedish study, results were reported separately for prenatal and postnatal smoke exposure in 55 former bronchiolitis patients at 17-20 years of age (192). Prenatal smoke exposure was associated with lower post-bd FEV1/FVC ratio and airway hyper-responsiveness; in contrast, postnatal smoke exposure had no impact on spirometry findings but increased the risk of own personal smoking (192). The authors suggested that maternal smoking during pregnancy may induce airway hyper-responsiveness, leading to development of adult asthma and lung function abnormalities, and passive smoking may predispose to asthma as a result of active smoking (192). In our present cohort study, tobacco smoke exposure during

pregnancy or infancy was not associated with lung function at 5-7 years of age (18), or post-bronchiolitis asthma at 5-7 or 10-13 years of ages (180, 181).

2.8.2 Effects of overweight

A relationship between overweight and lung function is well recognised in adults. Overweight adults tend to have decreased functional residual capacity and expiratory reserve volume; instead, total lung capacity has been diminished only in massively obese patients (193, 194). In spirometry, most adult studies have documented reduced FEV1 and FVC values, but FEV1/FVC has been normal (193-195). The proposed mechanisms for these changes are chest-wall adiposity and relocation of diaphragm due to abdominal fat that decreases lung volumes without changed ability to inflate or deflate the lungs (194).

In children, available literature suggests reductions in baseline (43, 196) and post-bd spirometry (197, 198). In a large meta-analysis from year 2017, the most commonly detected spirometry finding in children and adolescents was reduced FEV1/FVC with normal or even increased FEV1 and FVC (195). This can be related to airway obstruction or, alternatively, overweight may trigger a dysanaptic increase in lung growth in relation to airway growth (40, 195). A study from Pennsylvania, US summarised lung function results of 4,521 children and adolescents aged 6-20 years from six different cohort studies, and airway dysanapsis was defined as low FEV1/FVC with normal to high FVC and normal FEV1 (41). In that study, obesity was associated with dysanapsis, and in obese asthmatic children, the presence of dysanapsis was associated with more severe disease (41). There is evidence on reductions of FEV1 and FVC in overweight children (199, 200), suggesting that restrictive pattern of lung function may exist as in adults.

Overweight is associated with increased risk of wheezing symptoms and asthma (201, 202). In asthmatics, overweight is related to lower responsiveness to ICSs (203, 204) and more severe disease (205). Obesity-associated childhood asthma may be modified by age at asthma onset: the early-onset (<12 years) phenotype was characterised by higher IgE levels, lower FEV1/FVC values, greater airway reactivity, greater bronchodilator responsiveness, and more frequent emergency visits compared to late-onset phenotype (≥12 years) (206). Some genes have been identified as regulators in both asthma and overweight (40). Interestingly, genetic variations of asthma-associated locus were related to body mass index (BMI) only in

asthmatic patients (207). In addition, asthma may predispose children to excessive weight gain, possibly due to limitation of physical activity (40).

There are some findings on association between overweight and lung function in adolescence after infant bronchiolitis. In a Finnish study, 27 overweight or obese and 53 normal weight former bronchiolitis patients were compared at the median age of 12.3 years, and overweight and obesity were defined as standard deviation (SD) scores for BMI of >1.3 SD and >2.0 SD, respectively (43). The risk of reduced baseline FEV1/FVC was 4.0-fold in overweight and 8.1-fold in obese children, but overweight or obesity did not show associations with decreased FEV1 or FVC and post-bd measurements were not performed (43). In the post-bronchiolitis study from Missouri, US, higher BMI percentile by the age of six years was associated with increase of height-adjusted FEV1 and FVC from five to 16 years of age, but not with FEV1/FVC (17).

In the present post-bronchiolitis follow-up at the age of 5-7 years, weight status was evaluated as zBMIs, and the authors used national cut-off limits corresponding to BMI >25 kg/m^2 for overweight and BMI >30 kg/m^2 for obesity in adults. Significant but low-to-modest correlations were found between zBMI and baseline and post-bd IOS variables measured from 99 children (44). In addition, overweight showed an association with preschool-age asthma (208).

2.8.3 The role of Toll-like receptors

TLRs are a group of transmembrane glycoproteins that are involved in innate immunity and further adaptive responses by recognising pathogens. These recognition receptors are expressed in numerous cell types, including cells in the respiratory system, and each of them senses a specific ligand derived from pathogens. Besides activation of host defence in infections, TLRs also play a role in non-infectious airway diseases (83). In humans, ten functional TLRs have been recognized: TLRs 1, 2, 4, 5, 6 and 10 act in the plasma membrane, while TLRs 3, 7, 8 and 9 are located intracellularly in the endoplasmic reticulum (209).

Genetic mutations of TLR encoding genes may lead to altered TLR function (83). Those mutations where one base is replaced with another base during replication are referred to as single-nucleotide polymorphisms (SNP). This dissertation includes data on SNPs of genes encoding the TLR2 subfamily, TLR4 and TLR7. There are no other post-bronchiolitis follow-ups that have evaluated the associations between *TLR* gene variations and lung function.

The TLR2 subfamily consists of TLRs 1, 2, 6 and 10, which share common mechanisms of innate immunity sensing (210, 211) and are able to recognise pathogens of respiratory infections including RSV and rhinoviruses (212-214). Of the TLR2 subfamily members, TLR1 and 6 form functional heterodimers with TLR2 (TLR2/1, TLR2/6) and may not work as homodimers (210). TLR10 is able to act without TLR2, but it can form heterodimers with TLR1 and TLR2 (TLR10/1, TLR10/2) (215). TLR10 is the only human TLR that has an inhibitory function on immune responses and inflammation (216-218). TLR4 is able to recognise the membrane protein F of RSV (219). TLR7 recognises single-stranded ribonucleic acid (RNA) of viruses, including RNA of RSV and rhinoviruses (83).

TLR polymorphisms are associated with development of asthma and allergic diseases in children (220), but findings are not concordant (221), and the effect may require certain environmental factors or childhood infections (221, 222).

The data on associations between *TLR* polymorphisms and lung function are limited. There is some evidence of such relations in *TLR1* rs5743618, *TLR2* rs5743708, *TLR4* rs4986790, *TLR6* rs5743810, *TLR7* rs179008 and *TLR10* rs4129009, which were included in the present dissertation. A Canadian study reported association between *TLR2* rs5743708 polymorphism and better lung function in 275 swine operation workers, but such association was not seen for *TLR4* rs4986790 (223). A Belgian study evaluated 34 SNPs in relation to lung function in 89 cystic fibrosis patients (224), four of which are included in the present dissertation. The authors found association between *TLR2* rs5743708 polymorphism and FEV1 decline, but findings on *TLR4* rs4986790, *TLR6* rs5743810 and *TLR10* rs4129009 were negative (224). In 110 Dutch patients with moderate or severe COPD, *TLR4* rs4986790 and *TLR*2 rs5743708 polymorphisms showed no associations with lung function even though some other SNPs of *TLR2* and *TLR4* were associated with the level and decline of FEV1 (225). In addition, there is evidence on association between *TLR4* rs4986790 polymorphisms and reduced COPD progression via decreased reactivity of those microbial components that are recognised by TLRs during infections (83).

Previously in the present study, *TLR4* rs4986790, *TLR6* rs5743810 and *TLR7* rs179008 showed exploratory associations with IOS measured at 5-7 years of age (226), while *TLR1* rs5743618 and *TLR10* rs4129009 were associated with post-bronchiolitis asthma at 5-7 and 10-13 years of ages (227-229).

2.8.4 Risk factors for irreversible airway obstruction

Five other post-bronchiolitis follow-ups have found an association between infant bronchiolitis and post-bd FEV1, FEV1/FVC or MEF50 (12, 14, 17, 21, 230), and most of them showed a link between current post-bronchiolitis asthma and irreversible reductions (14, 17, 21). Three studies evaluated risk factors other than asthma (14, 17, 230). A Finnish post-bronchiolitis study evaluating lung function outcomes at the age of 28-31 years defined irreversible airway obstruction as reduced post-bd FEV1/FVC and, in that study, parental asthma and blood eosinophilia during bronchiolitis increased the risk for irreversible airway obstruction after bronchiolitis (230). In a study from Missouri, USA, lung function after bronchiolitis was reported as trajectories through school age and loss of lung function was associated with male gender, asthma diagnosis by age six and atopy; however, post-bd measurements were rare (17). In a Swedish post-bronchiolitis study, early-life and current risk factors were assessed for reduced FEV1, FEV1/FVC ratio, MEF50 or MEF25 at the age of 17-20 years (14). In that study, female gender and prenatal smoke exposure increased the risk for post-bd abnormality after bronchiolitis; however, prenatal smoke exposure was not a significant risk factor after adjusting with current confounders including asthma, atopy, hyper-responsiveness and active smoking (14).

3 AIMS OF THE STUDY

The principal aims of the present study were to evaluate lung function in children aged 10-13 years after bronchiolitis in infancy and to identify factors predicting lung function at that age. The specific aims were:

1. To evaluate baseline and post-bronchodilator lung function measured with flow-volume spirometry in a controlled setting at the age of 10-13 years after hospitalisation for bronchiolitis at less than six months of age (Article I).

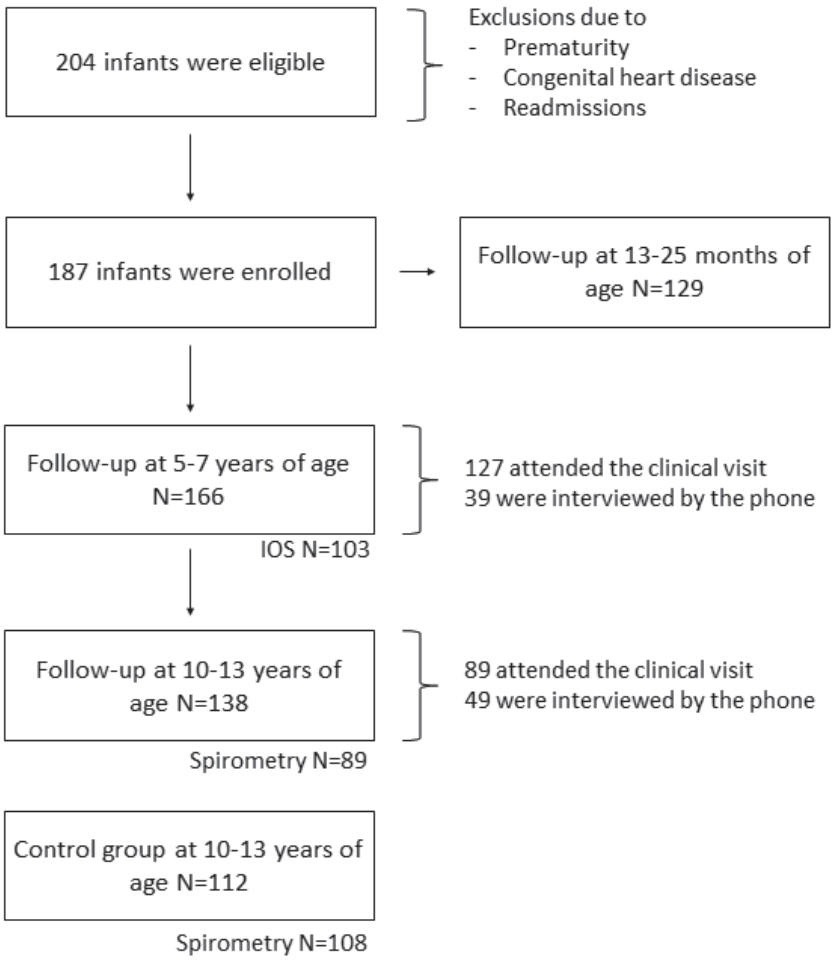
2. To investigate effects of overweight or obesity at the age of 10-13 years on lung function abnormalities by spirometry in former bronchiolitis patients (Article II).

3. To explore the role of single nucleotide polymorphisms in the genes encoding TLRs 1, 2, 6 and 10 (TLR2 subfamily), and TLR4 and TLR7 in post-bronchiolitis lung function at 10-13 years of age (Articles III, IV).

4. To identify early-life and preschool-age risk factors for irreversible airway obstruction, assessed with reduced post-bd FEV1 or post-bd FEV1/FVC, at 10-13 years of age after infant bronchiolitis (Article V).

4 MATERIALS AND METHODS

Figure 6. Study subjects



4.1 Patient enrolment and previous follow-ups

4.1.1 Infant enrolment and hospitalisation data

Originally, 204 infants with hospitalisation for bronchiolitis were eligible for this study between 1st December 2001 and 31st May 2002, and between 28th October 2002 and 31st May 2004 in the Department of Paediatrics, Tampere University Hospital, Finland. In all, 187 children who were less than six months of age, born full-term and previously healthy, and who had been hospitalised for their first episode of acute LRTI, were enrolled to the study. The diagnosis of bronchiolitis was clinical and based on presence of respiratory tract infection associated with clinical findings of rhinitis, cough, tachypnoea and crackles or wheezes on auscultation.

Nasopharyngeal aspirates were obtained from all infants during hospitalisation to determine viral aetiology of bronchiolitis. Indirect immunofluorescence was used to identify RSV, influenza A and B virus, parainfluenza type 1, 2 and 3 virus and adenovirus from fresh samples. Reverse-transcription PCR was performed to supplement the findings from frozen samples for the same seven viruses, as well as for rhinovirus, metapneumovirus and human bocavirus.

Clinical data registered during the hospitalisation included age on admission, length of hospital stay, need for oxygen and need for feeding support. None of the study subjects needed intensive care. A structured questionnaire was used to record family history of asthma and allergies as well as exposure to tobacco smoke during pregnancy or infancy.

4.1.2 Follow-up visit at the age of 13-25 months

The first follow-up visit was arranged from May 2003 to June 2005, and 129 children attended at a mean age of 18 months. A paediatrician performed clinical examination, and symptom diaries completed at home were reviewed during the control visit. The diaries included records of doctor-diagnosed respiratory infections and wheezing episodes as well as use of ICS after bronchiolitis during the first 18 months of life.

4.1.3 Follow-up visit at the age of 5-7 years

The second follow-up visit at the age of 5-7 years was arranged in October 2008, in January and March 2009, and in October 2009. In all, 166 children attended; 127 children attended the clinical examination, and 39 families answered only the questionnaire. The structured questionnaires were sent to homes before follow-up visits, and they were completed, if needed, together by the study physician and the families during the follow-up visit or by phone. The questionnaire charted family history of asthma, allergies and tobacco smoking, previous and current asthma-presumptive symptoms, allergic diseases and doctor-diagnosed asthma in children, and in addition, previous or current use of bronchodilators or ICSs. At the age of 5-7 years, skin prick tests were performed on 124 children, and the test panel included eight airborne allergens: birch pollen, timothy grass pollen, mugwort pollen, cat dander, dog dander, spores of the mould *Alternaria alternate* and house dust mites *Dermatophagoides pteronyssimus* and *D. farinae*. Skin prick tests were regarded positive if the diameter of the wheal was 3 mm or more.

4.1.4 Lung function by impulse oscillometry at the age of 5-7 years

Lung function was measured by IOS at the 5-7-year follow-up. In all, 107 children performed IOS, but two children were excluded from the analyses due to technical reasons and two more children due to insufficient co-operation. Thus, IOS results were available from 103 preschool aged children. Antihistamines had to be interrupted five days in advance and short-acting beta agonists 12 hours before lung function measurements. ICS medication, if present, was not interrupted.

IOS was performed at the Department of Clinical Physiology, Tampere University Hospital, using impulse oscillometer (Master Screen IOS; Jaeger, Hochberg, Germany). IOS was registered during quiet tidal breathing, with the child sitting still in an upright position. The nose was sealed with a clip, and the technician supported cheeks with hands to minimise pressure loss from upper airways. An experienced clinical physiologist checked the output curves, and measurements were repeated until three acceptable curves were obtained. Only curves that were graphically solid and artefact-free for the whole recording time of 30 seconds were accepted and the curves with best coherence reflecting the reliability of IOS were

chosen (coherence limit >0.6 at 5 Hz and >0.9 at 10 Hz). The frequencies of the input signals in the present study were 5-20 Hz.

Three separate IOS recordings were obtained from each child. First, baseline measurements were registered at the beginning of the protocol. Thereafter, children underwent free-running exercise challenge test after which IOS measurements were registered again. Bronchodilation test was performed after the exercise challenge test. In bronchodilation test, the children received 0.1 mg salbutamol (Ventoline®, GlaxoSmithKline, Brentford, London, UK) three times through a spacer (Babyhaler®, GlaxoSmithKline, Brentford, London, UK), and post-bd parameters were registered 15 minutes after bronchodilator administration. The parameters in IOS measurements were reported as z-scores from national, height-adjusted reference values (231).

4.2 Follow-up at the age of 10-13 years

4.2.1 Follow-up visit

The follow-up visit at the age of 10-13 years was arranged between 1st June 2014 and 31st January 2015. All 166 children who had participated in the control visit at the age of 5-7 years were invited. In all, 138 children attended; 89 attended the clinical examination, and 49 answered only the questionnaire. Structured questionnaires were sent before the follow-up visits, completed by the families, and checked, if needed, by the study physician together with the family or by phone, as at the 5-7 years visit. The questionnaires consisted of the same questions as five years earlier at the 5-7 years follow-up visits, supplemented with data on symptoms, diseases, diagnoses and treatments from six to 13 years of age. Weights and heights were measured for all children during the follow-up visit.

4.2.2 Flow-volume spirometry

Lung function was measured with flow-volume spirometry at the 10-13 years follow-up. All 89 children who attended the clinical examination underwent spirometry, and all 89 recordings were included in the analyses. Antihistamines had to be interrupted five days in advance and short-acting beta agonists 12 hours before lung function measurements. ICS medication, if present, was not interrupted.

Spirometry was performed at the Department of Clinical Physiology, Tampere University Hospital using a calibrated spirometer (Vmax™ Carefusion; Becton, Dickinson and Company, Franklin Lakes, NJ, USA). During the procedure, the child was in a sitting position and wearing a nose clip. Before the procedure, the technician demonstrated correct technique for forced expiratory flows. Children repeated the blows until three, artefact-free, similar and technically acceptable curves were received. Only curves with rapid rise from beginning to peak flow and descending curves lasting at least 6 seconds were accepted. The highest values of FVC and FEV1 were chosen for the analyses while the second highest values had to sit within 0.15 L of the highest value.

Two separate recordings of flow-volume spirometry were obtained from each child. First, baseline measurements were registered at the beginning of the protocol. Thereafter, children received 0.1 mg salbutamol (Ventoline®, GlaxoSmithKline, Brentfort, London, UK) four times and post-bd parameters were registered 15 minutes after bronchodilator administration.

4.2.3 Control group

At the 10-13 years follow-up, four controls for each of the 166 study subjects, totalling 664 control patients, matched by age and sex, were collected from the population register of the Pirkanmaa (Tampere) Hospital District and invited to the clinical visit. Exclusion criteria were hospitalisation for any medical reason and any outpatient treatment for lower respiratory tract illnesses, including bronchiolitis, in infancy. In all, 112 control patients attended the follow-up visit and 108 of them performed flow-volume spirometry.

4.3 Genetics

4.3.1 Genetic methods

Genetic studies were performed from whole blood samples taken during hospitalisation for clinical reasons and frozen for further studies. If such samples were missing, supplementary samples were collected during the follow-up visits. In all, frozen samples for genetic studies were available from 135 children.

The present dissertation includes analyses of SNPs of *TLR1* rs5743618, *TLR2* rs5743708, *TLR4* rs4986790, *TLR6* rs5743810, *TLR7* rs179008 and *TLR10* rs4129009 genes. These SNPs were chosen based on previously found associations with asthma at 5-7 and 10-13 years of age and with impulse oscillometry at 5-7 years of age in this post-bronchiolitis cohort study (226-229).

Deoxyribonucleic acid (DNA) extraction from whole blood was performed using a commercial kit (Qiagen QiAmp DNA blood Mini Kit 250, Qiagen Inc., Hilden, Germany), and genotyping of *TLR* polymorphisms were performed at the Department of Clinical Microbiology in Tampere University Hospital, at the Department of Medical Microbiology and Immunology in Turku University Hospital, and at the National Institute of Health and Welfare in Turku, Finland.

4.3.2 Genotyping of *TLR1, 2, 4, 6, 7* and *10* polymorphisms

TLR1 rs5743618, *TLR2* rs5743708 and *TLR6* rs5743810 genotyping were performed by pyrosequencing with the PSQ™96MA Pyrosequencer (Biotage, Uppsala, Sweden). PSQ™96 Pyro Golf Q96 reagent kit was used for recognising PCR products with potential SNPs according to manufacturer protocol.

TLR4 rs4986790 genotyping was performed by pyrosequencing with the ABIPRISM 7000 Sequence Detection System (Applied Biosystems, CA). Genotyping was later supplemented with the PSQ™96MA Pyrosequencer (Biotage, Uppsala, Sweden) with the use of PSQ™96 Pyro Golf Q96 reagent kit.

TLR7 rs179008 genotyping was performed by PCR-based sequencing. First, the PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to manufacturer protocol. After purification, PCR products were pipetted into a 96-well plate together with *TLR7* rs179008 forward primer, and this plate was sent for final sequencing to the Institute for Molecular Medicine laboratory in Helsinki, Finland.

TLR10 rs4129009 genotyping was performed by high resolution melting analysis with the Light Cycler 480 (Roche Diagnostics, Basel, Switzerland). PCR reactions were run at 95 ºC for 10 min following 45 cycles of amplification at 95 ºC for 10 secs, at 60 ºC for 10 secs and at 72 ºC for 15 secs. After completing the PCR process, the final melting cycle conditions were performed according to manufacturer protocol and known *TLR10* rs4129009 standards were used at each run.

4.4 Definitions

In the flow-volume spirometry, FVC, FEV1, FEV1/FVC and MEF50 were reported as percentages of population-based, sex-specific, height-related references (percentages of predicted) (232). Bronchodilation test results were reported as changes in FEV1. The findings of flow-volume spirometry were considered pathological with the following limits: FVC ≤81% for boys and ≤82% for girls, FEV1 ≤80% for boys and ≤82% for girls, FEV1/FVC ≤87% for boys and ≤88% for girls, and MEF50 ≤64% for boys and ≤63% for girls, corresponding to the lower 95% tolerance limits in the national reference data (232).

Weight and height data were transformed into age- and sex-specific, height-related body mass index z scores (zBMI) according to Finnish growth reference data. We used national zBMI cut-off values corresponding to threshold BMIs <17 kg/m^2 for underweight, >25 kg/m^2 for overweight and >30 kg/m^2 for obesity, respectively (233).

Current asthma was defined as current use of ICSs or, alternatively, as the presence of asthma-presumptive symptoms with FEV1 change of 12% and 0.2l or more in the bronchodilation test. Asthma-presumptive symptoms were repeated wheezing, prolonged cough or night cough over four weeks during the last 12 months. Persistent asthma was defined as the presence of physician-diagnosed asthma at 5-7 years of age and, in addition, current asthma at 10-13 years of age. Current ICS use was defined as regular use of ICSs during the last 12 months; similarly, current bronchodilator use was defined as regular use of bronchodilators during the last 12 months. Current atopic dermatitis and current allergic rhinitis needed to be symptomatic during the last 12 months. Skin prick test positivity was defined as at least one positive finding for eight airborne allergens tested at 5-7 years of age.

Irreversible airway obstruction was defined as pathological finding in post-bd FEV1 or post-bd FEV1/FVC measurement with FEV1 change of less than 12% in the bronchodilation test. Risk factor data on atopic dermatitis in infancy, presence of maternal or parental asthma, presence of maternal smoking during pregnancy or infancy, and previous physician-made asthma diagnoses from birth to 5-7 year control visit were based on structured questionnaires.

4.5 Statistical analyses

SPSS for Windows versions 23.0 and 25.0 (IBM Corp, Armonk, NY, USA) were used for the statistical analyses. Continuous variables were expressed as means, standard deviations (SD) and 95% confidence intervals (95%CI), and categorised variables as numbers and frequencies. Differences between groups in crude analyses were calculated with t-test for continuous variables and with Fisher's exact test or Chi-square test for categorised variables. The difference was considered statistically significant if the 95%CIs did not overlap, or alternatively, if the p value was less than 0.05.

Analysis of covariance (ANCOVA) was used in adjusted analyses for continuous variables and logistic regression in adjusted analyses for categorised variables. The results of ANCOVA were expressed as adjusted p values and the results of logistic regression as adjusted odds ratios (OR) and their 95%CIs. The used confounding factors were current asthma, maternal smoking in infancy, RSV aetiology of bronchiolitis and zBMI, when appropriate.

The adjusted correlations between zBMI and continuous spirometry parameters were calculated using multivariate linear regression analysis. Results of significant correlations were expressed as unstandardised coefficients (B) for zBMI, reflecting the degree of decrease or increase in spirometry parameter during one unit increase in zBMI. Adjusted R^2 presents the predictive strength of the linear regression model.

The normality of the continuous variables and residuals of linear regression analysis were estimated visually and using Kolmogorov-Smirnov and Shapiro-Wilkinson tests.

4.6 Ethics

The study was approved by the Ethics Committee of the Tampere University Hospital District. The enrollment phase and all control visits were processed separately by the Ethics Committee.

Participation in the study was approved by parents with written consent before enrollment and at all follow-ups at 18 months, 5-7 years and 10-13 years of ages. At the latest follow-up, participation was also approved by study subjects. Information about the control visit and structured questionnaire was sent to homes before each control visit, together with an invitation letter that included the information on

voluntary participation. The invited study subjects and controls could choose to attend the clinical study or to complete the questionnaire study only.

The use of genetic data was limited to genes potentially associated with asthma and lung function. Genetic data were assigned to laboratory in coded form, and laboratory workers received no other information about the study subjects. The personalised genetic data were stored carefully and separately from other research data, and access to this data was limited only to the principal investigator at each study phase.

5 RESULTS

5.1 Background factors

Follow-up data were available in 89 former bronchiolitis patients (cases) at the mean age of 11.6 (SD 0.9) years and from 108 controls at the mean age of 11.8 (SD 1.0) years, and 50.6% cases vs. 44.4% controls were girls.

Maternal smoking during pregnancy was present in 15.9% cases and in 2.8% controls ($p=0.001$), and the figures for maternal smoking during infancy were 27.0% and 5.6% ($p<0.001$), respectively. There were no significant differences between cases and controls in terms of presence of maternal asthma (11.2% vs. 13.0%, $p=0.734$). Current asthma was present in 13.5% of cases and in 11.1% of controls ($p=0.612$), and 9% of cases and 8.3% of controls had used ICSs during the last 12 months ($p=0.870$).

Viral data were available from 88/89 former bronchiolitis patients. RSV was positive in 71.6% of cases, rhinovirus in 11.4% of cases, and 13.6% of cases had other viruses. Multiple viruses were found in 9.1% of cases. Those 11.4% of cases without positive viral findings were added to the analyses as non-RSV bronchiolitis cases.

5.2 Post-bronchiolitis lung function at the age of 10-13 years (I)

Baseline and post-bd parameters of flow-volume spirometry were compared between cases who had been hospitalised for bronchiolitis at less than six months of age and age- and sex-matched controls without hospitalisation of any LRTIs in infancy. Parameters of the spirometry were expressed as percentages of population-based, sex-specific, height-related references (percentages of predicted).

5.2.1 Comparison to control data, crude analyses

Mean values and 95%CIs of baseline and post-bd FVC, FEV1, FVC/FEV1 and MEF50 were all within the normal limits. Post-bd FEV1/FVC was significantly lower in cases than in controls. There were no significant differences between cases and controls in other baseline or post-bd parameters as continuous variables (Table 1).

Table 1. Baseline and post-bronchodilator parameters in flow-volume spirometry in 89 former bronchiolitis patients (cases) and 108 controls, analysed as continuous variables.

		Cases		Controls	
Spirometry parameters		Mean (SD)	95%CI	Mean (SD)	95%CI
FVC	Baseline	95.9 (11.6)	93.4-98.3	96.5 (9.5)	94.7-98.3
	Post-bd	96.5 (11.9)	94.0-99.0	96.9 (8.8)	95.2-98.5
FEV1	Baseline	89.0 (11.4)	86.6-91.4	91.7 (9.6)	89.9-93.6
	Post-bd	92.4 (11.9)	89.9-94.9	95.9 (9.1)	94.1-97.6
FEV1/FVC	Baseline	93.3 (7.8)	91.6-94.9	95.2 (6.0)	94.0-96.3
	Post-bd	**96.1 (6.7)**	**94.6-97.5**	**99.0 (5.6)**	**98.0-100.1**
MEF50	Baseline	85.5 (21.3)	81.0-90.0	93.0 (22.6)	88.7-97.4
	Post-bd	97.4 (22.9)	92.6-102.2	105.7 (22.9)	101.3-110.0
Bronchodilation test					
FEV1 change	%	3.9 (6.3)	2.6-5.3	4.7 (4.3)	3.8-5.5

CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; MEF50, maximal expiratory flow at 50% of FVC; Post-bd, post-bronchodilator; SD, standard deviation. Parameters of spirometry are expressed as percentages of predicted values. Statistically significant findings are marked with bold font.

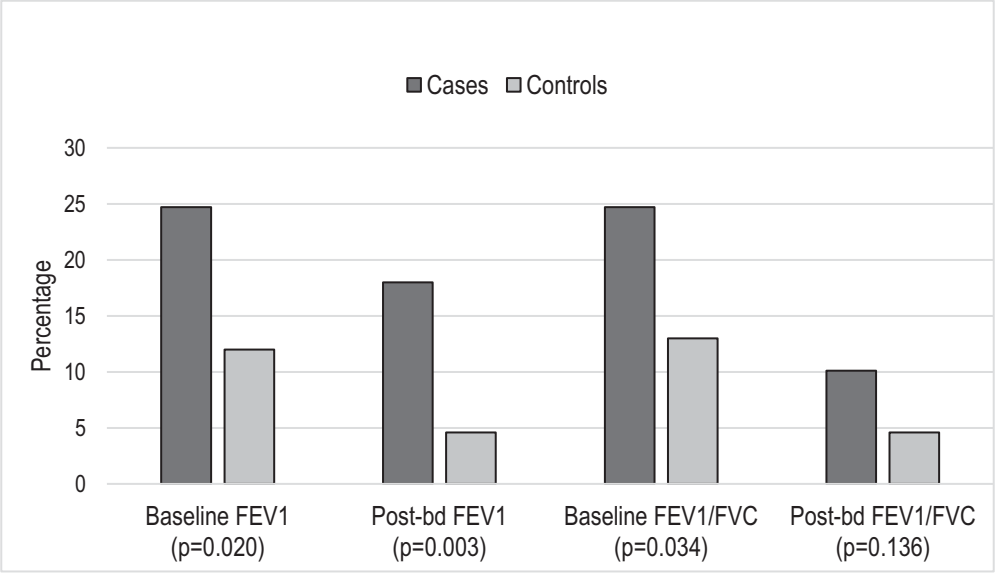
Baseline FEV1 was pathological in 24.7% of cases and 12.0% of controls (p=0.020), post-bd FEV1 was pathological in 18.0% of cases and 4.6% of controls (p=0.003) and baseline FEV1/FVC was pathological in 24.7% of cases and 13.0% of controls (p=0.034) (Table 2, Figure 7). There were no other significant differences between cases and controls in other baseline or post-bd parameters or in the bronchodilation test results (Table 2).

Table 2. Pathological findings in flow-volume spirometry in 89 former bronchiolitis patients (cases) and 108 controls, analysed as categorised variables.

		Cases	Controls	
Pathological parameters†		No (%)	No (%)	p value‡
FVC	Baseline	8 (9.0)	5 (4.6)	0.220
	Post-bd	9 (10.1)	6 (5.6)	0.230
FEV1	Baseline	**22 (24.7)**	**13 (12.0)**	**0.020**
	Post-bd	**16 (18.0)**	**5 (4.6)**	**0.003**
FEV1/FVC	Baseline	**22 (24.7)**	**14 (13.0)**	**0.034**
	Post-bd	9 (10.1)	5 (4.6)	0.136
MEF50	Baseline	16 (18.0)	11 (10.2)	0.113
	Post-bd	6 (6.7)	4 (3.7)	0.352
Bronchodilation test				
FEV1 change	≥12%	7 (7.9)	6 (5.6)	0.516

FEV1, forced expiratory volume in one second; FVC, forced vital capacity; MEF50, maximal expiratory flow at 50% of FVC; Post-bd, post-bronchodilator. Statistically significant findings are marked with bold font. †FVC ≤81% for boys and ≤82% for girls, FEV1 ≤80% for boys and ≤82% for girls, FEV1/FVC ≤87% for boys and ≤88% for girls and MEF50 ≤64% for boys and ≤63% for girls (232), expressed as percentages of predicted. ‡Chi-square test.

Figure 7. Pathological baseline and post-bronchodilator FEV1 and FEV1/FVC in cases and controls.

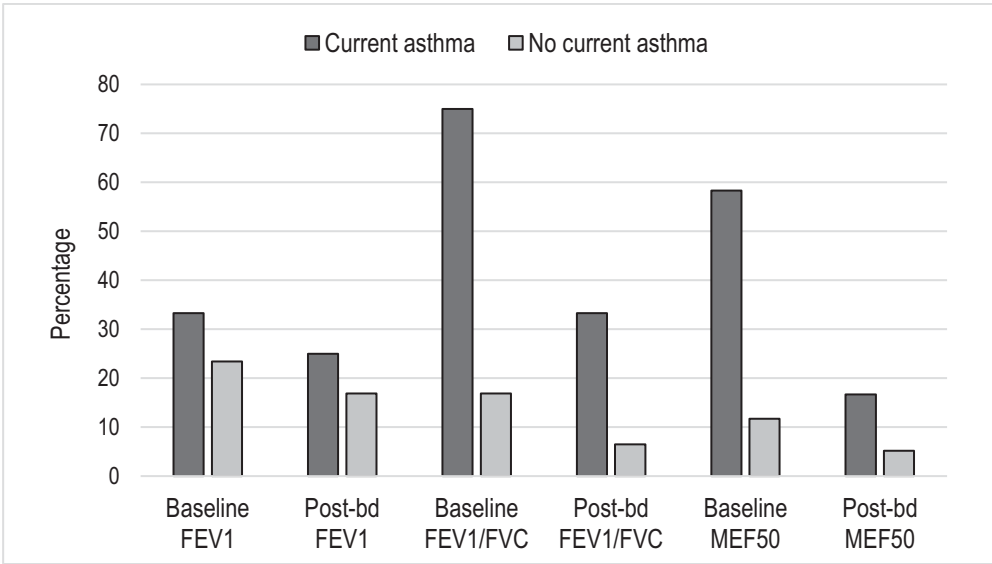


5.2.2 Current asthma, maternal asthma, exposure for smoking

When cases and controls were analysed in combination, there were more pathological findings in 24 children with current asthma compared to 173 without in baseline FEV1/FVC (58.3% vs. 12.7%, p<0.001), in baseline MEF50 (50.0% vs. 8.7%, p<0.001), and in bronchodilation test ≥12% (33.3% vs. 2.9%, p<0.001). No other baseline parameters nor any post-bd parameters showed significant associations with current asthma.

When the same analyses were performed within the bronchiolitis group, 12 cases with current asthma had pathological findings more often in relation to 77 cases without current asthma in spirometry. The figures were 75.0% vs. 16.9% (p<0.001) for baseline FEV1/FVC, 33.3% vs. 6.5% (p=0.017) for post-bd FEV1/FVC, 58.3% vs. 11.7% (p=0.001) for baseline MEF50, and 41.7% vs. 2.6% (p<0.001) for bronchodilation test ≥12% (unpublished data, Figure 8). Other spirometry parameters did not differ significantly between cases with or without current asthma.

Figure 8. Pathological baseline and post-bronchodilator FEV1, FEV1/FVC and MEF50 in 89 cases with or without current asthma.



There were no significant differences in the presence of pathological spirometry parameters between 24 cases or controls with and 173 without maternal asthma, nor between 30 cases or controls with and 167 without maternal smoking during infancy.

5.2.3 Comparison to control data, multivariate analyses

Logistic regression was used for multivariate analyses to compare pathological findings in spirometry between cases and controls. Since current asthma was associated with pathological baseline spirometry and maternal smoking during infancy was more common in cases than in controls, current asthma and maternal smoking during infancy were used as covariates in the analyses. Cases had a 2.4-fold risk (95%CI 1.1-5.3) of pathological baseline FEV1 and a 4.4-fold risk (95%CI 1.5-12.8) of pathological post-bd FEV1, but the risk of pathological FVC, FEV1/FVC or MEF50 did not differ significantly between cases and controls.

The same multivariate model was used to study pathological findings in spirometry in relation to the aetiology of bronchiolitis, and children with RSV and children with non-RSV aetiology were compared with controls. RSV bronchiolitis was associated with a 2.9-fold risk of pathological baseline FEV1 and with a 4.6-fold risk of pathological post-bd FEV1. Non-RSV bronchiolitis showed no significant associations with pathological findings in spirometry (Table 3).

Table 3. Multivariate logistic regression: Pathological baseline or post-bronchodilator parameters in flow-volume spirometry in 63 cases with RSV bronchiolitis and 25 cases with non-RSV bronchiolitis, compared to 108 controls.

		RSV bronchiolitis		**Non-RSV bronchiolitis**	
Pathological parameters†		N (%)	OR (95%CI)‡	N (%)	OR (95%CI)‡
FVC	Baseline	7 (11.1)	3.0 (0.9-10.3)	1 (4.0)	1.1 (0.1-10.8)
	Post-bd	8 (12.7)	2.8 (0.9-8.9)	1 (4.0)	1.0 (0.1-8.8)
FEV1	Baseline	**17 (27.0)**	**2.9 (1.2-6.7)**	5 (20.0)	1.7 (0.5-5.8)
	Post-bd	**12 (19.0)**	**4.6 (1.5-14.2)**	4 (16.0)	4.1 (0.9-18.7)
FEV1/FVC	Baseline	13 (20.6)	2.1 (0.8-5.6)	9 (36.0)	2.4 (0.8-7.6)
	Post-bd	6 (9.5)	2.1 (0.6-7.8)	3 (12.0)	2.1 (0.4-11.2)
MEF50	Baseline	10 (15.9)	2.3 (0.8-6.8)	6 (24.0)	1.4 (0.4-5.3)
	Post-bd	5 (7.9)	2.8 (0.7-11.5)	1 (4.0)	1.0 (0.1-10.3)
Bronchodilation test					
FEV1 change	≥12 %	5 (7.9)	2.6 (0.6-11.5)	2 (8.0)	1.0 (0.2-6.4)

FEV1, forced expiratory volume in one second; FVC, forced vital capacity; MEF50, maximal expiratory flow at 50% of FVC; Post-bd, post-bronchodilator. Statistically significant findings are marked with bold font. †FVC ≤81% for boys and ≤82% for girls, FEV1 ≤80% for boys and ≤82% for girls, FEV1/FVC ≤87% for boys and ≤88% for girls and MEF50 ≤64% for boys and ≤63% for girls (232), expressed as percentages of predicted. ‡Adjusted for current asthma and maternal smoking in infancy.

5.3 Weight status in relation to post-bronchiolitis lung function (II)

The analyses of 88 children with early-life bronchiolitis in relation to weight status (zBMI) included 65 (73.9%) normal weight, 20 (22.7%) overweight and three (3.4%) obese children. The mean age was 11.7 years and 44 (50.0%) were girls. One girl was excluded from the analyses due to underweight. Overweight and obese children were analysed as a combined group (overweight or obese) due to the small number of obese children. There were no significant differences between normal weight and obese or overweight children in the prevalence of current asthma or maternal smoking during infancy.

In crude analyses, overweight or obese children had lower baseline and post-bd FEV1/FVC than normal weight children. Other spirometry parameters did not show significant associations with overweight or obesity as continuous variables (Table 4).

Table 4. Baseline and post-bronchodilator parameters in flow-volume spirometry in 65 normal weight and 23 overweight or obese former bronchiolitis patients.

		Normal weight n=65		**Overweight or obese n=23**	
Spirometry parameters		Mean (SD)	95%CI	Mean (SD)	95%CI
FVC	Baseline	94.4 (11.3)	91.6-97.2	99.6 (12.0)	94.4-104.8
	Post-bd	95.4 (12.0)	92.4-98.4	99.2 (11.6)	94.2-104.2
FEV1	Baseline	88.9 (9.9)	86.5-91.4	88.4 (14.5)	82.1-94.7
	Post-bd	92.6 (10.9)	89.9-95.3	91.0 (14.3)	84.8-97.2
FEV1/FVC	Baseline	**94.7 (6.8)**	**93.0-96.4**	**88.9 (8.9)**	**85.0-92.7**
	Post-bd	**97.5 (6.2)**	**96.0-99.0**	**91.6 (6.5)**	**88.8-94.4**
MEF50	Baseline	87.7 (18.6)	83.1-92.3	78.2 (26.6)	66.7-89.7
	Post-bd	99.3 (22.2)	93.8-104.8	90.9 (24.1)	80.4-101.3

CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; MEF50, maximal expiratory flow at 50% of FVC; Post-bd, post-bronchodilator; SD, standard deviation. Parameters of spirometry are expressed as percentages of predicted. Statistically significant findings are highlighted with bold font.

As categorised variables, 23 overweight or obese children had pathological findings more often than 65 normal weight children in terms of baseline FEV1/FVC (52.2% vs 15.4%, p<0.001), post-bd FEV1/FVC (26.1% vs 4.6%, p=0.009), baseline MEF50 (34.8% vs 12.3%, p=0.026) and post-bd MEF50 (17.4% vs 3.1%, p=0.038). There were no significant differences in other spirometry parameters between overweight or obese and normal weight former bronchiolitis patients.

In multivariate linear regression, adjusted for current asthma and maternal smoking in infancy, zBMI showed modest correlations between decreasing baseline and post-bd FEV1/FVC. One unit increase in zBMI predicted 2.3% decrease in baseline FEV1/FVC and 2.5% decrease in post-bd FEV1/FVC. In addition, zBMI showed a low correlation with increasing baseline FVC, but the adjusted R^2, reflecting the predictive strength of the model, was only 2.8% (Table 5). There were no other significant correlations between zBMI and spirometry parameters.

Table 5. Multivariate linear regression: Association of zBMI with parameters of flow-volume spirometry in 88 former bronchiolitis patients.

Spirometry parameters		B for zBMI	p value for B	Pearson's R	Adjusted R^2
FVC	Baseline	2.9	0.033	0.25	0.028
	Post-bd	2.3	0.093	0.22	0.016
FEV1	Baseline	0.5	0.680	0.19	0.001
	Post-bd	-0.1	0.941	0.10	-0.025
FEV1/FVC	Baseline	-2.3	0.006	0.46	0.180
	Post-bd	-2.5	<0.001	0.50	0.227
MEF50	Baseline	-3.4	0.149	0.34	0.084
	Post-bd	-4.2	0.106	0.28	0.047

FEV1, forced expiratory volume in one second; FVC, forced vital capacity; MEF50, maximal expiratory flow at 50% of FVC; Post-bd, post-bronchodilator; zBMI, body mass index z-scores. Parameters of spirometry are expressed as percentages of predicted. B = coefficient B. Correlations (Crude R): Strong correlation: >0.60 or <-0.60, modest correlation: 0.3 to 0.6 or -0.3 to -0.6, low correlation: 0 to 0.3 or 0 to -0.3.

In multivariate logistic regression, overweight or obese children had a 5.4-fold risk (95%CI 1.7-17.8) of pathological baseline FEV1/FVC and a 5.6-fold risk (95%CI 1.2-26.4) of pathological post-bd FEV1/FVC compared to normal weight children. The risk of pathogical FVC, FEV1 or MEF50 did not differ significantly between overweight or obese and normal weight children.

5.4 Genetic variation of Toll-like receptors in relation to post-bronchiolitis lung function (III and IV)

Genetic data on *TLR1, TLR2, TLR4, TLR6,* and *TLR7* polymorphisms were available from 82 former bronchiolitis patients (50.0% girls), and on *TLR10* polymorphism from 81 former bronchiolitis patients (49.4% girls), who performed flow-volume spirometry at the mean age of 11.6 years. Current asthma was present in 10 (12.2%) children, maternal smoking during infancy was present in 24 (29.3%) children, and RSV was positive in 58 (70.7%) children. Mean zBMI was 0.28 (SD 0.97).

Genotypes and minor allele frequencies of the analysed *TLR* polymorphisms are presented in Table 6. Wild genotype refers those without the presence of minor allele and variant genotype refers those with the presence of minor allele. Variant homozygous and heterozygous genotypes were analysed in combination due to low numbers of variant homozygous genotypes. Genes encoding TLR7 are located in X-chromosome and, for that reason, *TLR7* genotypes were analysed separately for boys and girls. Current asthma, maternal smoking during infancy, RSV aetiology of bronchiolitis and current zBMI were used as confounding factors in adjusted analyses.

Table 6. *Toll-like receptor 1, 2, 4, 6, 7* and *10* genotypes and minor allele frequencies in 82 former bronchiolitis patients compared to Finnish population data.

SNP	Wild (Major/Major)	Variant (Major/Minor)	Variant (Minor/Minor)	MAF	FIN
TLR1 rs5743618	GG 0.76	GT 0.20	TT 0.05	0.15	0.17
TLR2 rs5743708	CC 0.94	CT 0.06	TT 0	0.03	0.03
TLR4 rs4986790	AA 0.84	AG 0.16	TT 0	0.09	0.12
TLR6 rs5743810	CC 0.31	CT 0.46	TT 0.23	0.46	0.42
TLR7 rs179008 Girls†	AA 0.56	AT 0.39	TT 0.05	0.32‡	0.31
TLR7 rs179008 Boys†	AA 0.81	AT 0	TT 0.20		
TLR10 rs4129009§	AA 0.82	AG 0.19	GG 0	0.09	0.08

FIN, minor allele frequency in the Finnish data of the 1000 Genome Project (234); MAF, minor allele frequency in the study population; SNP, single nucleotide polymorphism. †N=41 for girls and 41 for boys. ‡MAF is calculated including both girls and boys. §N=81 for TLR10.

5.4.1 TLR2 subfamily

Associations of *TLR1, TLR2, TLR6* and *TLR10* (encoding respective TLR2 subfamily members) wild and variant genotypes, and associations of genotype combinations within the TLR2 subfamily, were analysed in relation to post-bronchiolitis lung function.

There were no significant associations between *TLR1* rs5743618, *TLR2* rs5743708 or *TLR6* rs5743810 wild or variant genotypes and baseline or post-bd spirometry parameters.

Concerning *TLR2* rs5743708 and *TLR1* rs5743618 combinations, the difference between three possible combinations was significant in adjusted analyses for baseline FEV1/FVC. Children with *TLR2* variant and *TLR1* wild genotypes had the highest and children with *TLR2* wild and *TLR1* wild genotypes had the lowest baseline FEV1/FVC. The *TLR2* rs5743708 and *TLR1* rs5743618 or *TLR6* rs5743810 genotype combinations showed no other significant associations with spirometry parameters (Table 7).

Table 7. Combinations of *TLR2* rs5743708 and *TLR1* rs5743618 genotypes in relation to baseline and post-bronchodilator FEV1/FVC in 81 former bronchiolitis patients.

	***TLR2* wild** ***TLR1* wild N=58**	***TLR2* wild** ***TLR1* variant N=19**	***TLR2* variant** ***TLR1* wild N=4**	
FEV1/FVC	Mean (SD) [95%CI]	Mean (SD) [95%CI]	Mean (SD) [95%CI]	Adjusted p†
Baseline	92.6 (7.3) [90.7-94.6]	95.9 (8.2) 91.9-99.8	97.8 (6.7) [87.1-108.5]	**0.034**
Post-bd	95.8 (6.4) [94.1-97.4]	97.3 (6.9) [94.0-100.7]	100.0 (7.7) [87.7-112.3]	0.114

CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; Post-bd, post-bronchodilator; SD, standard deviation. Parameters of spirometry are expressed as percentages of predicted. Statistically significant findings are marked with bold font. Only one study subject had the combination of variant *TLR2* rs5743708 and variant *TLR1* rs5743618 genotypes. †ANCOVA, adjusted for current asthma, maternal smoking in infancy, RSV aetiology of bronchiolitis and zBMI (for definitions, see main text).

Concerning *TLR10* rs4129009, 15 former bronchiolitis patients with the variant genotype had significantly higher baseline and post-bd FEV1/FVC and baseline MEF50 in adjusted analyses than those 66 with the wild genotype. The *TLR10* rs4129009 showed no significant associations with other spirometry parameters (Table 8).

Table 8. Wild and variant genotypes of *TLR10* rs4129009 in relation to results of flow-volume spirometry in 81 former bronchiolitis patients.

		Wild (AA) N=66		**Variant (AG) N=15**		
Spirometry parameters		Mean (SD)	95%CI	Mean (SD)	95%CI	Adjusted p†
FVC	Baseline	96.7 (11.5)	93.9-99.5	92.6 (11.8)	86.1-99.1	0.102
	Post-bd	97.4 (12.1)	94.5-100.4	92.9 (11.0)	86.8-99.0	0.089
FEV1	Baseline	89.2 (11.2)	86.5-92.0	88.3 (11.8)	81.8-94.9	0.832
	Post-bd	93.0 (11.7)	90.2-95.9	89.8 (12.9)	82.7-96.9	0.361
FEV1/FVC	Baseline	92.4 (7.6)	90.5-94.2	97.4 (8.0)	93.0-101.9	**0.002**
	Post-bd	95.5 (6.6)	93.9-97.1	98.6 (7.1)	94.7-102.5	**0.011**
MEF50	Baseline	83.7 (20.7)	78.7-88.8	93.6 (21.4)	81.8-105.4	**0.035**
	Post-bd	95.8 (22.5)	90.3-101.3	105.9 (24.7)	92.2-119.5	0.065

CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; MEF50, maximal expiratory flow at 50% of FVC; Post-bd, post-bronchodilator; SD, standard deviation. Parameters of spirometry are expressed as percentages of predicted. Statistically significant findings are marked with bold font. None had the variant GG genotype. †ANCOVA, adjusted for current asthma, maternal smoking in infancy, RSV aetiology of bronchiolitis and zBMI (for definitions, see main text).

Concerning *TLR10* rs4129009 and *TLR1* rs5743618 combinations, the difference between three possible combinations was significant in adjusted analyses for baseline and post-bd FEV1/FVC. Children with *TLR10* variant and *TLR1* variant genotypes had the highest and children with *TLR10* wild and *TLR1* variant had the lowest baseline and post-bd FEV1/FVC. The *TLR10* rs4129009 and *TLR1* rs5743618 genotype combinations showed no significant associations with other spirometry parameters (Table 9).

Table 9. Combinations of *TLR10* rs4129009 and *TLR1* rs5743618 genotypes in relation to baseline and post-bronchodilator FEV1/FVC in 81 former bronchiolitis patients.

	***TLR10* wild *TLR1* wild N=61**	***TLR10* wild *TLR1* variant N=5**	***TLR10* variant *TLR1* variant N=15**	
FEV1/FVC	Mean (SD) [95%CI]	Mean (SD) [95%CI]	Mean (SD) [95%CI]	Adjusted p†
Baseline	92.8 (7.3) [90.9-94.7]	86.9 (9.4) 75.3-98.5	97.4 (8.0) [93.0-101.9]	**0.003**
Post-bd	95.9 (6.4) [94.3-97.6]	90.3 (6.5) [82.2-98.3]	98.6 (7.1) [94.7-102.5]	**0.012**

CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; Post-bd, post-bronchodilator; SD, standard deviation. Parameters of spirometry are expressed as percentages of predicted. Statistically significant findings are marked with bold font. None had the combination of variant *TLR10* rs412900 and wild *TLR1* rs5743618 genotypes †ANCOVA, adjusted for current asthma, maternal smoking in infancy, RSV aetiology of bronchiolitis and zBMI (for definitions, see main text).

Concerning *TLR10* rs4129009 and *TLR2* rs5743708 combinations, the difference between three possible combinations was significant in the adjusted analyses in baseline and post-bd FEV1/FVC. Children with *TLR10* variant and *TLR2* wild genotypes had the highest and children with *TLR10* wild and *TLR2* wild genotypes had the lowest baseline and post-bd FEV1/FVC. The *TLR10* rs4129009 and *TLR2* rs5743708 genotype combinations did not show significant associations with other spirometry parameters (Table 10).

Table 10. Combinations of *TLR10* rs4129009 and *TLR2* rs5743708 genotypes in relation to baseline and post-bronchodilator FEV1/FVC in 81 former bronchiolitis patients.

	***TLR10* wild *TLR2* wild N=61**	***TLR10* wild *TLR2* variant N=5**	***TLR10* variant *TLR2* wild N=15**	
FEV1/FVC	Mean (SD) [95%CI]	Mean (SD) [95%CI]	Mean (SD) [95%CI]	Adjusted p†
Baseline	92.3 (7.2) [90.5-94.2]	93.0 (12.2) [77.9-108.1]	97.4 (8.0) [93.0-101.9]	**0.007**
Post-bd	95.4 (6.2) [93.8-97.0]	96.1 (10.9) [82.6-109.7]	98.6 (7.1) [94.7-102.5]	**0.037**

CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; Post-bd, post-bronchodilator; SD, standard deviation. Parameters of spirometry are expressed as percentages of predicted. Statistically significant findings are marked with bold font. None had the combination of variant *TLR10* rs412900 and variant *TLR2* rs5743708 genotypes. †ANCOVA, adjusted for current asthma, maternal smoking in infancy, RSV aetiology of bronchiolitis and zBMI (for definitions, see main text).

5.4.2 TLR4 and 7

TLR4 rs4986790 and *TLR7* rs179008 wild and variant genotypes were analysed in relation to post-bronchiolitis lung function. There were no significant differences between 69 children with *TLR4* rs4986790 wild and those 13 with variant genotypes in baseline or post-bd spirometry parameters. There were no significant differences between *TLR7* rs179008 wild and variant genotypes in baseline or post-bd spirometry parameters in girls or boys.

5.5 Irreversible airway obstruction after bronchiolitis (V)

Irreversible airway obstruction was present in 21 (23.9%) of the 88 former bronchiolitis patients. Ten children (47.6%) with irreversible airway obstruction had a history of asthma.

The children with and without irreversible airway obstruction did not differ for gender or mean age, height or zBMI. Repeated wheezing during the last 12 months was more common in 21 children with than in those 67 without irreversible airway obstruction, but such association was not seen for prolonged cough or night cough. Current ICS use, but not current or persistent asthma, was significantly associated with irreversible airway obstruction. There were no significant differences between children with or without irreversible airway obstruction in presence of current atopic dermatitis, current allergic rhinitis or current use of bronchodilators (Table 11).

Table 11. Current characteristics of 21 former bronchiolitis patients with versus those 67 without irreversible airway obstruction at 10-13 years of age.

	Irreversible airway obstruction N=21		**Normal post-BD FEV1 and FEV1/FVC N=67**		
Continuous variables	Mean	SD	Mean	SD	p value†
Age (years)	11.7	0.9	11.6	1.0	0.925
Height (cm)	153.5	7.7	150.6	9.1	0.201
zBMI (kg)	0.56	1.1	0.16	0.9	0.103
Categorised variables	n	%	n	%	p value‡
Girls	9	42.9	35	52.2	0.453
Current atopic dermatitis	8	38.1	19	28.4	0.399
Current allergic rhinitis	12	57.1	29	43.3	0.267
Repeated wheezing	9	42.9	14	20.9	**0.046**
Prolonged cough	3	14.3	7	10.4	0.697
Night cough	6	28.6	8	11.9	0.090
Bronchodilator use	8	38.1	12	17.9	0.074
ICS use	**5**	**23.8**	**3**	**4.5**	**0.017**
Current asthma	5	23.8	7	10.4	0.148
Persistent asthma	3	14.3	3	4.5	0.145

SD, standard deviation; FEV1, forced expiratory volume in one second; FVC, forced vital capacity. For definitions, see Materials and Methods section. Statistically significant findings are marked with bold font. †Calculated by T-test. ‡Calculated by Chi-square test or Fisher's exact test.

5.5.1 Early-life and preschool-age risk factors

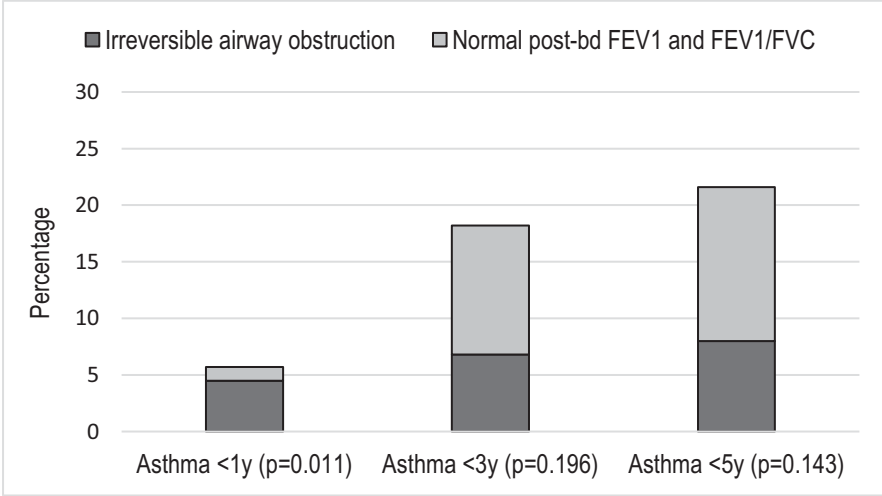
Early-life risk factors that were included in the analyses are presented in Table 12. Hospitalisation ages of less than four or 12 weeks, or RSV or rhinovirus aetiologies of bronchiolitis, were not associated with irreversible airway obstruction. In addition, atopic dermatitis at less than 12 months of age, maternal asthma, parental asthma and maternal smoking during pregnancy or infancy did not show significant associations with irreversible airway obstruction. Doctor-diagnosed asthma at less than 12 months of age was associated with irreversible airway obstruction, but such association was not seen for asthma diagnoses at less than three years or five years of age (Table 12, Figure 9).

Table 12. Early-life risk factors in relation to irreversible airway obstruction at the age of 10-13 years after early-life bronchiolitis.

	Irreversible airway obstruction N=21		**Normal post-BD FEV1 and FEV1/FVC N=67**		
Early-life risk factors	n	%	n	%	p value †
Hospitalisation age ≤4 weeks	4	19.0	9	13.4	0.501
Hospitalisation age ≤12 weeks	11	52.4	43	64.2	0.333
RSV aetiology ‡	15	71.4	47	71.2	0.985
Rhinovirus aetiology ‡	2	9.5	8	12.1	1.0
Atopic dermatitis <12 months of age	9	42.9	16	23.9	0.092
Maternal asthma	1	4.8	9	13.6	0.440
Parental asthma	3	14.3	11	16.4	1.0
Maternal smoking during pregnancy	5	23.8	9	13.4	0.308
Maternal smoking <12 months of age	7	33.3	17	25.4	0.475
Asthma diagnosis <12 months of age	**4**	**19.0**	**1**	**1.5**	**0.011**
Asthma diagnosis <3 years of age	6	28.6	10	14.9	0.196
Asthma diagnosis <5 years of age	7	33.3	12	17.9	0.143

FEV1, forced expiratory volume in one second; FVC, forced vital capacity. For definitions, see Material and Methods section. Statistically significant findings are marked with bold font. †Calculated by Chi-square test or Fisher's exact test. ‡N=87 for data of viral aetiology.

Figure 9. Irreversible airway obstruction, assessed by post-bronchodilator FEV1 and FEV1/FVC at 10-13 years of age, in 88 former bronchiolitis patients with asthma diagnosis at less than 12 months, three years and five years.



In multivariate logistic regression, adjusted for current asthma and zBMI, doctor-diagnosed asthma at less than 12 months of age remained an independently significant risk factor for irreversible airway obstruction (adjusted OR 12.6, 95%CI 1.3-126.4). Other early-life risk factors showed no significant associations with irreversible airway obstruction in multivariate logistic regression.

Preschool-age risk factors, such as current atopic dermatitis, current allergic rhinitis, physician-made asthma diagnosis at 5-7 years of age, or skin prick test positivity at 5-7 years of age, did not show significant associations with irreversible airway obstruction.

5.5.2 Role of *TLR* polymorphisms

There was a significant association between *TLR4* rs4986790 and irreversible airway obstruction after infant bronchiolitis. Irreversible airway obstruction was present in 20 (29.0%) of 69 children with wild AA genotype and in none of those 13 with variant AG genotype of *TLR4* rs4986790 (p=0.031). However, this significance was lost in multivariate logistic regression, adjusted for current asthma and zBMI (adjusted OR 0.2, 95%CI 0.0-1.6). Wild or variant genotypes of *TLR1* rs5743618, *TLR2* rs5743708, *TLR6* rs5743810, *TLR7* rs179008 or *TLR10* rs4129009 did not show significant associations with irreversible airway obstruction.

5.5.3 Impulse oscillometry at the age of 5-7 years in relation to irreversible airway obstruction at the age of 10-13 years

Results of both lung function measurements, including IOS at 5-7 years of age and spirometry at 10-13 years of age, were available from 62 former bronchiolitis patients, 17 (27.4%) of whom had irreversible airway obstruction. Higher post-bd Rrs5 and lower baseline and post-bd Xrs5 were significantly associated with irreversible airway obstruction, and these findings remained significant after adjustments for current asthma and zBMI (Table 13).

Table 13. Baseline and post-bronchodilator respiratory system resistance at 5 Hz and respiratory system reactance at 5 Hz measured with impulse oscillometry at the age of 5-7 years of age in relation to irreversible airway obstruction at the age of 10-3 years of age.

		Irreversible airway obstruction N=17		**Normal post-BD FEV1 and FEV1/FVC N=45**		
IOS parameters		Mean (SD)	95%CI	Mean (SD)	95%CI	Adjusted p value†
Rrs5	Baseline	0.18 (0.99)	-0.33 to 0.69	-0.22 (0.93)	-0.50 to 0.06	0.230
	Post-bd	**-1.22 (0.84)**	**-1.65 to -0.78**	**-1.92 (0.75)**	**-2.1 to -1.70**	**0.004**
Xrs5	Baseline	**-1.63 (1.53)**	**-2.4 to -0.85**	**-0.43 (0.99)**	**-0.72 to -0.13**	**0.001**
	Post-bd	**-0.21 (0.70)**	**-0.57 to 0.15**	**0.56 (0.56)**	**0.40 to 0.73**	**<0.001**

CI, confidence interval; Rrs5, respiratory system resistance at 5 Hz; SD, standard deviation; Xrs5, respiratory system reactance at 5 Hz. For definitions, see Material and Methods section. Parameters of impulse oscillometry are expressed as z scores. Statistically significant findings are marked with bold font. †ANCOVA between groups, adjusted for current asthma and zBMI.

6 DISCUSSION

6.1 Lung function after bronchiolitis

This thesis evaluated baseline and post-bd lung function measured with flow-volume spirometry in adolescence after hospitalisation for bronchiolitis at the age of less than 6 months in a controlled setting. Originally, 187 infants were enrolled, and 89 former bronchiolitis patients and 108 controls attended the study at the age of 10-13 years.

6.1.1 Outcome at the age of 10-13 years

Hospitalisation for bronchiolitis at the age of less than 6 months was associated with decreased baseline and post-bd FEV1 and FEV1/FVC at the mean age of 11.6 years compared with age- and sex-matched controls. In analyses of continuous data, infant bronchiolitis showed a significant association with post-bd FEV1/FVC, and in analyses of categorised data, with baseline and post-bd FEV1 and with baseline FEV1/FVC. More precisely, baseline FEV1 was pathological in 25% of cases versus 12% of controls, and post-bd FEV1 in 18% of cases versus 5% of controls. In multivariate logistic regression, the risk of irreversible FEV1 reduction was more than four-fold after infant bronchiolitis. This increased risk was independent of current asthma and significant after RSV positive but not after RSV negative bronchiolitis. In addition, baseline FEV1/FVC was pathological in 25% of cases versus 13% of controls, and post-bd FEV1/FVC in 10% and 5%, respectively. The association between bronchiolitis and FEV1/FVC was not significant in analyses adjusted for asthma, which implies that FEV1/FVC reduction was dependent on asthma, or that the analyses were underpowered due to small figures.

Four previous post-bronchiolitis studies have evaluated lung function at 10-13 years of age (Table 14). Three studies were controlled, age on admission was under 12 months, RSV was the only or main causative agent, and findings were reported as continuous variables only (13, 15, 16). In all three studies, bronchiolitis was significantly associated with decreased baseline FEV1 (13, 16) or FEV1/FVC (15,

16). This contradicts findings of this thesis, since we found no significant differences between cases and controls in baseline spirometry as continuous variables. Obviously, our controls were biased, as those with asthmatic symptoms were over-represented and, in addition, prevalence of asthma in cases and controls was rather similar (181). In previous post-bronchiolitis studies, the prevalence of asthma in cases has been higher (21-39%) than in controls (3-13%) and higher than prevalence of post-bronchiolitis asthma in the present cohort (13%). Only a Swedish study reported post-bd results (15). In that study, hospitalisation for RSV bronchiolitis was associated with decreased post-bd FEV1/FVC, but such association was not found after excluding study subjects with current asthma or recurrent wheezing symptoms (15). These findings are in line with the present results and suggest that FEV1/FVC reduction in early adolescence after bronchiolitis depends on asthma.

A non-controlled study from Finland reported baseline spirometry as categorised variables at the age of 12 years in children who were hospitalised for bronchiolitis at the age of less than 24 months (Table 14) (176). In that study, FEV1 and FEV1/FVC were pathological in 18% and 16% of adolescents, respectively (176), compared to 25% in the current thesis. That Finnish cohort was followed up until the age of 17-20 years (11), at which point the study was controlled and both baseline and post-bd spirometry were performed; however, the results were reported only as continuous variables. Surprisingly, there were no significant differences in baseline or post-bd FEV1 or FEV1/FVC between cases and controls. In addition, the finding remained similar in RSV positive and rhinovirus positive groups, although both groups showed higher asthma prevalence, 43% and 64%, respectively, compared to 12% asthma prevalence in controls (11). In the present thesis, former bronchiolitis patients showed lower post-bd FEV1/FVC values than controls, and those with RSV positive bronchiolitis were at increased risk for irreversible FEV1 reduction. This opposes the findings of the older Finnish cohort study, but instead, is supported by other previous follow-ups demonstrating decreased post-bd FEV1/FVC after hospitalisation for bronchiolitis (10, 14, 21) and decreased post-bd FEV1 after hospitalisation for RSV bronchiolitis (17, 175).

In the Finnish study, RSV aetiology of bronchiolitis was associated with reduced FVC at 12 years age suggesting restrictive changes (11, 176). An older Finnish cohort study evaluated lung function outcome at the age of 30 years in 70 participants with RSV bronchiolitis or RSV pneumonia in infancy, and, similarly, there was a link between severe RSV infection and reduced FVC. The present results on adolescents did not confirm these findings since we found no association between bronchiolitis

and FVC. Due to small numbers of study subjects, the present and previous long-term follow-ups were probably underpowered to find all existing associations.

Table 14. Summary of post-bronchiolitis studies evaluating lung function outcome at the age of 10-13 years.

Study No (enrolled)	No cases/ controls	Age at enrollment, months	RSV, %	Asthma, % cases/ controls	Post-bd spirometry	Categori-sed variables
Noble (13) Nottingham, UK 1979-81 N=101	61/47	<12	66	39/13	No	No
Mikalsen (16) Bergen, Norway 1997-98 N=131	108/89	<12	74	21/9	No	No
Sigurs (15) Borås, Sweden 1989-90 N=47	44/86	<12	100	28/3	Yes	No
Hyvärinen (176) Kuopio, Finland 1992-93 N=100	80/-	<24	30	40/-	No	Yes
Present study Tampere, Finland 2000-04 N=187	89/108	<6	72	13/11	Yes	Yes

6.1.2 Present results of spirometry in early adolescence in relation to impulse oscillometry at preschool age

In the present post-bronchiolitis follow-up, baseline and post-bd lung functions were measured twice: by IOS at preschool age and by spirometry in early

adolescence. Of those who underwent spirometry, 70% had participated in follow-up visit with measurements of IOS at preschool age.

At the age of 5-7 years, 20% of children presented with abnormal findings in baseline IOS, but only one of them in post-bd IOS, as published previously (18). Five years later, prevalence of abnormal findings in baseline spirometry was rather similar, but prevalence of abnormal findings in spirometry after bronchodilation was markedly higher: 18% for post-bd FEV1 and 10% for post-bd FEV1/FVC. Thus, school age may be the critical period for progression of changes in lung function of children with bronchiolitis in infancy. A study from Missouri, USA demonstrated a steep decline of FEV1 from seven to 12 years of age after hospitalisation for RSV bronchiolitis (17). However, asthma was common and post-bd measurements were rarely taken in that study, which means that the identified FEV1 trajectories mainly reflected baseline lung function in post-bronchiolitis asthma. Still, the results suggest that there was a decline in post-bronchiolitis FEV1 during school age.

In the present follow-up, the use of different measuring techniques at preschool age and in early adolescence brings a risk of bias. In addition, present observations in IOS were based on Rrs5 and Xrs5 and did not include those IOS measures that are thought as most sensitive for small airways. Maximal expiratory flows, assessed with spirometry, are modified by lung elastic recoil in addition to airflow resistance (93). In contrast, Rrs assessed with IOS is mainly dependent on airway calibre (109). This means that higher prevalence of irreversible abnormalities in spirometry compared to Rrs may be due to changes in lung elastic recoil and could not be seen in IOS at preschool age although potentially present. Interestingly, abnormalities in IOS at preschool age accentuated on Xrs5 (18), that is described to reflect elastic properties of the lung periphery in addition to peripheral obstruction (113).

Previously in this cohort study, we have demonstrated moderate to strong correlations between IOS at preschool age and spirometry in adolescence (235). The observations of lung function in asthmatic children followed from preschool age to adolescence in Helsinki, Finland, were similar (236). In the present analyses, higher post-bd Rrs5 and lower baseline and post-bd Xrs5 in IOS at preschool age increased the risk of irreversible changes in lung function in adolescence. Thus, the process leading to irreversible reduction of lung function probably starts before school age.

6.1.3 Mechanisms of reduced lung function

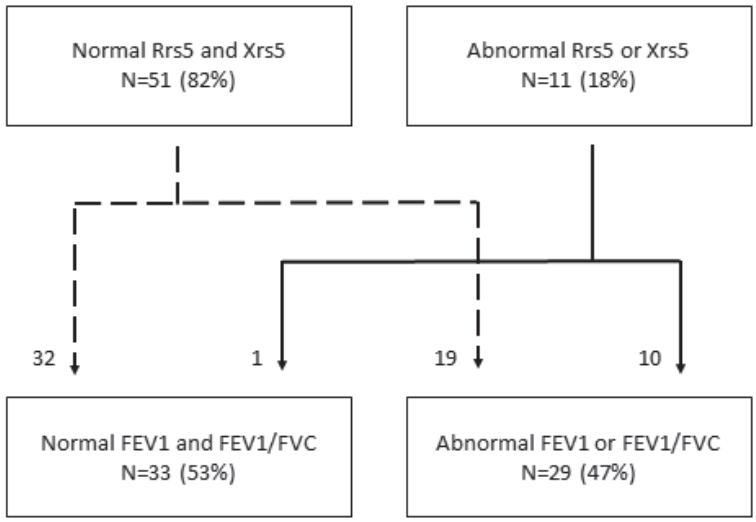
Origins of reduced lung function in children and the roles of early-childhood LRTIs have been under active discussion since lung function growth was found to be associated with wheezing phenotypes in the TCRS birth cohort study (164). Children with wheezing LRTI during the first three years of life were classified as transient wheezers, who no longer wheezed at six years of age, and as persistent wheezers, who still wheezed at six years of age. In relation to never wheezers, both wheeze groups showed lower maximal expiratory flows from preschool age to adolescence, but only transient wheezers already presented with decreased flows in infancy before any LRTI (33, 134).

Airway remodelling is thought to be the primary reason for lung function decline in asthma and persistent wheeze (26, 27). Reduced lung function in transient wheezers, most obviously, has its origin prenatally or postnatally in early infancy (164). Possible mechanisms for reduced maximal expiratory flows in infancy include at least persistently reduced airway calibre, variations in lung elastic properties, and increased airway reactivity. Irrespective of mechanism, reduced expiratory flows in infancy increases the risk of LRTIs in early childhood (30-32) and decreased expiratory flows later in childhood (34, 35). The design of the present study did not allow any comparisons between these mechanisms since data on infant lung function was not available. However, lung function reduction was seen in asthmatic and non-asthmatic participants, which suggests that both mechanisms, airway remodelling and premorbid origin, were likely to be present.

In the present thesis, over half of adolescents who had irreversible reduction of post-bd FEV1 or post-bd FEV1/FVC had no history of asthma. Due to low, 1%, prevalence of abnormal post-bd IOS at preschool age, the present findings in non-asthmatic children suggest a reduction in growth of the lung function after bronchiolitis. Our findings are in disagreement with those of the TCRS birth cohort study that documented persistently reduced expiratory flows in transient wheezers after early childhood LRTIs without progression during school years (134). Compared to the present study, TCRS evaluated influences of mild respiratory infections on the outcome and reported findings only as continuous variables within the whole, probably heterogeneous, group of transient wheezers. The discrepancy between the present results and those of the TCRS call for further, longitudinal studies on lung function development after severe LRTIs with analyses stratified for asthma and standardized for used measuring techniques.

Interesting findings were made in a large study combining findings of two population-based cohorts from Perth, Australia and from Manchester, UK (34). The authors demonstrated that even 75% of children with reduced expiratory flows in infancy moved to average or above-average lung function trajectories in later childhood or in adolescence (34), meaning that improvement in childhood lung function was rather common. In the present study, the majority of children with abnormal Xrs5 or Rrs5 in IOS at preschool age presented with abnormal FEV1 or FEV1/FVC function in spirometry in early adolescence, suggesting that the shift to normal was not common at school age after severe bronchiolitis (Figure 10).

Figure 10. Normal and abnormal Rrs5 and Xrs5 measured with impulse oscillometry at 5-7 years of age compared to normal and abnormal FEV1 and FEV1/FVC measured with flow-volume spirometry at the age of 10-13 years in 62 study subjects.



FEV1, forced expiratory volume in one second; FVC, forced vital capacity; Rrs5, respiratory system resistance at 5 Hz; Xrs5, respiratory system reactance at 5 Hz. Thresholds for abnormal impulse oscillometry as z scores were Rrs5 >+1.65 or Xrs5 <-1.65. Threshold for abnormal spirometry as percent of predicted were FEV1 ≤80% for boys and ≤82% for girls or FEV1/FVC ≤87% for boys and ≤88% for girls.

6.2 Tobacco smoke exposure in relation to bronchiolitis

The present results revealed no associations between tobacco smoke exposure and post-bronchiolitis lung function at the age of 10-13 years. Similarly, in an older Finnish post-bronchiolitis study, exposure to smoking during pregnancy or infancy

did not affect lung function at the age of 12 years although it was associated with airway hyper-responsiveness at the same age (176).

In the present follow-up, 27% of the mothers smoked during the child's first living year. The rate of maternal smoking in early childhood was rather similar to those of 28-29% reported from other Finnish post-bronchiolitis cohorts (191, 237). Maternal smoking was clearly more common in former bronchiolitis patients than in controls in the present cohort. Similarly, maternal smoking has been identified as a risk factor for early childhood LRTIs in several studies (33, 127, 182). Previous post-bronchiolitis studies have not reported numbers of early-life smoke exposures in comparison with controls.

6.3 Effects of overweight in post-bronchiolitis lung function

A large meta-analysis concluded that the most common pattern of spirometry in overweight or obese children is reduced FEV1/FVC with normal or increased FEV1 and FVC (195). The present results in former bronchiolitis patients are in line; overweight (obese included) was associated with reduced FEV1/FVC at the age of 10-13 years, but not with FEV1 or FVC. This result was similar as continuous and as categorised variables, and it remained significant after adjustments with current asthma and maternal smoking.

In the present cohort at 10-13 years of age, 52% of overweight children had reduced FEV1/FVC, and half of them presented with irreversible reduction. This probably means that effects of overweight are not similar in all subjects and may reflect two separate connections between overweight and lung function in children, as described previously in literature. First, overweight is associated with increased airway reactivity and asthma, and second, overweight may induce dysanaptic increase in lung growth (40), and both of these mechanisms may finally lead to decreased FEV1/FVC.

Only one previous study has evaluated impacts of overweight or obesity on post-bronchiolitis lung function. In that Finnish study, association between overweight and baseline spirometry was evaluated at the age of 12.3 years in 74 children who had been hospitalised for infection associated wheezing at less than 24 months of age (43). In line with our results, FEV1/FVC, but not other spirometry parameters, was decreased in overweight subjects, and the findings remained significant after adjusting for anti-inflammatory medication of asthma (43). In the same Finnish study, 33% of children with current overweight or obesity had reduced baseline

FEV1/FVC (43). In the present cohort, prevalence of baseline FEV1/FVC reduction at the age of 10-13 years was higher, since about half of overweight children showed reduced FEV1/FVC. However, post-bd measurements were not performed in the other Finnish study, and for this reason, more detailed comparison cannot be done.

Interesting findings were reported in a study from Brazil that evaluated the association between overweight and lung function in 114 asthmatic and 74 non-asthmatic children at school age (42). In that study, asthma and overweight independently reduced baseline FEV1/FVC, but after bronchodilation, overweight contributed more to FEV1/FVC ratio than asthma (42). Dysanaptic discrepancy between airways and the lungs may be increased in those overweight children, whose post-bd FEV1/FVC is reduced before from other reason than overweight. This may be one explanation for high prevalence of FEV1/FVC reduction in overweight children in the present study.

6.4 The role of *TLR* polymorphisms

In this thesis, only one SNP was determined for each *TLR* gene based on previously reported findings on their functional properties and impacts on post-bronchiolitis outcome previously in this cohort study.

A link between *TLR* polymorphisms and pulmonary diseases is evident (220), but studies evaluating associations between *TLR* polymorphisms and lung function are rare. Few previous studies have evaluated those SNPs included in the present thesis, showing inconsistent findings and variable designs (223-225). Variant genotype of *TLR2* rs5743708 was associated with better lung function in swine operation workers in a Canadian study (223), and with a faster decline of FEV1 in patients with cystic fibrosis in a Belgian study (224). In a Dutch study, the same SNP of *TLR2* polymorphism and *TLR4* rs4986790 showed no association with FEV1 in COPD patients (225).

In the present cohort, *TLR4* rs4986790 and *TLR6* rs5743810 showed preliminary associations with airway reactivity measured by IOS at the age of 5-7 years. Airway reactivity was not measured in the follow-up at 10-13 years, but these SNPs did not show associations with baseline or post-bd spirometry. The findings suggest that although an association existed between *TLR4* rs4986790 or *TLR6* rs5743810 and airway reactivity at preschool age after bronchiolitis in infancy, it was not followed by significant changes in lung function measured with spirometry by the age of 10-

13 years. The present results did not confirm an association between *TLR7* rs179008 and lung function measured with IOS at the age of 5-7 years.

In the present analyses of the TLR2 subfamily, the most distinct association was found in terms of *TLR10* rs4129009: variant genotype was associated with higher baseline and post-bd FEV1/FVC, and the association was seen in all studied genotype combinations that included variant genotype of *TLR10* rs4129009. In contrast, variant genotypes of *TLR10* rs4129009 were associated with asthma treated with ICSs or continued from preschool age to adolescence previously in the present follow-up (228).

Previous literature has described TLR10 mainly as an inhibitory receptor (216-218). Several possible anti-inflammatory mechanisms for TLR10 include hetero-dimerisation with other TLRs, competing over same ligands with other TLRs and regulating the production of anti-inflammatory cytokines. TLR10 probably uses different intra-cellular binding proteins than other TLRs, which may lead to antagonist signalling pathways against the pro-inflammatory effects of other TLRs. In addition, TLR10 may reduce interactions between immune cells and T lymphocytes (212). Recent evidence suggests that TLR10 can also activate pro-inflammatory cytokine production, and TLR10 polymorphisms may change the balance between pro- and anti-inflammatory responses, leading to altered vulnerability to infections and asthma (212).

The present results at the age of 10-13 years after hospitalisation for bronchiolitis suggest that both pro-inflammatory and anti-inflammatory mechanisms are present, since variant genotype of *TLR10* rs4129009 predisposed children to severe manifestations of asthma but had a protective effect against lung function reductions. This discrepancy may reflect the complex modulatory effects of TLR10, or it may result from different TLR10 interactions with different pathogens.

The results from the present cohort study revealed, in line with available literature (15, 21, 175), irreversible changes in lung function, especially after RSV bronchiolitis. In contrast, rhinovirus aetiology has been associated with increased risk of post-bronchiolitis asthma (162). Unlike rhinoviruses, RSV is able to cause direct epithelial damage in the airways (57). There is some evidence that TLRs may have a role in recognising endogenous molecules of necrotic cells and in inducing the regeneration of injured tissue (238), but these findings are not confirmed for TLR10 or, in particular, for bronchiolitis pathology.

TLR1 rs5743618 or *TLR2* rs5743708 were not related to post-bronchiolitis lung function, but interestingly, genotype combinations of these two SNPs showed

significant association with baseline FEV1/FVC. This suggests an altered function via hetero-dimerisation between TLR1 and TLR2.

6.5 Risk factors for irreversible airway obstruction after bronchiolitis

Smoking is the major reason for irreversible airway obstruction and COPD in adults. However, early-life origins for COPD have attracted increasing interest in recent years. Early-childhood asthma and respiratory infections may have a crucial role in development of persistently decreased lung function, especially in non-smokers (123, 124). The definitions of irreversible airway obstruction vary which complicates comparison of findings from different studies. The present definition comprises irreversible changes in FEV1 and in FEV1/FVC.

In the present cohort at the age of 10-13 years, irreversible airway obstruction was present in 24% of children who were hospitalised for bronchiolitis in early infancy. The prevalence of irreversible airway obstruction was rather high and may reflect the outcome of those children who were hospitalised for bronchiolitis in the very first months of life. In an older Finnish post-bronchiolitis cohort, prevalence of irreversible airway obstruction, defined by reduced post-bd FEV1/FVC, was 21% in early adulthood (10) compared to 10% of post-bd FEV1/FVC reduction in the present cohort. However, the older Finnish study did not report prevalence of post-bd FEV1 reduction.

Childhood asthma is a well-known cause of lung function abnormalities. Persistent, severe childhood asthma may trigger reduced airway growth that leads to irreversible changes in lung function and increases the risk of later COPD (24). In the present cohort, children with irreversible airway obstruction at the age of 10-13 more often reported repeated wheezing and used ICSs compared to those without irreversible obstruction. Likewise, physician-diagnosed asthma increased the risk of significant FEV1 decline in a post-bronchiolitis cohort from Missouri, USA (17).

The present findings revealed a significant association between irreversible airway obstruction and age at asthma diagnosis. The risk of irreversible airway obstruction was 13-fold in those with physician-diagnosed asthma by the age of 12 months. In contrast, physician-diagnosed asthma by the age of three or five years did not show such a significant association. Interestingly, 80% of those who were diagnosed with asthma by the age of 12 months had irreversible airway obstruction at the age of 10-13 years. The respective figure was 38% for those who were diagnosed with asthma

by the age of three years, and two thirds of the children with irreversible airway obstruction had already been diagnosed with asthma during the first year of life. Noteworthy, physician-diagnosed asthma by age 12 months may reflect either transient or persistent respiratory symptoms assessed as six years of age, which is the most used practice in epidemiological studies. Still, special attention should be paid to this small group of children with very early onset of respiratory symptoms associated with asthma to start sufficient asthma management and to prevent known environmental factors that may inhibit later lung growth.

We found preliminary evidence, that variant genotype of *TLR4* rs4986790 may be protective against irreversible airway obstruction after early-life bronchiolitis. Previously, *TLR4* rs4986790 polymorphisms have been linked to reduced COPD severity; interestingly, the described mechanisms include decreased reactivity of those microbial patterns that are recognised by TLRs during infections (83).

6.6 Methodological aspects

The design of the present prospective cohort study, consisting of lung function measurement in adolescents hospitalised for bronchiolitis at less than six months of age, is unique. Due to hospitalisation and the young hospitalisation age, the results present the outcome of high-risk infants with genetic, prenatal and early postnatal factors that predispose children to severe manifestation of bronchiolitis. This design is likely to have increased the prevalence of pathological findings, including those of categorised parameters in post-bd spirometry. On the other hand, this design decreased the comparability of results to those of other post-bronchiolitis studies that have used older upper age limits at enrolment and usually reported baseline spirometry expressed as continuous variables only.

It is important to note that categorised variables, that were used to define pathological lung function, are dependent on reference values and selected threshold limits. In addition, the Finnish reference values (232) used in the present analyses were stratified for height and sex, but not for age. However, the use of categorised variables allowed us to assess lung function reductions in heterogenous group of former bronchiolitis patients of whom most presented with normal lung function.

Long-term follow-up with a prospective design, and the controlled setting at the age of 10-13 years, stand as the strengths of the present cohort study. Due to carefully collected data during hospitalisation in infancy and at three follow-ups visits until 10-13 years of age, we were able to compare the early-life and preschool-age

risk factors in relation to lung function abnormalities in adolescence after bronchiolitis. Viral testing at admission confirmed predominance of RSV and enabled outcome assessment especially after RSV bronchiolitis.

The number of study subjects was low for genetic studies, which increased the risk of false-negative findings. Although all ten TLRs were included, only one SNP for each was studied, and the results remained mainly negative. The drop-out rate 48% was high and may have caused a selection bias, since symptomatic subjects attend studies more often than non-symptomatic subjects. However, drop-outs are understandable in long-term follow-up studies. It is noteworthy, that the prevalence of current asthma in the present study was lower than in any other post-bronchiolitis cohort studies. In contrast, the prevalence of asthma among controls was higher than in the Finnish general population, which implies that symptomatic controls were particularly interested to attend. Finally, the asthma rates were relatively similar among cases and controls. This has probably diminished the differences in lung function outcomes between cases and controls, which highlights those differences we were able to confirm.

Baseline and post-bd lung function were measured twice during the follow-up: at the age of 5-7 years with IOS and at the age of 10-13 years with spirometry. Our cohort study is the only post-bronchiolitis follow-up that has evaluated post-bd lung function before and after early school years. As many as 70% of study subjects who participated in the clinical follow-up at the age of 10-13 years had performed IOS five years earlier. However, longitudinal comparisons may not be reliable due different measuring techniques applied at 5-7 and 10-13 years of ages. In addition, post-bd measurements at 5-7 years of age were performed after exercise test.

7 CONCLUSIONS

Hospitalisation for bronchiolitis at the age of less than six months was associated with decreased baseline and post-bd FEV1 and FEV1/FVC at the mean age of 11.6 years. The prevalence of pathological findings in cases and controls were 25% vs. 12% for baseline FEV1, 18% vs. 5% for post-bd FEV1, 25% vs. 13% for baseline FEV1/FVC and 10% vs. 5% for post-bd FEV1/FVC. The risk of FEV1 reduction existed especially after RSV bronchiolitis and it was not dependent on asthma status.

Overweight was associated with reduced baseline and post-bd FEV1/FVC after bronchiolitis. About half of overweight cases had pathological baseline FEV1/FVC and one quarter of overweight cases had pathological post-bd FEV1/FVC.

The variant genotype of *TLR10* rs4129009 was associated with higher baseline and post-bd FEV1/FVC after bronchiolitis. The results remained similar when *TLR10* rs4129009 genotypes were combined with *TLR1* rs5743618 or *TLR2* rs5743708 genotypes.

Irreversible airway obstruction after bronchiolitis was assessed by reduced post-bd FEV1 or post-bd FEV1/FVC and it was more common in those who used current ICS medication for asthma. Children who were diagnosed with asthma during the first year of life were at increased risk for irreversible airway obstruction. Higher Rrs5 in post-bd IOS and lower Xrs5 in baseline and post-bd IOS five years earlier predicted current irreversible changes in spirometry.

8 FUTURE CONSIDERATIONS

Prospective long-term post-bronchiolitis follow-ups are still needed to clarify the risks of later asthma and COPD. The assessment of the COPD risk means that the studies should continue for more than 50 years. The first recruited subjects in the prospective Finnish and Swedish post-bronchiolitis cohorts are now 40 years old.

Lung function growth during childhood determines the maximal level of lung function in early adulthood, and factors that affect that growth plays a role in development of reduced lung function. The outcomes suggesting COPD risk, such as development of irreversible reductions in spirometry should be assessed during this growth period. Due to association between RSV bronchiolitis and irreversible lung function abnormalities, post-bd measurements should be included in the RSV follow-ups.

In future, lung function trajectories at school age and the reached/attributable levels of lung function might be used (for example by the help of machine learning methods) for prediction significant lung function decline and COPD risk in later life. If the risk and risk factors for reduction of lung function and for COPD are known, it is possible to focus preventive measures specifically on those who are at the greatest risk. Avoidance of tobacco smoking, good physical condition, avoidance of overweight, and appropriate diagnosis and treatment of asthma if present are among these preventive tools.

The proportion of study drop-outs increases in relation to follow-up time, which clearly form a problem in long-term follow-ups. It may be impossible to identify different patterns of lung function growth after hospitalisation for bronchiolitis with such low number of study subjects reported in the follow-ups continued until young adulthood. In the birth cohort studies, the problem is smaller number of pathological outcomes, such as asthma, COPD or lung function deficits, compared to post-bronchiolitis studies with an enrichment of such outcomes. In response, there is a need to combine different materials to gain enough power for multivariable calculations, which are no doubt needed. In the analyses of genetic factors, there is a need to combine data from various studies and to compare the data with large population-based genetic datasets.

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REFERENCES

1. Rodríguez-Martínez CE, Castro-Rodriguez JA, Nino G and Midulla F. The impact of viral bronchiolitis phenotyping: is it time to consider phenotype-specific responses to individualize pharmacological management? Paediatr Respir Rev 2020; 34: 53-58.
2. Nenna R, Frassanito A, Petrarca L, Di Mattia G and Midulla F. Age limit in bronchiolitis diagnosis: 6 or 12 months? Front Pediatr 2020; 8: 144.
3. Jartti T, Lehtinen P, Vuorinen T and Ruuskanen O. Bronchiolitis: age and previous wheezing episodes are linked to viral etiology and atopic characteristics. Pediatr Infect Dis J 2009; 28: 311-317.
4. Jartti T, Smits HH, Bønnelykke K, Bircan O, Elenius V, Konradsen JR, et al. Bronchiolitis needs a revisit: distinguishing between virus entities and their treatments. Allergy 2019; 74: 40-52.
5. Korppi M, Kotaniemi-Syrjänen A, Waris M, Vainionpää R and Reijonen TM. Rhinovirus-associated wheezing in infancy: comparison with respiratory syncytial virus bronchiolitis. Pediatr Infect Dis J 2004; 23: 995-999.
6. Broberg EK, Waris M, Johansen K, Snacken R and Penttinen P. Seasonality and geographical spread of respiratory syncytial virus epidemics in 15 European countries, 2010 to 2016. Euro surveill 2018; 23: 17-00284.
7. Collins PL and Graham BS. Viral and host factors in human respiratory syncytial virus pathogenesis. J Virol 2008; 82: 2040-2055.
8. Shi T, McAllister DA, O'Brien KL, Simoes EAF, Madhi SA, Gessner BD, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet 2017; 390: 946-958.
9. Ong BA, Li J, McDonough JM, Wei Z, Kim C, Chiavacci R, et al. Gene network analysis in a pediatric cohort identifies novel lung function genes. PLoS One 2013; 8: e72899.
10. Backman K, Piippo-Savolainen E, Ollikainen H, Koskela H and Korppi M. Irreversible airway obstruction in adulthood after bronchiolitis in infancy: evidence from a 30-year follow-up study. Respir Med 2014; 108: 218-223.
11. Backman K, Ollikainen H, Piippo-Savolainen E, Nuolivirta K and Korppi M. Asthma and lung function in adulthood after a viral wheezing episode in early childhood. Clin Exp Allergy 2018; 48: 138-146.
12. Fjaerli H, Farstad T, Rød G, Ufert GK, Gulbrandsen P and Nakstad B. Acute bronchiolitis in infancy as risk factor for wheezing and reduced pulmonary function by seven years in Akershus County, Norway. BMC Pediatr 2005; 5: 31.
13. Noble V, Murray M, Webb MS, Alexander J, Swarbrick AS and Milner AD. Respiratory status and allergy nine to 10 years after acute bronchiolitis. Arch Dis Child 1997; 76: 315-319.
14. Goksör E, Gustafsson PM, Alm B, Amark M and Wennergren G. Reduced airway function in early adulthood among subjects with wheezing disorder before two years of age. Pediatr Pulmonol 2008; 43: 396-403.

15. Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. Am J Respir Crit Care Med 2005; 171: 137-141.
16. Mikalsen IB, Halvorsen T and Øymar K. The outcome after severe bronchiolitis is related to gender and virus. Pediatr Allergy Immunol 2012; 23: 391-398.
17. Kitcharoensakkul M, Bacharier LB, Schweiger TL, Wilson B, Goss CW, Lew D, et al. Lung function trajectories and bronchial hyperresponsiveness during childhood following severe RSV bronchiolitis in infancy. Pediatr Allergy Immunol 2021; 32: 457-464.
18. Lauhkonen E, Koponen P, Nuolivirta K, Paassilta M, Toikka J and Korppi M. Lung function by impulse oscillometry at age 5-7 years after bronchiolitis at age 0-6 months. Pediatr Pulmonol 2015; 50: 389-395.
19. Sigurs N, Bjarnason R, Sigurbergsson F and Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am J Respir Crit Care Med 2000; 161: 1501-1507.
20. Korppi M, Kuikka L, Reijonen T, Remes K, Juntunen-Backman K and Launiala K. Bronchial asthma and hyperreactivity after early childhood bronchiolitis or pneumonia. An 8-year follow-up study. Arch Pediatr Adolesc Med 1994; 148: 1079-1084.
21. Sigurs N, Aljassim F, Kjellman B, Robinson PD, Sigurbergsson F, Bjarnason R, et al. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. Thorax 2010; 65: 1045-1052.
22. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. Updated 2020. http://www.ginasthma.org/. Date last accessed: April 9th, 2021.
23. Vonk JM, Jongepier H, Panhuysen CIM, Schouten JP, Bleecker ER and Postma DS. Risk factors associated with the presence of irreversible airflow limitation and reduced transfer coefficient in patients with asthma after 26 years of follow up. Thorax 2003; 58: 322-327.
24. McGeachie MJ, Yates KP, Zhou X, Guo F, Sternberg AL, Van Natta ML, et al. Patterns of growth and decline in lung function in persistent childhood asthma. N Engl J Med 2016; 374: 1842-1852.
25. Sousa AW, Barros Cabral AL, Arruda Martins M and Carvalho CRF. Risk factors for fixed airflow obstruction in children and adolescents with asthma: 4-Year follow-up. Pediatr Pulmonol 2020; 55: 591-598.
26. Rasmussen F, Taylor DR, Flannery EM, Cowan JO, Greene JM, Herbison GP, et al. Risk factors for airway remodeling in asthma manifested by a low postbronchodilator FEV1/vital capacity ratio: a longitudinal population study from childhood to adulthood. Am J Respir Crit Care Med 2002; 165: 1480-1488.
27. Pascual RM and Peters SP. Airway remodeling contributes to the progressive loss of lung function in asthma: an overview. J Allergy Clin Immunol 2005; 116: 477-486.
28. Tillie-Leblond I, de Blic J, Jaubert F, Wallaert B, Scheinmann P and Gosset P. Airway remodeling is correlated with obstruction in children with severe asthma. Allergy 2008; 63: 533-541.
29. Lambert L, Sagfors AM, Openshaw PJM and Culley FJ. Immunity to RSV in early-life. Front Immunol 2014; 5: 1-14.

30. Young S, Arnott J, O'Keeffe PT, Le Souef PN and Landau LI. The association between early life lung function and wheezing during the first 2 yrs of life. Eur Respir J 2000; 15: 151-157.
31. Håland G, Lødrup Carlsen KC, Mowinckel P, Munthe-Kaas MC, Devulapalli CS, Berntsen S, et al. Lung function at 10 yr is not impaired by early childhood lower respiratory tract infections. Pediatr Allergy Immunol 2009; 20: 254-260.
32. Murray CS, Pipis SD, McArdle EC, Lowe LA, Custovic A and Woodcock A. Lung function at one month of age as a risk factor for infant respiratory symptoms in a high risk population. Thorax 2002; 57: 388-392.
33. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M and Morgan WJ. Asthma and wheezing in the first six years of life. N Engl J Med 1995; 332: 133-138.
34. Belgrave DCM, Granell R, Turner SW, Curtin JA, Buchan IE, Le Souëf PN, et al. Lung function trajectories from pre-school age to adulthood and their associations with early life factors: a retrospective analysis of three population-based birth cohort studies. Lancet Respir Med 2018; 6: 526-534.
35. Stern DA, Morgan WJ, Wright AL, Guerra S and Martinez FD. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. Lancet 2007; 370: 758-764.
36. Becnel D, You D, Erskin J, Dimina DM and Cormier SA. A role for airway remodeling during respiratory syncytial virus infection. Respir Res 2005; 6: 122.
37. Tourdot S, Mathie S, Hussell T, Edwards L, Wang H, Openshaw PJM, et al. Respiratory syncytial virus infection provokes airway remodelling in allergen-exposed mice in absence of prior allergen sensitization. Clin Exp Allergy 2008; 38: 1016-1024.
38. You D, Becnel D, Wang K, Ripple M, Daly M and Cormier SA. Exposure of neonates to respiratory syncytial virus is critical in determining subsequent airway response in adults. Respir Res 2006; 7: 107.
39. Rossi GA and Colin AA. Respiratory syncytial virus - Host interaction in the pathogenesis of bronchiolitis and its impact on respiratory morbidity in later life. Pediatr Allergy Immunol 2017; 28: 320-331.
40. di Palmo E, Filice E, Cavallo A, Caffarelli C, Maltoni G, Miniaci A, et al. Childhood obesity and respiratory diseases: which link? Children (Basel) 2021; 8.
41. Forno E, Weiner DJ, Mullen J, Sawicki G, Kurland G, Han YY, et al. Obesity and airway dysanapsis in children with and without asthma. Am J Respir Crit Care Med 2017; 195: 314-323.
42. Jones MH, Roncada C, Fernandes MTC, Heinzmann-Filho JP, Sarria Icaza EE, Mattiello R, et al. Asthma and obesity in children are independently associated with airway dysanapsis. Front Pediatr 2017; 5: 270.
43. Sidoroff V, Hyvärinen M, Piippo-Savolainen E and Korppi M. Lung function and overweight in school aged children after early childhood wheezing. Pediatr Pulmonol 2011; 46: 435-441.
44. Lauhkonen E, Koponen P, Nuolivirta K, Paassilta M, Toikka J, Saari A, et al. Obesity and bronchial obstruction in impulse oscillometry at age 5-7 years in a prospective post-bronchiolitis cohort. Pediatr Pulmonol 2015; 50: 908-914.
45. Kanagaratham C, Camateros P, Flaczyk A and Radzioch D. Polymorphisms in Toll-like receptor genes and their roles in allergic asthma and atopy. Recent Pat Inflamm Allergy Drug Discov 2011; 5: 45-56.
46. Colby TV. Bronchiolitis. Pathologic considerations. Am J Clin Pathol 1998; 109: 101-109.

47. Meissner HC. Viral Bronchiolitis in Children. N Engl J Med 2016; 374: 62-72.
48. Martinez FD, Morgan WJ, Wright AL, Holberg CJ and Taussig LM. Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. N Engl J Med 1988; 319: 1112-1117.
49. Turunen R, Koistinen A, Vuorinen T, Arku B, Söderlund-Venermo M, Ruuskanen O, et al. The first wheezing episode: respiratory virus etiology, atopic characteristics, and illness severity. Pediatr Allergy Immunol 2014; 25: 796-803.
50. Brand PLP, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, et al. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur Respir J 2008; 32: 1096-1110.
51. Tapiainen T, Aittoniemi J, Immonen J, Jylkkä H, Meinander T, Nuolivirta K, et al. Finnish guidelines for the treatment of laryngitis, wheezing bronchitis and bronchiolitis in children. Acta Paediatr 2016; 105: 44-49.
52. Hancock DG, Charles-Britton B, Dixon D and Forsyth KD. The heterogeneity of viral bronchiolitis: A lack of universal consensus definitions. Pediatr Pulmonol 2017; 52: 1234-1240.
53. Kirolos A, Manti S, Blacow R, Tse G, Wilson T, Lister M, et al. A systematic review of clinical practice guidelines for the diagnosis and management of bronchiolitis. J Infect Dis 2020; 222: S672-S679.
54. Ralston SL, Lieberthal AS, Meissner HC, Alverson BK, Baley JE, Gadomski AM, et al. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. Pediatrics 2014; 134: 1474.
55. Randolph AG, Meert KL, O'Neil ME, Hanson JH, Luckett PM, Arnold JH, et al. The feasibility of conducting clinical trials in infants and children with acute respiratory failure. Am J Respir Crit Care Med 2003; 167: 1334-1340.
56. Mecklin M, Heikkilä P and Korppi M. Low age, low birthweight and congenital heart disease are risk factors for intensive care in infants with bronchiolitis. Acta Paediatr 2017; 106: 2004-2010.
57. Vandini S, Calamelli E, Faldella G and Lanari M. Immune and inflammatory response in bronchiolitis due to respiratory syncytial virus and rhinovirus infections in infants. Paediatr Respir Rev 2017; 24: 60-64.
58. Miller EK, Gebretsadik T, Carroll KN, Dupont WD, Mohamed YA, Morin L, et al. Viral etiologies of infant bronchiolitis, croup and upper respiratory illness during 4 consecutive years. Pediatr Infect Dis J 2013; 32: 950-955.
59. Kennedy JL, Turner RB, Braciale T, Heymann PW and Borish L. Pathogenesis of rhinovirus infection. Curr Opin Virol 2012; 2: 287-293.
60. Dumas O, Mansbach JM, Jartti T, Hasegawa K, Sullivan AF, Piedra PA, et al. A clustering approach to identify severe bronchiolitis profiles in children. Thorax 2016; 71: 712-718.
61. Papoff P, Moretti C, Cangiano G, Bonci E, Roggini M, Pierangeli A, et al. Incidence and predisposing factors for severe disease in previously healthy term infants experiencing their first episode of bronchiolitis. Acta Paediatr 2011; 100: 17.
62. García CG, Bhore R, Soriano-Fallas A, Trost M, Chason R, Ramilo O, et al. Risk factors in children hospitalized with RSV bronchiolitis versus non-RSV bronchiolitis. Pediatrics 2010; 126: 1453.
63. Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, et al. The burden of respiratory syncytial virus infection in young children. N Engl J Med 2009; 360: 588-598.

64. Smyth RL and Openshaw PJM. Bronchiolitis. Lancet 2006; 368: 312-322.
65. Di Cicco M, Kantar A, Masini B, Nuzzi G, Ragazzo V and Peroni D. Structural and functional development in airways throughout childhood: children are not small adults. Pediatr Pulmonol 2021; 56: 240-251.
66. Papastamelos C, Panitch HB, England SE and Allen JL. Developmental changes in chest wall compliance in infancy and early childhood. J Appl Physiol (1985) 1995; 78: 179-184.
67. Purcell K and Fergie J. Driscoll Children's Hospital respiratory syncytial virus database: risk factors, treatment and hospital course in 3308 infants and young children, 1991 to 2002. Pediatr Infect Dis J 2004; 23: 418-423.
68. Lanari M, Prinelli F, Adorni F, Di Santo S, Vandini S, Silvestri M, et al. Risk factors for bronchiolitis hospitalization during the first year of life in a multicenter Italian birth cohort. Ital J Pediatr 2015; 41: 40.
69. Law BJ, Langley JM, Allen U, Paes B, Lee DSC, Mitchell I, et al. The Pediatric Investigators Collaborative Network on Infections in Canada study of predictors of hospitalization for respiratory syncytial virus infection for infants born at 33 through 35 completed weeks of gestation. Pediatr Infect Dis J 2004; 23: 806-814.
70. Haataja P, Korhonen P, Ojala R, Hirvonen M, Korppi M, Gissler M, et al. Hospital admissions for lower respiratory tract infections after early-, late-, and post-term birth. Paediatr Perinat Epidemiol 2020; 34: 139-149.
71. Ghosh S and Klein RS. Sex drives dimorphic immune responses to viral infections. J Immunol 2017; 198: 1782-1790.
72. Klein SL and Flanagan KL. Sex differences in immune responses. Nat Rev Immunol 2016; 16: 626-638.
73. Hoo A, Dezateux C, Hanrahan JP, Cole TJ, Tepper RS and Stocks J. Sex-specific prediction equations for Vmax(FRC) in infancy: a multicenter collaborative study. Am J Respir Crit Care Med 2002; 165: 1084-1092.
74. Homaira N, Mallitt K, Oei J, Hilder L, Bajuk B, Lui K, et al. Risk factors associated with RSV hospitalisation in the first 2 years of life, among different subgroups of children in NSW: a whole-of-population-based cohort study. BMJ Open 2016; 6: e011398.
75. Semple MG, Taylor-Robinson DC, Lane S and Smyth RL. Household tobacco smoke and admission weight predict severe bronchiolitis in infants independent of deprivation: prospective cohort study. PLoS One 2011; 6: e22425.
76. Johnson J, Gonzales R, Olson S, Wright P and Graham B. The histopathology of fatal untreated human respiratory syncytial virus infection. Mod Pathol 2007; 20: 108-119.
77. Everard ML, Swarbrick A, Wrightham M, McIntyre J, Dunkley C, James PD, et al. Analysis of cells obtained by bronchial lavage of infants with respiratory syncytial virus infection. Arch Dis Child 1994; 71: 428-432.
78. Yasui K, Baba A, Iwasaki Y, Kubo T, Aoyama K, Mori T, et al. Neutrophil-mediated inflammation in respiratory syncytial viral bronchiolitis. Pediatr Int 2005; 47: 190-195.
79. Bohmwald K, Espinoza JA, Rey-Jurado E, Gómez RS, González PA, Bueno SM, et al. Human respiratory syncytial virus: infection and pathology. Semin Respir Crit Care Med 2016; 37: 522-537.
80. Renko M and Tapiainen T. Change in respiratory syncytial virus seasonality in Finland. Acta Paediatr 2020; 109: 202-203.
81. Laham FR, Mansbach JM, Piedra PA, Hasegawa K, Sullivan AF, Espinola JA, et al. Clinical profiles of respiratory syncytial virus subtypes A and B among children hospitalized with bronchiolitis. Pediatr Infect Dis J 2017; 36: 808-810.

82. Simon AK, Hollander GA and McMichael A. Evolution of the immune system in humans from infancy to old age. Proc Biol Sci 2015; 282: 20143085.
83. Arora S, Ahmad S, Irshad R, Goyal Y, Rafat S, Siddiqui N, et al. TLRs in pulmonary diseases. Life Sci 2019; 233: 116671.
84. Alvarez AE, Marson FAL, Bertuzzo CS, Bastos JCS, Baracat ECE, Brandão MB, et al. Association between single nucleotide polymorphisms in TLR4, TLR2, TLR9, VDR, NOS2 and CCL5 genes with acute viral bronchiolitis. Gene 2018; 645: 7-17.
85. Tal G, Mandelberg A, Dalal I, Cesar K, Somekh E, Tal A, et al. Association between common Toll-like receptor 4 mutations and severe respiratory syncytial virus disease. J Infect Dis 2004; 189: 2057-2063.
86. Zhou J, Zhang X, Liu S, Wang Z, Chen Q, Wu Y, et al. Genetic association of TLR4 Asp299Gly, TLR4 Thr399Ile, and CD14 C-159T polymorphisms with the risk of severe RSV infection: a meta-analysis. Influenza Other Respir Viruses 2016; 10: 224-233.
87. Legg JP, Hussain IR, Warner JA, Johnston SL and Warner JO. Type 1 and type 2 cytokine imbalance in acute respiratory syncytial virus bronchiolitis. Am J Respir Crit Care Med 2003; 168: 633-639.
88. Kidd P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. Altern Med Rev 2003; 8: 223-246.
89. Annunziato F, Romagnani C and Romagnani S. The 3 major types of innate and adaptive cell-mediated effector immunity. J Allergy Clin Immunol 2015; 135: 626-635.
90. Lutfi MF. The physiological basis and clinical significance of lung volume measurements. Multidiscip Respir Med 2017; 12: 3.
91. Voets, PJGM and van Helvoort, HAC. The role of equal pressure points in understanding pulmonary diseases. Adv Physiol Educ 2013; 37: 266-267.
92. Allen JL. Airway function throughout the lifespan: Pediatric origins of adult respiratory disease. Pediatr Investig 2019; 3: 236-244.
93. Pedersen O and Butler J. Expiratory flow limitation. Compr Physiol 2011; 1: 1861-1882.
94. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005; 26: 319-338.
95. Mitzner W. Mechanics of the lung in the 20th century. Compr Physiol 2011; 1: 2009-2027.
96. Vogt B, Falkenberg C, Weiler N and Frerichs I. Pulmonary function testing in children and infants. Physiol Meas 2014; 35: 59.
97. Tepper RS, Wise RS, Covar R, Irvin CG, Kercsmar CM, Kraft M, et al. Asthma outcomes: pulmonary physiology. J Allergy Clin Immunol 2012; 129: 65.
98. Bar-Yishay E, Amirav I and Goldberg S. Comparison of maximal midexpiratory flow rate and forced expiratory flow at 50% of vital capacity in children. Chest 2003; 123: 731-735.
99. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J 2005; 26: 948-968.
100. Hoesterey D, Das N, Janssens W, Buhr RG, Martinez FJ, Cooper CB, et al. Spirometric indices of early airflow impairment in individuals at risk of developing COPD: Spirometry beyond FEV1/FVC. Respir Med 2019; 156: 58-68.
101. Johns DP, Das A, Toelle BG, Abramson MJ, Marks GB, Wood-Baker R, et al. Improved spirometric detection of small airway narrowing: concavity in the expiratory flow-volume curve in people aged over 40 years. Int J Chron Obstruct Pulmon Dis 2017; 12: 3567-3577.

102. Calverley PMA, Albert P and Walker PP. Bronchodilator reversibility in chronic obstructive pulmonary disease: use and limitations. Lancet Respir Med 2013; 1: 564-573.
103. Janson C, Malinovschi A, Amaral AFS, Accordini S, Bousquet J, Buist AS, et al. Bronchodilator reversibility in asthma and COPD: findings from three large population studies. Eur Respir J 2019; 54.
104. Zhang L, He L, Gong J and Liu C. Risk factors associated with irreversible airway obstruction in asthma: a systematic review and meta-analysis. Biomed Res Int 2016; 2016: 9868704.
105. Elias JA. Airway remodeling in asthma. Unanswered questions. Am J Respir Crit Care Med 2000; 161: 168.
106. Limb SL, Brown KC, Wood RA, Wise RA, Eggleston PA, Tonascia J, et al. Irreversible lung function deficits in young adults with a history of childhood asthma. Journal of allergy and clinical immunology 2005; 116: 1213-1219.
107. Global Initiative for Chronic Obstructive Pulmonary Disease. Global Strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. Updated 2020. https://www.goldcopd.org/. Date last accessed: April 7th, 2021.
108. Postma DS, Brusselle G, Bush A and Holloway JW. I have taken my umbrella, so of course it does not rain. Thorax 2012; 67: 88-89.
109. Kaminsky DA. What does airway resistance tell us about lung function? Respir Care 2012; 57: 85-99.
110. Macklem PT. The physiology of small airways. Am J Respir Crit Care Med 1998; 157: 181.
111. Brashier B and Salvi S. Measuring lung function using sound waves: role of the forced oscillation technique and impulse oscillometry system. Breathe (Sheff) 2015; 11: 57-65.
112. Bickel S, Popler J, Lesnick B and Eid N. Impulse oscillometry: interpretation and practical applications. Chest 2014; 146: 841-847.
113. Smith H, Reinhold P and Goldman M. Chapter 5. Forced oscillation technique and impulse oscillometry. Eur Respir Mon 2005; 31: 72-105.
114. Nair A, Ward J and Lipworth BJ. Comparison of bronchodilator response in patients with asthma and healthy subjects using spirometry and oscillometry. Ann Allergy Asthma Immunol 2011; 107: 317-322.
115. Vink GR, Arets HGM, van der Laag J and van der Ent, Cornelis K. Impulse oscillometry: a measure for airway obstruction. Pediatr Pulmonol 2003; 35: 214-219.
116. Manoharan A, Anderson WJ, Lipworth J and Lipworth BJ. Assessment of spirometry and impulse oscillometry in relation to asthma control. Lung 2015; 193: 47-51.
117. Björn L, Erik M, Per T, Mikael N and Jenny H. Agreement between spirometry and impulse oscillometry for lung function assessment in 6-year-old children born extremely preterm and at term. Pediatr Pulmonol 2020; 55: 2745-2753.
118. Shi Y, Aledia AS, Tatavoosian AV, Vijayalakshmi S, Galant SP and George SC. Relating small airways to asthma control by using impulse oscillometry in children. J Allergy Clin Immunol 2012; 129: 671-678.
119. Komarow HD, Skinner J, Young M, Gaskins D, Nelson C, Gergen PJ, et al. A study of the use of impulse oscillometry in the evaluation of children with asthma: analysis of lung parameters, order effect, and utility compared with spirometry. Pediatr Pulmonol 2012; 47: 18-26.
120. Schittny JC. Development of the lung. Cell Tissue Res 2017; 367: 427-444.

121. Joshi S and Kotecha S. Lung growth and development. Early Hum Dev 2007; 83: 789-794.
122. Merkus PJ, ten Have-Opbroek AA and Quanjer PH. Human lung growth: a review. Pediatr Pulmonol 1996; 21: 383-397.
123. Bush A. COPD: a pediatric disease. COPD 2008; 5: 53-67.
124. Martinez FD. Early-life origins of chronic obstructive pulmonary disease. N Engl J Med 2016; 375: 871-878.
125. Bui DS, Lodge CJ, Burgess JA, Lowe AJ, Perret J, Bui MQ, et al. Childhood predictors of lung function trajectories and future COPD risk: a prospective cohort study from the first to the sixth decade of life. Lancet Respir Med 2018; 6: 535-544.
126. Klimentidis YC, Vazquez AI, de Los Campos G, Allison DB, Dransfield MT and Thannickal VJ. Heritability of pulmonary function estimated from pedigree and whole-genome markers. Front Genet 2013; 4: 174.
127. Jayes L, Haslam PL, Gratziou CG, Powell P, Britton J, Vardavas C, et al. SmokeHaz: Systematic reviews and meta-analyses of the effects of smoking on respiratory health. Chest 2016; 150: 164-179.
128. Gold DR, Wang X, Wypij D, Speizer FE, Ware JH and Dockery DW. Effects of cigarette smoking on lung function in adolescent boys and girls. N Engl J Med 1996; 335: 931-937.
129. Quanjer P, Borsboom G, Kivastik J, Merkus P, Hankinson J, Houthuijs D, et al. Cross-sectional and longitudinal spirometry in children and adolescents: interpretative strategies. Am J Respir Crit Care Med 2008; 178: 1262-1270.
130. Wang X, Dockery DW, Wypij D, Fay ME and Ferris BG. Pulmonary function between 6 and 18 years of age. Pediatr Pulmonol 1993; 15: 75-88.
131. Green M, Mead J and Turner JM. Variability of maximum expiratory flow-volume curves. J Appl Physiol 1974; 37: 67-74.
132. Martin TR, Feldman HA, Fredberg JJ, Castile RG, Mead J and Wohl ME. Relationship between maximal expiratory flows and lung volumes in growing humans. J Appl Physiol (1985) 1988; 65: 822-828.
133. Smith BM, Kirby M, Hoffman EA, Kronmal RA, Aaron SD, Allen NB, et al. Association of dysanapsis with chronic obstructive pulmonary disease among older adults. JAMA 2020; 323: 2268-2280.
134. Morgan WJ, Stern DA, Sherrill DL, Guerra S, Holberg CJ, Guilbert TW, et al. Outcome of asthma and wheezing in the first 6 years of life: Follow-up through adolescence. Am J Respir Crit Care Med 2005; 172: 1253-1258.
135. Guerra S, Lombardi E, Stern DA, Sherrill DL, Gilbertson-Dahdal D, Wheatley-Guy CM, et al. Fetal origins of asthma: a longitudinal study from birth to age 36 years. Am J Respir Crit Care Med 2020; 202: 1646-1655.
136. Hallas HW, Chawes BL, Rasmussen MA, Arianto L, Stokholm J, Bønnelykke K, et al. Airway obstruction and bronchial reactivity from age 1 month until 13 years in children with asthma: a prospective birth cohort study. PLoS Med 2019; 16: e1002722.
137. Hallas HW, Chawes BL, Arianto L, Rasmussen MA, Kunøe A, Stokholm J, et al. Children with asthma have fixed airway obstruction through childhood unaffected by exacerbations. J Allergy Clin Immunol Pract 2020; 8: 1263-1271.e3.
138. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med 2003; 349: 1414-1422.

139. Tai A, Tran H, Roberts M, Clarke N, Gibson A, Vidmar S, et al. Outcomes of childhood asthma to the age of 50 years. J Allergy Clin Immunol 2014; 133: 1572-1578.e3.
140. Gauderman WJ, Urman R, Avol E, Berhane K, McConnell R, Rappaport E, et al. Association of improved air quality with lung development in children. N Engl J Med 2015; 372: 905-913.
141. Kozlowska WJ, Bush A, Wade A, Aurora P, Carr SB, Castle RA, et al. Lung function from infancy to the preschool years after clinical diagnosis of cystic fibrosis. Am J Respir Crit Care Med 2008; 178: 42-49.
142. Pfeffer G and Povitz M. Respiratory management of patients with neuromuscular disease: Current perspectives. Degener Neurol Neuromuscul Dis 2016; 6: 111-118.
143. Al-Muhsen S, Johnson JR and Hamid Q. Remodeling in asthma. J Allergy Clin Immunol 2011; 128: 451-464.
144. Jeffery PK. Remodeling in asthma and chronic obstructive lung disease. Am J Respir Crit Care Med 2001; 164: 28.
145. Fehrenbach H, Wagner C and Wegmann M. Airway remodeling in asthma: What really matters. Cell Tissue Res 2017; 367: 551-569.
146. Hoshino M, Nakamura Y, Sim JJ, Yamashiro Y, Uchida K, Hosaka K, et al. Inhaled corticosteroid reduced lamina reticularis of the basement membrane by modulation of insulin-like growth factor-I expression in bronchial asthma. Clin Exp Allergy 1998; 28: 568-77.
147. Olivieri D, Chetta A, Del Donno M, Bertorelli G, Casalini A, Pesci A, et al. Effect of short-term treatment with low-dose inhaled fluticasone propionate on airway inflammation and remodeling in mild asthma: a placebo-controlled study. Am J Respir Crit Care Med 1997; 155: 1864-71.
148. Trigg CJ, Manolitsas ND, Wang J, Calderón MA, McAulay A, Jordan SE, et al. Placebo-controlled immunopathologic study of four months of inhaled corticosteroids in asthma. Am J Respir Crit Care Med 1994; 150: 17-22.
149. Grainge CL, Lau LCK, Ward JA, Dulay V, Lahiff G, Wilson S, et al. Effect of bronchoconstriction on airway remodeling in asthma. N Engl J Med 2011; 364: 2006-2015.
150. de Blic J, Tillie-Leblond I, Tonnel AB, Jaubert F, Scheinmann P and Gosset P. Difficult asthma in children: An analysis of airway inflammation. J Allergy Clin Immunol 2004; 113: 94-100.
151. Regamey N, Ochs M, Hilliard TN, Mühlfeld C, Cornish N, Fleming L, et al. Increased airway smooth muscle mass in children with asthma, cystic fibrosis, and non-cystic fibrosis bronchiectasis. Am J Respir Crit Care Med 2008; 177: 837-843.
152. Turato G, Barbato A, Baraldo S, Zanin ME, Bazzan E, Lokar-Oliani K, et al. Nonatopic children with multitrigger wheezing have airway pathology comparable to atopic asthma. Am J Respir Crit Care Med 2008; 178: 476-482.
153. Saglani S, Payne DN, Zhu J, Wang Z, Nicholson AG, Bush A, et al. Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. Am J Respir Crit Care Med 2007; 176: 858-864.
154. Payne DNR, Rogers AV, Adelroth E, Bandi V, Guntupalli KK, Bush A, et al. Early thickening of the reticular basement membrane in children with difficult asthma. Am J Respir Crit Care Med 2003; 167: 78-82.
155. Saglani S, Malmström K, Pelkonen AS, Malmberg LP, Lindahl H, Kajosaari M, et al. Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. Am J Respir Crit Care Med 2005; 171: 722-727.

156. Malmström K, Pelkonen AS, Malmberg LP, Sarna S, Lindahl H, Kajosaari M, et al. Lung function, airway remodelling and inflammation in symptomatic infants: Outcome at 3 years. Thorax 2011; 66: 157-162.
157. Aghasafari P, George U and Pidaparti R. A review of inflammatory mechanism in airway diseases. Inflamm Res 2019; 68: 59-74.
158. Barnes PJ. Small airway fibrosis in COPD. Int J Biochem Cell Biol 2019; 116: 105598.
159. Chawes BLK, Poorisrisak P, Johnston SL and Bisgaard H. Neonatal bronchial hyperresponsiveness precedes acute severe viral bronchiolitis in infants. J Allergy Clin Immunol 2012; 130: 354-361.e3.
160. Zomer-Kooijker K, Uiterwaal, Cuno S. P. M., van der Gugten, Anne C., Wilbrink B, Bont LJ and van der Ent, Cornelis K. Decreased lung function precedes severe respiratory syncytial virus infection and post-respiratory syncytial virus wheeze in term infants. Eur Respir J 2014; 44: 666-674.
161. Henry RL, Milner AD, Stokes GM, Hodges IG and Groggins RC. Lung function after acute bronchiolitis. Arch Dis Child 1983; 58: 60-63.
162. Kotaniemi-Syrjänen A, Vainionpää R, Reijonen TM, Waris M, Korhonen K and Korppi M. Rhinovirus-induced wheezing in infancy - The first sign of childhood asthma? J Allergy Clin Immunol 2003; 111: 66-71.
163. Simões EAF, Carbonell-Estrany X, Rieger CHL, Mitchell I, Fredrick L and Groothuis JR. The effect of respiratory syncytial virus on subsequent recurrent wheezing in atopic and nonatopic children. J Allergy Clin Immunol 2010; 126: 256-262.
164. Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ and Martinez FD. Tucson Children's Respiratory Study: 1980 to present. J Allergy Clin Immunol 2003; 111: 661-675; quiz 676.
165. Lucas JS, Foreman CT and Clough JB. Measuring lung function in infancy. Respir Med 2000; 94: 641-647.
166. Stein RT, Holberg CJ, Morgan WJ, Wright AL, Lombardi E, Taussig L, et al. Peak flow variability, methacholine responsiveness and atopy as markers for detecting different wheezing phenotypes in childhood. Thorax 1997; 52: 946-952.
167. Collins SA, Pike KC, Inskip HM, Godfrey KM, Roberts G, Holloway JW, et al. Validation of novel wheeze phenotypes using longitudinal airway function and atopic sensitization data in the first 6 years of life: evidence from the Southampton Women's survey. Pediatr Pulmonol 2013; 48: 683-692.
168. Henderson J, Granell R, Heron J, Sherriff A, Simpson A, Woodcock A, et al. Associations of wheezing phenotypes in the first 6 years of life with atopy, lung function and airway responsiveness in mid-childhood. Thorax 2008; 63: 974-980.
169. Golding J, Pembrey M and Jones R. ALSPAC--the Avon Longitudinal Study of Parents and Children. I. Study methodology. Paediatr Perinat Epidemiol 2001; 15: 74-87.
170. Young S, O'Keeffe PT, Arnott J and Landau LI. Lung function, airway responsiveness, and respiratory symptoms before and after bronchiolitis. Arch Dis Child 1995; 72: 16-24.
171. Turner SW, Young S, Landau LI and Le Souëf PN. Reduced lung function both before bronchiolitis and at 11 years. Arch Dis Child 2002; 87: 417-420.
172. Custovic A, Simpson BM, Murray CS, Lowe L and Woodcock A. The National Asthma Campaign Manchester Asthma and Allergy Study. Pediatr Allergy Immunol 2002; 13: 32-37.
173. Belgrave DCM, Buchan I, Bishop C, Lowe L, Simpson A and Custovic A. Trajectories of lung function during childhood. Am J Respir Crit Care Med 2014; 189: 1101-1109.

174. Håland G, Carlsen K, Devulapalli CS, Pettersen M, Mowinckel P and Lødrup Carlsen KC. Lung function development in the first 2 yr of life is independent of allergic diseases by 2 yr. Pediatr Allergy Immunol 2007; 18: 528-534.
175. Backman K, Piippo-Savolainen E, Ollikainen H, Koskela H and Korppi M. Adults face increased asthma risk after infant RSV bronchiolitis and reduced respiratory health-related quality of life after RSV pneumonia. Acta Paediatr 2014; 103: 850-855.
176. Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen TM, Korhonen K and Korppi MO. Lung function and bronchial hyper-responsiveness 11 years after hospitalization for bronchiolitis. Acta Paediatr 2007; 96: 1464-1469.
177. Sørensen KG, Øymar K, Dalen I, Halvorsen T and Mikalsen IB. Lung function and bronchial hyper-reactivity from 11 to 18 years in children with bronchiolitis in infancy. Pediatr Allergy Immunol 2020; 31: 57-65.
178. Kotaniemi-Syrjänen A, Reijonen TM, Korhonen K, Waris M, Vainionpää R and Korppi M. Wheezing due to rhinovirus infection in infancy: Bronchial hyperresponsiveness at school age. Pediatr Int 2008; 50: 506-510.
179. Kotaniemi-Syrjänen A, Laatikainen A, Waris M, Reijonen TM, Vainionpää R and Korppi M. Respiratory syncytial virus infection in children hospitalized for wheezing: virus-specific studies from infancy to preschool years. Acta Paediatr 2005; 94: 159-165.
180. Koponen P, Helminen M, Paassilta M, Luukkaala T and Korppi M. Preschool asthma after bronchiolitis in infancy. Eur Respir J 2012; 39: 76-80.
181. Törmänen S, Lauhkonen E, Riikonen R, Koponen P, Huhtala H, Helminen M, et al. Risk factors for asthma after infant bronchiolitis. Allergy 2018; 73: 916-922.
182. Stein RT, Holberg CJ, Sherrill D, Wright AL, Morgan WJ, Taussig L, et al. Influence of parental smoking on respiratory symptoms during the first decade of life: the Tucson Children's Respiratory Study. Am J Epidemiol 1999; 149: 1030-1037.
183. Hanrahan JP, Tager IB, Segal MR, Tosteson TD, Castile RG, Van Vunakis H, et al. The effect of maternal smoking during pregnancy on early infant lung function. Am Rev Respir Dis 1992; 145: 1129-1135.
184. Bisgaard H, Loland L, Holst KK and Pipper CB. Prenatal determinants of neonatal lung function in high-risk newborns. J Allergy Clin Immunol 2009; 123: 651-4.
185. Stick SM, Burton PR, Gurrin L, Sly PD and LeSouëf PN. Effects of maternal smoking during pregnancy and a family history of asthma on respiratory function in newborn infants. Lancet 1996; 348: 1060-1064.
186. Milner AD, Marsh MJ, Ingram DM, Fox GF and Susiva C. Effects of smoking in pregnancy on neonatal lung function. Arch Dis Child Fetal Neonatal Ed 1999; 80: 8.
187. Tepper RS, Williams-Nkomo T, Martinez T, Kisling J, Coates C and Daggy J. Parental smoking and airway reactivity in healthy infants. Am J Respir Crit Care Med 2005; 171: 78-82.
188. Bisgaard H, Jensen SM and Bønnelykke K. Interaction between asthma and lung function growth in early life. Am J Respir Crit Care Med 2012; 185: 1183-1189.
189. Turner S, Fielding S, Mullane D, Cox DW, Goldblatt J, Landau L, et al. A longitudinal study of lung function from 1 month to 18 years of age. Thorax 2014; 69: 1015-1020.
190. Guerra S, Stern DA, Zhou M, Sherrill DL, Wright AL, Morgan WJ, et al. Combined effects of parental and active smoking on early lung function deficits: a prospective study from birth to age 26 years. Thorax 2013; 68: 1021-1028.
191. Piippo-Savolainen E, Remes S, Kannisto S, Korhonen K and Korppi M. Early predictors for adult asthma and lung function abnormalities in infants hospitalized

for bronchiolitis: a prospective 18- to 20-year follow-up. Allergy Asthma Proc 2006; 27: 341-349.
192. Goksör E, Amark M, Alm B, Gustafsson PM and Wennergren G. The impact of pre- and post-natal smoke exposure on future asthma and bronchial hyper-responsiveness. Acta Paediatr 2007; 96: 1030-1035.
193. Littleton SW. Impact of obesity on respiratory function. Respirology 2012; 17: 43-49.
194. Dixon AE and Peters U. The effect of obesity on lung function. Expert Rev Respir Med 2018; 12: 755-767.
195. Forno E, Han Y, Mullen J and Celedón JC. Overweight, obesity, and lung function in children and adults-a meta-analysis. J Allergy Clin Immunol Pract 2018; 6: 570-581.e10.
196. Bekkers MBM, Wijga AH, de Jongste JC, Kerkhof M, Postma D, Gehring U, et al. Waist circumference, BMI, and lung function in 8-year-old children: the PIAMA birth cohort study. Pediatr Pulmonol 2013; 48: 674-682.
197. Lang J, Holbrook J, Wise R, Dixon A, Teague W, Wei C, et al. Obesity in children with poorly controlled asthma: sex differences. Pediatr Pulmonol 2013; 48: 847-856.
198. Tantisira KG, Litonjua AA, Weiss ST and Fuhlbrigge AL. Association of body mass with pulmonary function in the Childhood Asthma Management Program (CAMP). Thorax 2003; 58: 1036-1041.
199. Ulger Z, Demir E, Tanaç R, Gökşen D, Gülen F, Darcan S, et al. The effect of childhood obesity on respiratory function tests and airway hyperresponsiveness. Turk J Pediatr 2006; 48: 43-50.
200. Spathopoulos D, Paraskakis E, Trypsianis G, Tsalkidis A, Arvanitidou V, Emporiadou M, et al. The effect of obesity on pulmonary lung function of school aged children in Greece. Pediatr Pulmonol 2009; 44: 273-280.
201. Egan KB, Ettinger AS and Bracken MB. Childhood body mass index and subsequent physician-diagnosed asthma: A systematic review and meta-analysis of prospective cohort studies. BMC Pediatr 2013; 13: 121.
202. Papoutsakis C, Priftis KN, Drakouli M, Prifti S, Konstantaki E, Chondronikola M, et al. Childhood overweight/obesity and asthma: is there a link? A systematic review of recent epidemiologic evidence. J Acad Nutr Diet 2013; 113: 77-105.
203. Forno E, Lescher R, Strunk R, Weiss S, Fuhlbrigge A and Celedón JC. Decreased response to inhaled steroids in overweight and obese asthmatic children. J Allergy Clin Immunol 2011; 127: 741-749.
204. Klepaker G, Svendsen MV, Hertel JK, Holla ØL, Henneberger PK, Kongerud J, et al. Influence of obesity on work ability, respiratory symptoms, and lung function in adults with asthma. Respiration 2019; 98: 473-481.
205. Lang JE, Hossain J, Smith K and Lima JJ. Asthma severity, exacerbation risk, and controller treatment burden in underweight and obese children. J Asthma 2012; 49: 456-463.
206. Holguin F, Bleecker ER, Busse WW, Calhoun WJ, Castro M, Erzurum SC, et al. Obesity and asthma: an association modified by age of asthma onset. J Allergy Clin Immunol 2011; 127: 1486-1493.e2.
207. Wang L, Murk W and DeWan AT. Genome-wide gene by environment interaction analysis identifies common SNPs at 17q21.2 that are associated with increased body mass index only among asthmatics. PLoS One 2015; 10: e0144114.

208. Törmänen S, Lauhkonen E, Saari A, Koponen P, Korppi M and Nuolivirta K. Excess weight in preschool children with a history of severe bronchiolitis is associated with asthma. Pediatr Pulmonol 2015; 50: 424-430.
209. Kawasaki T and Kawai T. Toll-like receptor signaling pathways. Front Immunol 2014; 5: 461.
210. Tapping RI, Omueti KO and Johnson CM. Genetic polymorphisms within the human Toll-like receptor 2 subfamily. Biochem Soc Trans 2007; 35: 1445-1448.
211. Guan Y, Ranoa DRE, Jiang S, Mutha SK, Li X, Baudry J, et al. Human TLRs 10 and 1 share common mechanisms of innate immune sensing but not signaling. J Immunol 2010; 184: 5094-5103.
212. Su S, Tao L, Deng Z, Chen W, Qin S and Jiang H. TLR10: insights, controversies and potential utility as a therapeutic target. Scand J Immunol 2021; 93: e12988.
213. Triantafilou K, Vakakis E, Richer EAJ, Evans GL, Villiers JP and 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: 22-29.
214. Murawski MR, Bowen GN, Cerny AM, Anderson LJ, Haynes LM, Tripp RA, et al. Respiratory syncytial virus activates innate immunity through Toll-like receptor 2. J Virol 2009; 83: 1492-1500.
215. Hasan U, Chaffois C, Gaillard C, Saulnier V, Merck E, Tancredi S, et al. Human TLR10 is a functional receptor, expressed by B cells and plasmacytoid dendritic cells, which activates gene transcription through MyD88. J Immunol 2005; 174: 2942-2950.
216. Oosting M, Cheng S, Bolscher JM, Vestering-Stenger R, Plantinga TS, Verschueren IC, et al. Human TLR10 is an anti-inflammatory pattern-recognition receptor. Proc Natl Acad Sci USA 2014; 111: 4478.
217. Hess NJ, Felicelli C, Grage J and Tapping RI. TLR10 suppresses the activation and differentiation of monocytes with effects on DC-mediated adaptive immune responses. J Leukoc Biol 2017; 101: 1245-1252.
218. Jiang S, Li X, Hess NJ, Guan Y and Tapping RI. TLR10 is a negative regulator of both MyD88-dependent and -independent TLR signaling. J Immunol 2016; 196: 3834-3841.
219. Mukherjee S, Karmakar S and Babu SPS. TLR2 and TLR4 mediated host immune responses in major infectious diseases: a review. Braz J Infect Dis 2016; 20: 193-204.
220. Tesse R, Pandey RC and Kabesch M. Genetic variations in Toll-like receptor pathway genes influence asthma and atopy. Allergy 2011; 66: 307-316.
221. Klaassen EM, Thönissen BE, van Eys G, Dompeling E and Jöbsis Q. A systematic review of CD14 and Toll-like receptors in relation to asthma in Caucasian children. Allergy Asthma Clin Immunol 2013; 9: 10.
222. Teräsjärvi JT, Toivonen L, Vuononvirta J, Mertsola J, Peltola V and He Q. TLR4 polymorphism, nasopharyngeal bacterial colonization, and the development of childhood asthma: a prospective birth-cohort study in Finnish children. Genes (Basel) 2020; 11.
223. Gao Z, Dosman JA, Rennie DC, Schwartz DA, Yang IV, Beach J, et al. Association of Toll-like receptor 2 gene polymorphisms with lung function in workers in swine operations. Ann Allergy Asthma Immunol 2013; 110: 44-50.e1.
224. Haerynck F, Mahachie John JM, Van Steen K, Schelstraete P, Van daele S, Loeys B, et al. Genetic variations in Toll-like receptor pathway and lung function decline in Cystic fibrosis patients. Hum Immunol 2013; 74: 1649-1655.

225. Budulac SE, Boezen HM, Hiemstra PS, Lapperre TS, Vonk JM, Timens W, et al. Toll-like receptor (TLR2 and TLR4) polymorphisms and chronic obstructive pulmonary disease. PLoS One 2012; 7: e43124.
226. Lauhkonen E, Koponen P, Vuononvirta J, Teräsjärvi J, Nuolivirta K, Toikka JO, et al. Gene polymorphism of Toll-Like receptors and lung function at five to seven years of age after infant bronchiolitis. PLoS One 2016; 11: e0146526.
227. Törmänen S, Korppi M, Teräsjärvi J, Vuononvirta J, Koponen P, Helminen M, et al. Polymorphism in the gene encoding toll-like receptor 10 may be associated with asthma after bronchiolitis. Sci Rep 2017; 7: 2956.
228. Törmänen S, Korppi M, Lauhkonen E, Koponen P, Teräsjärvi J, Vuononvirta J, et al. Toll-like receptor 1 and 10 gene polymorphisms are linked to postbronchiolitis asthma in adolescence. Acta Paediatr 2018; 107: 134-139.
229. Koponen P, Vuononvirta J, Nuolivirta K, Helminen M, He Q and Korppi M. The association of genetic variants in Toll-like receptor 2 subfamily with allergy and asthma after hospitalization for bronchiolitis in infancy. Pediatr Infect Dis J 2014; 33: 463-466.
230. Backman K, Nuolivirta K, Ollikainen H, Korppi M and Piippo-Savolainen E. Low eosinophils during bronchiolitis in infancy are associated with lower risk of adulthood asthma. 2015; 26: 668-673.
231. Malmberg LP, Pelkonen A, Poussa T, Pohjanpalo A, Haahtela T and Turpeinen M. Determinants of respiratory system input impedance and bronchodilator response in healthy Finnish preschool children. Clinical Physiology and Functional Imaging 2002; 22: 64-71.
232. Koillinen H, Wanne O, Niemi V and Laakkonen E. Terveiden suomalaislasten spirometrian ja uloshengityksen huippuvirtauksen viitearvot. Suom Lääkäril 1998; 5: 395-405. In Finnish.
233. Saari A, Sankilampi U, Hannila M, Kiviniemi V, Kesseli K and Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: length/height-for-age, weight-for-length/height, and body mass index-for-age. Ann Med 2011; 43: 235-248.
234. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. Nature 2012; 491: 56-65.
235. Lauhkonen E, Riikonen R, Törmänen S, Koponen P, Nuolivirta K, Helminen M, et al. Impulse oscillometry at preschool age is a strong predictor of lung function by flow-volume spirometry in adolescence. Pediatr Pulmonol 2018; 53: 552-558.
236. Knihtilä H, Kotaniemi-Syrjänen A, Mäkelä MJ, Bondestam J, Pelkonen AS and Malmberg LP. Preschool oscillometry and lung function at adolescence in asthmatic children. Pediatr Pulmonol 2015; 50: 1205-1213.
237. Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen TM, Korhonen K and Korppi MO. Teenage asthma after severe early childhood wheezing: an 11-year prospective follow-up. Pediatr Pulmonol 2005; 40: 316-323.
238. Zhang Z and Schluesener HJ. Mammalian Toll-like receptors: from endogenous ligands to tissue regeneration. Cell Mol Life Sci 2006; 63: 2901-2907.

ORIGINAL PUBLICATIONS

PUBLICATION I

Prospective study confirms that bronchiolitis in early infancy increases the risk of reduced lung function at 10-13 years of age.

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REGULAR ARTICLE

Prospective study confirms that bronchiolitis in early infancy increases the risk of reduced lung function at 10–13 years of age

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ABSTRACT

Aim: This study evaluated children hospitalised for bronchiolitis at less than six months of age to see if they had reduced lung function in early adolescence.

Methods: We have prospectively followed 166 children hospitalised for infant bronchiolitis in 2001–2004 at Tampere University Hospital, Finland. At 10–13 years of age, flow-volume spirometry was measured in 89 cases and 108 controls without infant bronchiolitis from the local population register. Parameters of flow-volume spirometry before and after bronchodilation were analysed.

Results: Forced expiratory volume in one second/forced vital capacity (FEV1/FVC) after bronchodilation was lower in cases than controls. FEV1 was pathological – under the 5th percentile of the national references – in 25% of cases and 12% of controls ($p = 0.020$) before bronchodilation and in 18% of cases and 5% of controls ($p = 0.003$) after bronchodilation. FEV1/FVC was pathological in 25% of cases and 13% of controls ($p = 0.034$) before bronchodilation. Logistic regression, adjusted for current asthma and maternal smoking, showed that infant bronchiolitis was associated with pathological FEV1 before (odds ratio 2.4) and after (odds ratio 4.4) bronchodilation. The result was similar for positive respiratory syncytial virus cases.

Conclusion: Reduced FEV1 after bronchodilation was found in early adolescence after infant bronchiolitis, suggesting irreversible bronchial obstruction.

INTRODUCTION

Bronchiolitis is a viral respiratory tract infection that affects infants and the peak incidence occurs when children are less than six months of age (1). Bronchiolitis begins with rhinitis, which is followed some days later by lower airway obstruction with tachypnoea and wheezing or crackles on auscultation (2). The respiratory syncytial virus (RSV) is the predominant causative agent before 12 months of age, and rhinoviruses are the main cause after that age (3).

Hospitalisation for bronchiolitis in infancy has been associated with later lung function reductions in previous postbronchiolitis studies at school age (4,5), in adolescence (6–9) and in adulthood (10–12). Changes in lung function may be irreversible in adults who had bronchiolitis in infancy (10–12). However, fewer studies have looked at irreversibility in children after bronchiolitis in infancy. In early adolescence, flow-volume spirometry was examined after bronchodilation in a Swedish study, and RSV bronchiolitis in infancy was linked to irreversible reduction of forced expiratory volume in one second/forced vital capacity (FEV1/FVC) (7).

We have prospectively followed 166 children hospitalised for bronchiolitis in 2001–2004 at the age of less than six

Abbreviations
CI, Confidence interval; FEV1, Forced expiratory volume in one second; FVC, Forced vital capacity; ICS, Inhaled corticosteroids; MEF50, Mid-expiratory flow at 50% of forced vital capacity; OR, Odds ratio; RSV, Respiratory syncytial virus; SD, Standard deviation.

Key notes
- This study evaluated 89 children hospitalised for bronchiolitis at less than six months of age, to see if they had reduced lung function in early adolescence compared to 108 controls.
- Early-life bronchiolitis was associated with an irreversible reduction of forced expiratory volume in one second in multivariate analyses at 10–13 years of age.
- Our findings confirmed irreversible bronchial obstruction in early adolescence after early-life bronchiolitis.

months in the Department of Paediatrics, Tampere University Hospital, Finland (13). The viral aetiology of bronchiolitis was identified in nasopharyngeal aspirates by immunofluorescence and polymerase chain reaction (14). At the age of five to seven years, lung function was examined with impulse oscillometry, and complete data were available from 103 former bronchiolitis patients (15). Impulse oscillometry was considered abnormal in 21 of them, resistance was increased in eight and reactance was decreased in 19 compared to population-based references (15). However, irreversible lung function abnormality that did not respond to bronchodilators was only present in one of them (15).

We carried out a follow-up study when these children with bronchiolitis at less than six months of age were 10–13 years old. The earlier phases of the study were noncontrolled, but for this part of the study, we collected age-specific population-based controls from Pirkanmaa (Tampere) Hospital District. In this study, we measured lung function with flow-volume spirometry, as the children were now old enough to perform forced expiratory blows. We hypothesised that bronchiolitis in early infancy was associated with reduced lung function in early adolescence and that part of the changes would be irreversible. The aim of this study was to evaluate baseline and postbronchodilator lung function at 10–13 years of age after bronchiolitis in early infancy, with special focus on the RSV aetiology.

MATERIAL AND METHODS

Study design

This prospective postbronchiolitis study evaluated lung function by flow-volume spirometry before and after bronchodilator administration and by FEV1 response in the bronchodilation test at 10–13 years of age in children who were hospitalised for bronchiolitis at less than six months of age. The results were compared with population-based controls of the same age without a history of bronchiolitis in infancy. The available data also allowed us to study the association of lung function reduction with RSV aetiology of bronchiolitis.

Hospitalisation in infancy and follow-up visit at five to seven years of age

Full-term infants without any underlying illness or earlier history of wheezing who were hospitalised for viral bronchiolitis at the age of less than six months in the Department of Paediatrics, Tampere University Hospital, Pirkanmaa Hospital District, Finland, during three consecutive RSV epidemics in 2001–2004, were recruited in the follow-up study (14). Bronchiolitis was defined as a lower respiratory tract infection associated with wheezes or crackles on auscultation, and the viral aetiology – including RSV, rhinoviruses, adenovirus, human bocavirus, influenza A virus, metapneumovirus and parainfluenza type 1, 2 and 3 viruses – was assessed in nasopharyngeal aspirates by immunofluorescence and polymerase chain reaction (14).

As described earlier (13), 166 children attended a prospective follow-up study at the age of five to seven years: 39 were interviewed by phone, 127 attended a follow up-visit (13) and impulse oscillometry data were available from 103 children (15).

Follow-up visit at 10–13 years of age

At the age of 10–13 years, the original 166 children were invited to attend a control visit from June to August 2014 and in January 2015. We approached 664 controls – four for each case – who were matched by age and sex from the population register of the Pirkanmaa (Tampere) Hospital District. The exclusion criteria for the controls were a history of bronchiolitis and hospital admission for any respiratory infection during infancy.

As published in 2017 (16), the medical histories of the former bronchiolitis patients and the controls were collected using a structured questionnaire completed by the parents before the control visit. The questions charted maternal and paternal smoking when the children were infants, maternal smoking during pregnancy, the presence of doctor-diagnosed asthma in parents, the use of controller medication for asthma and the presence of asthma-suggestive symptoms. At the control visit, the children and parents were interviewed, the questionnaire data were checked, the children were clinically examined and then the children performed flow-volume spirometry before and after bronchodilator administration. Ongoing inhaled corticosteroid (ICS) medication, if present, was not interrupted before the lung function testing.

Current asthma was considered to be present if the child had used continuous ICS medication during the last 12 months, or alternatively, if the FEV1 change was 12% and 0.2 L or more in the bronchodilation test at the study visit and the child had also reported asthma-presumptive symptoms during the last 12 months (16). Such symptoms were repeated wheezing, or prolonged cough or night cough for four weeks or longer.

In all, 138 former bronchiolitis patients participated in the follow-up study at the age of 10–13 years, but 49 of the children only answered the questionnaire. The clinical characteristics of the children have previously been published (16). Children who just completed the questionnaire and children who underwent clinical examinations did not differ significantly in terms of allergic rhinitis, atopic dermatitis, current asthma, asthma diagnosis from 5–7 years to 11–13 years of ages or the recent use of bronchodilators or ICSs (16). Maternal asthma was present in 22/138 (15.9%) of the children who participated in the latest follow-up visit compared to 2/28 (7.1%) who did not participate ($p = 0.375$). The respective figures for maternal smoking in infancy were 37/138 (26.8%) and 10/28 (35.7%, $p = 0.340$).

Finally, 89 former bronchiolitis patients and 112 controls attended the clinical follow-up visit at 10–13 years of age, but four controls were not able to perform spirometry. Thus, this study presents the flow-volume spirometry results for

89 former bronchiolitis cases and 108 controls of the same age without bronchiolitis in infancy.

Spirometry

Both the cases and controls performed flow-volume spirometry with a calibrated spirometer Vmax Carefusion (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Forced expiratory blows were obtained in a sitting position wearing a nose clip. The technician demonstrated the procedure and the children repeated the manoeuvre until three acceptable flow-volume spirograms were obtained. These needed to show a rapid rise to peak flow and a long enough descending curve, of more than six-seconds, without artefacts, such as coughing, glottis closure or air leaking (17). In order for the spirograms to be accepted, the second highest FEV1 and FVC had to lie within 0.15 L of the highest value, and the highest FEV1 and FVC were picked for the analyses. FEV1/FVC and the mid-expiratory flow at 50% of FVC (MEF50) were printed from the spirogram with the highest sum of FEV_1 and FVC (17). The flow-volume spirometry parameters were given as percentages of population-based, sex-specific, height-related references, namely percentage of predicted. We used the pathological limits of FVC ≤81% for boys and ≤82% for girls, FEV1 ≤80% for boys and ≤82% for girls, FEV1/FVC ≤87% for boys and ≤88% for girls and MEF50 ≤64% for boys and ≤63% for girls corresponding to the 5th percentile in the national reference data (18).

After baseline measurements, the children received four separate 0.1 mg doses of salbutamol (Ventoline, GlaxoSmithKline, London, UK) through a spacer. Like the baseline manoeuvres, three acceptable flow-volume spirograms were recorded 15 minutes after bronchodilator administration and the percentage changes in FEV1 were calculated and compared to the baseline measurements.

Statistics

The data were analysed using SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). The results of the univariate analyses are expressed as means, standard deviations (SD) and 95% confidence intervals (95% CI) for continuous variables, and as frequencies and p values for categorised variables. The chi-square test or Fisher's exact test was used to calculate p values in the univariate analyses of categorised data, as appropriate. P values of <0.05 and 95% CIs that did not overlap were considered statistically significant. Logistic regression was used for the multivariate analyses of categorised flow-volume spirometry parameters, and the results are expressed as adjusted odds ratios (OR) and their 95% CIs.

Ethics

The Ethics Committee of the Tampere University Hospital (Pirkanmaa) District approved the study. Informed written consent was obtained from the parents before enrolling the children and at the control visits.

RESULTS

Subjects

The mean age was 11.6 ± 0.94 years (range 10.34–13.22) in the 89 former bronchiolitis patients versus 11.8 ± 0.98 years (range 10.24–13.49) in the 108 controls, and 44 (49.4%) cases versus 60 (55.6%) controls were boys. Viral data were available from 88/89 former bronchiolitis patients: RSV was identified in 63 (71.6%), rhinoviruses in 10 (11.4%) and other viruses in 12 (13.6%) cases. More than one virus was found in eight (9.1%) cases. The 10 (11.4%) cases who were negative for viruses were included in the non-RSV bronchiolitis group.

Univariate analyses

On average, FVC, FEV1, FEV1/FVC and MEF50 were within normal limits in both the baseline and postbronchodilator measurements, and even the lower limits of the 95% CIs were above the sex-specific, height-related limits of pathology (Table 1). Postbronchodilator FEV1/FVC was lower in cases than in controls as a continuous variable, but there were no significant differences between cases and controls in any other flow-volume spirometry parameter nor in the bronchodilation test result (Table 1). Baseline FEV1 and FEV1/FVC and postbronchodilator FEV1 were pathological more frequently in cases than in controls as categorised variables, but there were no significant differences between the groups in other flow-volume spirometry parameters or in the bronchodilation test result at any cut-off level of FEV1 (Table 2).

Table 1 Baseline and postbronchodilator parameters in flow-volume spirometry, expressed as continuous variables, in 89 former bronchiolitis patients (cases) and 108 controls

Parameters in spirometry	Cases		Controls	
	Mean (SD)	95% CI	Mean (SD)	95% CI
Baseline*				
FVC	95.9 (11.6)	93.4–98.3	96.5 (9.5)	94.7–98.3
FEV1	89.0 (11.4)	86.6–91.4	91.7 (9.6)	89.9–93.6
FEV1/FVC	93.3 (7.8)	91.6–94.9	95.2 (6.0)	94.0–96.3
MEF50	85.5 (21.3)	81.0–90.0	93.0 (22.6)	88.7–97.4
Postbronchodilator*				
FVC	96.5 (11.9)	94.0–99.0	96.9 (8.8)	95.2–98.5
FEV1	92.4 (11.9)	89.9–94.9	95.9 (9.1)	94.1–97.6
FEV1/FVC	**96.1 (6.7)**	**94.6–97.5**	**99.0 (5.6)**	**98.0–100.1**
MEF50	97.4 (22.9)	92.6–102.2	105.7 (22.9)	101.3–110.0
Bronchodilation test				
FEV1 change (in %)	3.9 (6.3)	2.6–5.3	4.7 (4.3)	3.8–5.5

CI = Confidence interval; FEV1 = Forced expiratory volume in one second; FVC = Forced vital capacity; MEF50 = Mid-expiratory flow at 50% of FVC; SD = Standard deviation.
Statistically significant findings are marked with bold font.
*Expressed as percentage of predicted.

Table 2 Pathological baseline and postbronchodilator parameters in flow-volume spirometry, expressed as categorised variables, in 89 former bronchiolitis patients (cases) and 108 controls

Parameters in spirometry	Cases No (%)	Controls No (%)	p value*
Baseline†			
FVC	8 (9.0)	5 (4.6)	0.220
FEV1	**22 (24.7)**	**13 (12.0)**	**0.020**
FEV1/FVC	**22 (24.7)**	**14 (13.0)**	**0.034**
MEF50	16 (18.0)	11 (10.2)	0.113
Postbronchodilator†			
FVC	9 (10.1)	6 (5.6)	0.230
FEV1	**16 (18.0)**	**5 (4.6)**	**0.003**
FEV1/FVC	9 (10.1)	5 (4.6)	0.136
MEF50	6 (6.7)	4 (3.7)	0.352
Bronchodilation test			
FEV1 change ≥9%	20 (22.5)	20 (18.5)	0.492
FEV1 change ≥12%	7 (7.9)	6 (5.6)	0.516
FEV1 change ≥15%	4 (4.5)	2 (1.9)	0.412

FEV1 = Forced expiratory volume in one second; FVC = Forced vital capacity; MEF50 = Mid-expiratory flow at 50% of FVC.
Statistically significant findings are marked with bold font.
*Chi-square test or Fisher's exact test.
†FVC ≤81% for boys and ≤82% for girls, FEV1 ≤80% for boys and ≤82% for girls, FEV1/FVC ≤87% for boys and ≤88% for girls and MEF50 ≤64% for boys and ≤63% for girls (Ref 18), expressed as percentage of predicted.

Asthma in children

Current asthma was present in 12/89 (13.5%) cases and 12/108 (11.1%) controls. Eight cases and nine controls had used ICSs continuously during the last 12 months, and four cases and three controls were diagnosed with asthma during the control visit. Baseline FEV1/FVC and MEF50 were pathological more frequently in asthmatic than in nonasthmatic children and the bronchodilation test was positive more frequently in asthmatic children. However, significant differences were not seen in other baseline or in any postbronchodilator parameters (data not shown).

Maternal smoking and asthma

Maternal asthma was present in 10/89 (11.2%) cases and 14/108 (13.0%) controls, and maternal smoking during infancy in 24/89 (27.0%) cases and 6/108 (5.6%) controls ($p < 0.001$). The differences between those children with or without asthmatic mothers, or between those with or without smoking mothers, were not statistically significant for any of the flow-volume spirometry parameters in either baseline or postbronchodilator measurements (data not shown).

Multivariate analyses

Finally, we studied the differences in categorised flow-volume spirometry parameters between cases and controls using multivariate logistic regression. As current asthma was associated with reduced baseline flow-volume spirometry parameters, and maternal smoking during infancy was significantly more common in cases than in controls, both current asthma and maternal smoking during infancy were included as covariates in the model. There was a statistically significant difference between cases and controls in both the baseline and postbronchodilator FEV1 in the multivariate logistic regression (Table 3).

When children with RSV bronchiolitis were compared with controls using the same multivariate model, there was still a statistically significant difference in both baseline and postbronchodilator FEV1 (Table 4). However, there were no significant differences between children with non-RSV bronchiolitis and controls in any flow-volume spirometry parameters or in the bronchodilation test (Table 4).

DISCUSSION

There were two main results from this prospective follow-up study on lung function at the mean age of 11.6 years after hospitalisation for bronchiolitis at less than six months of age. First, postbronchodilator FEV1/FVC was lower and baseline and postbronchodilator FEV1 were reduced more frequently in former bronchiolitis patients than in population-based controls without bronchiolitis in infancy. Second, infant bronchiolitis, especially RSV bronchiolitis, was an independently significant risk factor for pathological baseline and postbronchodilator FEV1 when maternal smoking during infancy and current asthma in children were included as confounders in the multivariate logistic regression. Thus, we found evidence that infant bronchiolitis at the age of less than six months may be associated with irreversible bronchial obstruction in early adolescence.

Table 3 Multivariate logistic regression: Pathological baseline and postbronchodilator parameters in flow-volume spirometry in 89 former bronchiolitis patients (cases) compared to 108 controls

Parameters in spirometry	Cases (No)	Adjusted OR* (95% CI)
Baseline†		
FVC (N = 13)	8	2.4 (0.7–7.8)
FEV1 (N = 35)	22	**2.4 (1.1–5.3)**
FEV1/FVC (N = 36)	22	2.2 (0.9–5.0)
MEF50 (N = 27)	16	1.8 (0.7–4.7)
Postbronchodilator†		
FVC (N = 15)	9	2.4 (0.8–7.1)
FEV1 (N = 21)	16	**4.4 (1.5–12.8)**
FEV1/FVC (N = 14)	9	2.1 (0.6–7.0)
MEF50 (N = 10)	6	2.0 (0.5–7.8)
Bronchodilation test		
FEV1 change ≥12% (N = 13)	7	1.5 (0.4–5.4)

CI = Confidence interval; FEV1 = Forced expiratory volume in one second; FVC = Forced vital capacity; MEF50 = Mid-expiratory flow at 50% of FVC; OR = Odds ratio.
Statistically significant findings are marked with bold font.
*Adjusted for maternal smoking in infancy and current asthma.
†FVC ≤81% for boys and ≤82% for girls, FEV1 ≤80% for boys and ≤82% for girls, FEV1/FVC ≤87% for boys and ≤88% for girls and MEF50 ≤64% for boys and ≤63% for girls (Ref 18), expressed as percentage of predicted.

Table 4 Multivariate logistic regression: Pathological baseline and postbronchodilator parameters in flow-volume spirometry in 63 former RSV bronchiolitis patients and 25 former non-RSV bronchiolitis patients compared to 108 controls

	RSV bronchiolitis N = 63		Non-RSV bronchiolitis N = 25	
Parameters in spirometry	No (%)	Adjusted OR* (95% CI)	No (%)	Adjusted OR* (95% CI)
Baseline†				
FVC	7 (11.1)	3.0 (0.9–10.3)	1 (4.0)	1.1 (0.1–10.8)
FEV1	17 (27.0)	**2.9 (1.2–6.7)**	5 (20.0)	1.7 (0.5–5.8)
FEV1/FVC	13 (20.6)	2.1 (0.8–5.6)	9 (36.0)	2.4 (0.8–7.6)
MEF50	10 (15.9)	2.3 (0.8–6.8)	6 (24.0)	1.4 (0.4–5.3)
Postbronchodilator†				
FVC	8 (12.7)	2.8 (0.9–8.9)	1 (4.0)	1.0 (0.1–8.8)
FEV1	12 (19.0)	**4.6 (1.5–14.2)**	4 (16.0)	4.1 (0.9–18.7)
FEV1/FVC	6 (9.5)	2.1 (0.6–7.8)	3 (12.0)	2.1 (0.4–11.2)
MEF50	5 (7.9)	2.8 (0.7–11.5)	1 (4.0)	1.0 (0.1–10.3)
Bronchodilation test				
FEV1 change ≥12%	5 (7.9)	2.6 (0.6–11.5)	2 (8.0)	1.0 (0.2–6.4)

CI = Confidence interval; FEV1 = Forced expiratory volume in one second; FVC = Forced vital capacity; MEF50 = Mid-expiratory flow at 50% of FVC; OR = Odds ratio; RSV = Respiratory syncytial virus.
Statistically significant findings are marked with bold font.
*Adjusted for maternal smoking in infancy and current asthma.
†FVC ≤81% for boys and ≤82% for girls, FEV1 ≤80% for boys and ≤82% for girls, FEV1/FVC ≤87% for boys and ≤88% for girls and MEF50 ≤64% for boys and ≤63% for girls (Ref 18), expressed as percentage of predicted.

In accordance with the present study, infant bronchiolitis was associated with reduced lung function in early adolescence in four previous prospective postbronchiolitis studies (6–9). Three of these studies were controlled studies, where RSV was the main causative virus of bronchiolitis, and the hospitalisation age was <12 months (6–8). Postbronchodilator measurements were only performed in the Swedish study (7), and baseline and postbronchodilator FEV1/FVC were lower in 44 cases than in controls. However, such differences were not seen in FEV1 (7). The British study reported that the baseline FEV1 was lower in 61 cases than in the controls, but FEV1/FVC was not reported (6). In the Norwegian study, baseline FEV1 and FEV1/FVC were lower in 108 cases than in controls (8). In the study from Kuopio, Finland, the hospitalisation age for bronchiolitis was <24 months, 29% of children were RSV positive (19) and 33% were rhinovirus positive (20). In that Finnish study, flow-volume spirometry parameters were analysed as categorised variables and it reported that in early adolescence baseline FEV1 was reduced in 17.5% of 80 former bronchiolitis patients and baseline FEV1/FVC in 16.3% compared to population-based sex-specific and height-related references (9).

In the Swedish cohort, the follow-up continued until 18 years of age and bronchiolitis in infancy was still associated with an increased risk of irreversible FEV1/FVC reduction in 42 former bronchiolitis patients compared to controls (11). In addition, lung function with postbronchodilator measurements was evaluated in two other postbronchiolitis studies in adulthood, and the findings were rather similar (10,12). The older Swedish study comprised infants hospitalised for bronchiolitis at <24 months of age in 1984–1985 (12), including an RSV epidemic (21) and baseline and postbronchodilator FEV1/FVC were lower in 55 cases than in controls at the age of 17–20 years (12). In the older Finnish study, 47 study subjects performed flow-volume spirometry 27–29 years after bronchiolitis at <24 months of age (10). The findings showed that 21% of the former bronchiolitis patients had postbronchodilator FEV1/FVC below 0.88 and 15% below the 5th percentile in the population-based references (10). In the present study, the irreversible reduction of FEV1/FVC was present in 10.1% and irreversible reduction of FEV1 in 18.0% of former bronchiolitis patients with the respective figures being 9.5 and 19.0% after RSV bronchiolitis. The figures are high and need to be revisited at future follow-up visits. On the other hand, the findings may reflect real lung function reduction after severe RSV bronchiolitis during the first months of life.

An increase of 12% or more in the bronchodilation test was only diagnosed in 7.9% of former bronchiolitis patients, in 7.9% of former RSV bronchiolitis patients and in 5.6% of controls in the present study. Our findings were in line with earlier studies, as bronchial responses in the bronchodilation test have been minor in those with bronchiolitis in infancy (7,10,11), and the difference between cases and controls was only significant in one Swedish study (11).

Current asthma was present in 13.5% of former bronchiolitis patients and in 11.1% of controls at 10–13 years of age in the present study. The prevalence of asthma at that age was higher in previous long-term postbronchiolitis studies, at 15–40% (6–8,21–23). The young age during initial hospitalisation and the high proportion of RSV positive bronchiolitis cases probably explain the low

postbronchiolitis asthma prevalence in our study. The prevalence of asthma has been reported to range from 4 to 7% in Finnish school-aged children (24,25). This means that children with asthma were over-represented in the controls in the present study. In analyses, this selection bias probably decreased the risk of a type-1 statistical error, which highlights the reliability of the differences found between the cases and controls. As expected, current asthma in children was associated with a reduction in baseline lung function. Therefore, the final analyses were adjusted for current asthma, and the association between bronchiolitis in infancy and baseline and postbronchodilator FEV1 reductions remained significant. This highlights the independent role of early-life bronchiolitis in the emergence of lung function reduction.

In the present postbronchiolitis cohort, 15.9% of the mothers reported that they had smoked during pregnancy, compared to 2.8% of the mothers of controls. The respective figures during infancy were 27.0 and 5.6%. Tobacco smoke exposure, during pregnancy and after pregnancy, is a well-known cause of reduced lung function in childhood (26,27). Surprisingly, maternal smoking had no significant association with children's lung function in early adolescence in the present study. Importantly, bronchiolitis was an independently significant risk factor for lung function reduction in the analyses that were adjusted for maternal smoking.

How often and at what age irreversible lung function reduction after bronchiolitis develops is not known. A prospective birth cohort study from the Netherlands evaluated lung function using a single occlusion technique in 2.133 healthy term infants before two months of age (28). The researchers found that respiratory system compliance was lower in those 18 infants who were hospitalised for RSV bronchiolitis before 12 months of age, compared to 84 nonhospitalised RSV positive children (28). In the present study, the lung function of former bronchiolitis patients was evaluated with impulse oscillometry at five to seven years of age and with one exception, the lung function reductions, although present in 20%, were reversible (15). Thus, the results of the present study suggest that the irreversible reduction in lung function after infant bronchiolitis develops after seven years of age. It is worth highlighting that the methods used for lung function testing were different at different ages: impulse oscillometry at five to seven years and flow-volume spirometry at 10–13 years. However, resistance and reactance in impulse oscillometry at preschool age correlated well with parameters of flow-volume spirometry in early adolescence (29). One reason for the emergence of irreversible lung function changes after preschool age may be that the subjects were exposed to many disease modifying factors, whose cumulative effect is not evident at an earlier age.

The strengths of the present study were the carefully collected data during hospitalisation for bronchiolitis in infancy and the long follow-up time, which included clinical studies at five to seven years and at 10–13 years of age. For the present study at 10–13 years of age, we enrolled population-based controls of the same age without history of bronchiolitis or hospital admission for any respiratory infection during infancy. The study group was special, as the infants were hospitalised for bronchiolitis when they were less than six months of age. In addition, viral diagnoses were confirmed in 98.9% of the cases, with RSV being identified in 71.6%. This allowed us to carry out a stratified analysis for former RSV bronchiolitis patients, but not for patients with other viral findings. The main limitation of the study was the small number of subjects, because only 89 study subjects and 108 controls underwent spirometry. In addition, the controls were selected, as discussed above, which, however, merely decreased, rather than increased the differences between the cases and controls.

CONCLUSION

This ongoing prospective study showed that being hospitalised for bronchiolitis at less than six months of age was associated with reduced lung function at 10–13 years of age. In addition, we found evidence that bronchiolitis, especially RSV bronchiolitis, in early infancy seemed to be associated with irreversible bronchial obstruction in early adolescence, which was not seen at seven years of age.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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References

1. Ralston SL, Lieberthal AS, Meissner HC, Alverson BK, Baley JE, Gadomski AM, et al. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. *Pediatrics* 2014; 134: e1474–502.
2. American Academy of Pediatrics Subcommittee on Diagnosis and Management of Bronchiolitis. Diagnosis and management of bronchiolitis. *Pediatrics* 2006; 118: 1774–93.
3. Jartti T, Korppi M. Rhinovirus-induced bronchiolitis and asthma development. *Pediatr Allergy Immunol* 2011; 22: 350–5.
4. Fjaerli H, Farstad T, Roed G, Ufert GK, Gulbrandsen P, Nakstad B. Acute bronchiolitis in infancy as risk factor for wheezing and reduced pulmonary function by seven years in Akershus County, Norway. *BMC Pediatr* 2005; 5: 31.
5. 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.

6. Noble V, Murray M, Webb MS, Alexander J, Swarbrick AS, Milner AD. Respiratory status and allergy nine to 10 years after acute bronchiolitis. *Arch Dis Child* 1997; 76: 315–9.
7. Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med* 2005; 171: 137–41.
8. Mikalsen IB, Halvorsen T, Øymar K. The outcome after severe bronchiolitis is related to gender and virus. *Pediatr Allergy Immunol* 2012; 23: 391–8.
9. Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen TM, Korhonen K, Korppi MO. Lung function and bronchial hyperresponsiveness 11 years after hospitalization for bronchiolitis. *Acta Paediatr* 2007; 96: 1464–9.
10. Backman K, Piippo-Savolainen E, Ollikainen H, Koskela H, Korppi M. Irreversible airway obstruction in adulthood after bronchiolitis in infancy: evidence from a 30-year follow-up study. *Respir Med* 2014; 108: 218–23.
11. Sigurs N, Aljassim F, Kjellman B, Robinson PD, Sigurbergsson F, Bjarnason R, et al. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. *Thorax* 2010; 65: 1045–52.
12. Goksör E, Gustafsson PM, Alm B, Amark M, Wennergren G. Reduced airway function in early adulthood among subjects with wheezing disorder before two years of age. *Pediatr Pulmonol* 2008; 43: 396–403.
13. Koponen P, Helminen M, Paassilta M, Luukkaala T, Korppi M. Preschool asthma after bronchiolitis in infancy. *Eur Respir J* 2012; 39: 76–80.
14. Nuolivirta K, Koponen P, He Q, Halkosalo A, Korppi M, Vesikari T, et al. Bordetella pertussis infection is common in nonvaccinated infants admitted for bronchiolitis. *Pediatr Infect Dis J* 2010; 29: 1013–5.
15. Lauhkonen E, Koponen P, Nuolivirta K, Paassilta M, Toikka J, Korppi M. Lung function by impulse oscillometry at age 5-7 years after bronchiolitis at age 0-6 months. *Pediatr Pulmonol* 2015; 50: 389–95.
16. Törmänen S, Lauhkonen E, Riikonen R, Koponen P, Huhtala H, Helminen M, et al. Risk factors for asthma after infant bronchiolitis. *Allergy* 2018; 73: 916–22.
17. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J* 2005; 26: 319–38.
18. Koillinen H, Wanne O, Niemi V, Laakkonen E. Terveiden suomalaislasten spirometrian ja uloshengityksen huippuvirtauksen viitearvot. *Suom Lääkäril* 1998; 5: 395–405.
19. Kotaniemi-Syrjänen A, Laatikainen A, Waris M, Reijonen TM, Vainionpää R, Korppi M. Respiratory syncytial virus infection in children hospitalized for wheezing: virus-specific studies from infancy to preschool years. *Acta Paediatr* 2005; 94: 159–65.
20. 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.
21. Wennergren G, Amark M, Amark K, Oskarsdóttir S, Sten G, Redfors S. Wheezing bronchitis reinvestigated at the age of 10 years. *Acta Paediatr* 1997; 86: 351–5.
22. 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.
23. Korppi M, Kuikka L, Reijonen T, Remes K, Juntunen-Backman K, Launiala K. Bronchial asthma and hyperreactivity after early childhood bronchiolitis or pneumonia. An 8-year follow-up study. *Arch Pediatr Adolesc Med* 1994; 148: 1079–84.
24. Hugg T, Ruotsalainen R, Jaakkola MS, Pushkarev V, Jaakkola JJK. Comparison of allergic diseases, symptoms and respiratory infections between Finnish and Russian school children. *Eur J Epidemiol* 2008; 23: 123–33.
25. Pekkanen J, Remes ST, Husman T, Lindberg M, Kajosaari M, Koivikko A, et al. Prevalence of asthma symptoms in video and written questionnaires among children in four regions of Finland. *Eur Respir J* 1997; 10: 1787–94.
26. Landau LI. Tobacco smoke exposure and tracking of lung function into adult life. *Paediatr Respir Rev* 2008; 9: 39–44.
27. Jayes L, Haslam PL, Gratziou CG, Powell P, Britton J, Vardavas C, et al. SmokeHaz: systematic reviews and meta-analyses of the effects of smoking on respiratory health. *Chest* 2016; 150: 164–79.
28. Zomer-Kooijker K, Uiterwaal CS, van der Gugten AC, Wilbrink B, Bont LJ, van der Ent CK. Decreased lung function precedes severe respiratory syncytial virus infection and post-respiratory syncytial virus wheeze in term infants. *Eur Respir J* 2014; 44: 666–74.
29. Lauhkonen E, Riikonen R, Törmänen S, Koponen P, Nuolivirta K, Helminen M, et al. Impulse oscillometry at preschool age is a strong predictor of lung function by flow-volume spirometry in adolescence. *Pediatr Pulmonol* 2018; 53: 552–8.

PUBLICATION II

Preliminary communication suggests overweight was associated with reduced lung function in adolescence after infant bronchiolitis.

Riikonen R, Törmänen S, Saari A, Koponen P, Helminen M, Nuolivirta K, Korppi M, Lauhkonen E.

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ACTA PÆDIATRICA
NURTURING THE CHILD

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BRIEF REPORT

Preliminary communication suggests overweight was associated with reduced lung function in adolescence after infant bronchiolitis

The risk for reduced lung function was increased after bronchiolitis in infancy (1). Contrary to adults, the effect of overweight in children has often been reduced forced expiratory volume in one second/ forced vital capacity (FEV1/FVC) with normal FEV1 and FVC (2).

We have prospectively followed 166 children hospitalised for viral bronchiolitis under six months of age. Lung function was studied with impulse oscillometry in 70 normal weight, 22 overweight and seven obese children at five to seven years of age, and obesity was associated with airway obstruction (3). At 10–13 years of age, 89 children with infant bronchiolitis and 108 controls performed flow-volume spirometry and former bronchiolitis was associated with FEV1 reduction before and after bronchodilation (1). The present paper evaluates lung function in relation to weight at 10–13 years of age after infant bronchiolitis.

Flow-volume spirometry was measured with a calibrated spirometer V_{max} Carefusion (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Asthma controller medication was not interrupted (1). After baseline blows, the children inhaled 0.1 mg salbutamol (Ventoline, Glaxo-SmithKline, London, UK) four times through a spacer before the post-bronchodilator blows (4). The spirometry parameters were presented as percentages of predicted of population-based, sex-specific, height-related references. The pathological limits for categorised FVC, FEV1 and FEV1/FVC are presented in Table 1. Current asthma was considered to be present if the child had used regularly inhaled corticosteroids during the preceding 12 months or if the child had suffered from asthma-presumptive symptoms during the preceding 12 months with an increase 12% or more in the bronchodilation test (4).

Weights and heights were measured and transformed into body mass index for age z-scores (zBMIs) according to Finnish growth reference data (5). Weight status was categorised using national zBMI cut-off values to normal weight, overweight and obesity. One child was excluded due to underweight.

SPSS Statistics for Windows, version 23.0 (IBM Corp, Armonk, NY, USA), was used in statistical analyses. The continuous variables were expressed as means, standard deviations (SD) and 95% confidence intervals (95% CIs). Differences between the groups were calculated with *t*-test for the continuous and with chi-square or Fisher's exact test, when appropriate, for the categorised variables.

Multivariate linear regression was used to study the association between zBMI and continuous spirometry parameters, and the result for each parameter was expressed as an unstandardised coefficient (B). The F-test was used to estimate p values for the model, and correlation coefficients (R) were transformed into adjusted R^2 values to present predictive strength of the model. Multivariate logistic regression was used to study the associations between the categorised zBMI and spirometry parameters. The results are expressed as adjusted odds ratios (ORs) and 95% CIs. Multivariate regression models were adjusted for current asthma and maternal smoking in infancy.

The study was approved by the Ethics Committee of the Tampere University Hospital District.

The mean age of the 88 former bronchiolitis patients was 11.7 years (range 10.3–13.2) and 44 (50.0%) were boys. There were 65 (73.9%) normal weight, 20 (22.7%) overweight and three (3.4%) obese children. There were no significant differences between normal weight and overweight or obese children in prevalence of current asthma or maternal smoking in infancy.

In continuous analyses, FEV1/FVC was lower in the overweight (obese included) than normal weight children before (mean 88.9, SD 8.9, 95% CI: 85.0–92.7, vs. 94.7, 6.8, 93.0–96.4) and after (mean 91.6, SD 6.5, 95% CI: 88.8–94.4, vs. 97.5, 6.2, 96.0–99.0) bronchodilation. There were no significant differences between the groups in FEV1 and FVC (data not shown).

Table 1 Comparison of 65 normal weight and 23 overweight or obese study subjects at 10–13 years of age

Basic and clinical data and pathological findings in spirometry	Normal weight* (N = 65)	Overweight or obese* (N = 23)	p value†
Basic data			
Age (years), mean (SD)	11.7 (0.96)	11.5 (0.89)	0.235
Male gender, n (%)	32 (49.2)	12 (52.2)	0.808
Height (cm), mean (SD)	151.8 (8.9)	150.0 (8.5)	0.423
Weight (kg), mean (SD)	41.5 (7.5)	53.9 (10.3)	<0.001
zBMI, mean (SD)	−0.17 (0.70)	1.47 (0.46)	<0.001
Current asthma, n (%)	6 (9.2)	6 (26.1)	0.072
Maternal smoking in infancy, n (%)	17 (26.2)	7 (30.4)	0.692
Pathologic parameters‡, baseline			
FVC, n (%)	6 (9.2)	2 (8.7)	1.0
FEV1, n (%)	13 (20.0)	9 (39.1)	0.069
FEV1/FVC, n (%)	10 (15.4)	12 (52.2)	<0.001
Pathologic parameters‡, post-bronchodilator			
FVC, n (%)	8 (12.3)	1 (4.3)	0.436
FEV1, n (%)	10 (15.4)	6 (26.1)	0.345
FEV1/FVC, n (%)	3 (4.6)	6 (26.1)	0.009

FVC = Forced vital capacity; FEV1 = Forced expiratory volume in one second.
*According to the national zBMI cut-off values for normal weight, overweight and obesity (Ref. (5)).
†*t*-Test for continuous variables, chi-square test or Fisher's exact test for categorised variables.
‡FVC ≤ 81% for boys and ≤82% for girls, FEV1 ≤ 80% for boys and ≤82% for girls and FEV1/FVC ≤ 87% for boys and ≤88% for girls (Ref. (1)).

In categorised analyses, FEV1/FVC was pathological more often in the overweight (obese included) than normal weight children before (52.2% vs. 15.4%, $p < 0.001$) and after (26.1% vs. 4.6%, $p = 0.009$) bronchodilatation. There were no significant differences between the groups in FVC or FEV1 (Table 1).

In multivariate linear regression, one unit increase in zBMI predicted 2.3% decrease in baseline FEV1/FVC ($p = 0.006$) and 2.5% decrease in post-bronchodilator FEV1/FVC ($p < 0.001$). The adjusted R^2 of the model was 18.0% in baseline and 22.7% in post-bronchodilator measurements. There was a positive correlation between increasing zBMI and increasing FVC ($B = 2.9\%$, $p = 0.033$), but the predictive strength of this model was low (adjusted $R^2 = 2.8\%$) (data not shown). Multivariate logistic regression revealed that categorised (pathological vs. normal) baseline FEV1/FVC (OR 5.4, 95% CI: 1.66–17.77) and post-bronchodilator FEV1/FVC (OR 5.6, 95% CI: 1.19–26.37) differed significantly between the 23 overweight (obese included) and 65 normal weight children (data not shown).

The findings of this communication in former bronchiolitis patients at 10–13 years of age are in line with the observations of the meta-analysis on weight and lung function in children (2), since the presence of overweight or obesity was associated with reduced FEV1/FVC, but not with reduced FEV1 or FVC when analysed separately. The results are preliminary, since only 53.6% of those invited attended the lung function study. The study suggests an increased risk for reduced FEV1/FVC in adolescence if overweight develops after bronchiolitis and highlights the importance to prevent overweight in this special group of children.

CONFLICT OF INTEREST

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References

1. Riikonen R, Lauhkonen E, Törmänen S, Backman K, Koponen P, Helminen M, et al. Prospective study confirms that bronchiolitis in early infancy increases the risk of reduced lung function at 10–13 years of age. *Acta Paediatr* 2019; 108: 124–30.
2. Forno E, Han Y, Mullen J, Celedón JC. Overweight, obesity, and lung function in children and adults - A meta-analysis. *J Allergy Clin Immunol Pract* 2018; 6: 570–81.
3. Lauhkonen E, Koponen P, Nuolivirta K, Paassilta M, Toikka J, Saari A, et al. Obesity and bronchial obstruction in impulse oscillometry at age 5–7 years in a prospective post-bronchiolitis cohort. *Pediatr Pulmonol* 2015; 50: 908–14.
4. Törmänen S, Lauhkonen E, Riikonen R, Koponen P, Huhtala H, Helminen M, et al. Risk factors for asthma after infant bronchiolitis. *Allergy* 2018; 73: 916–22.
5. Saari A, Sankilampi U, Hannila M, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: Length/height-for-age, weight-for-length/height, and body mass index-for-age. *Ann Med* 2011; 43: 235–48.

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PUBLICATION III

***Toll-like receptor 10* rs4129009 gene polymorphism is associated with post-bronchiolitis lung function in adolescence.**

Riikonen R, Korppi M, Törmänen S, Nuolivirta K, Helminen M, He Q, Lauhkonen E.

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REGULAR ARTICLE

ACTA PÆDIATRICA NURTURING THE CHILD WILEY

Toll-like receptor 10 rs4129009 gene polymorphism is associated with post-bronchiolitis lung function in adolescence

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Abstract

Aim: The aim was to evaluate the association of polymorphisms in the Toll-like receptor (TLR) 2 subfamily encoding genes with lung function by spirometry at 10-13 years of age in children who had been hospitalised for bronchiolitis at <6 months of age.

Methods: In a prospective cohort of 166 former bronchiolitis patients, 138 returned a structured questionnaire and 89 attended a clinical follow-up visit including spirometry before and after bronchodilation at 10-13 years of age. Data on polymorphisms of the *TLR1*, *TLR2*, *TLR6* and *TLR10* genes were available from 81-82 children.

Results: In the *TLR10* rs4129009, the wild (AA) genotype was associated with lower FEV1/FVC before (92.4 vs 97.4, P = .002) and after (95.5 vs 98.6, P = .011) bronchodilator administration, compared to those with the variant genotype. When the *TLR10* rs4129009 and *TLR2* rs5743708 genotypes, and the *TLR10* rs4129009 and *TLR1* rs5743618 genotypes, respectively, were analysed as combined, both baseline and post-bronchodilator FEV1/FVC were lowest in the subjects with the wild (AA) genotype of the *TLR10* rs4129009.

Conclusion: In this post-bronchiolitis follow-up, lung function in children with the variant *TLR10* rs4129009 genotype with potentially altered TLR10 function was superior to lung function in those with the wild genotype.

KEYWORDS

bronchiolitis, child, lung function, spirometry, toll-like receptor

1 | INTRODUCTION

Bronchiolitis is a viral lower respiratory tract infection (LRTI) in infants, and the main causative pathogen of LRTIs at that age is respiratory syncytial virus (RSV).[1] Globally, RSV bronchiolitis causes approximately 1.4 million hospitalisations every year in children younger than 6 months old.[1]

Abbreviations: ANOVA, analysis of variance; CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; ICS, inhaled corticosteroids; LRTI, lower respiratory tract infection; MAF, minor allele frequencies; MEF50, mid-expiratory flow at 50% of forced vital capacity; RSV, respiratory syncytial virus; SD, standard deviation; SNP, single nucleotide polymorphism; TLR, Toll-like receptor; zBMI, body mass index for age *z*-score.

Infant bronchiolitis increases the risk for later lung function abnormalities. There is evidence that a proportion of children with early-life bronchiolitis may have reduced lung function already at birth before bronchiolitis or any other infection.[2] However, irreversible post-bronchiolitis changes in lung function have not been reported earlier than from adolescence onwards and mainly in those with RSV bronchiolitis in infancy.[3,4] Evidently, development of lung function abnormalities is a multifactorial process depending on genes contributing to lung growth and host responses to various environmental factors including viruses from the prenatal period into adulthood.

Toll-like receptors (TLRs) initiate immune reactions against different pathogens.[5] TLR2 subfamily consists of TLR1, TLR2, TLR6 and

TLR10, which act on cell surface and are considered to function as co-receptors.[6,7] TLR1 and TLR6 form functional heterodimers with TLR2, and probably, do not work at all as homodimers.[6] TLR10 can be activated without TLR2, working thus as a homodimer, but TLR10 can also form heterodimers with TLR1 and TLR2.[8]

In the present study, we have prospectively followed 166 children hospitalised for bronchiolitis at the age of <6 months. Lung function was measured first time at the age 5-7 years by impulse oscillometry,[9] and no associations were found between TLR2 subfamily encoding genes and post-bronchiolitis lung function.[10] However, polymorphism in the *TLR1* rs5743618 was associated with asthma before 7 years of age,[11] and polymorphism in the *TLR10* rs4129009 was associated with asthma at preschool age[12] and with persistent asthma from 5-7 to 10-13 years of age.[13]

This study evaluates the associations between the single nucleotide polymorphisms (SNPs) of the *TLR1* rs5743618, *TLR2* rs5743708, *TLR6* rs5743810 or *TLR10* rs4129009 genes and lung function by flow-volume spirometry in adolescence after hospitalisation for bronchiolitis in early infancy. We hypothesised that polymorphisms of the *TLR2* subfamily genes have an impact on lung function in adolescence after early-life hospitalisation for bronchiolitis.

Key notes

- Toll-like receptor (TLR) 2 subfamily members have been linked to the post-bronchiolitis asthma development, but their role for post-bronchiolitis lung function is unknown.
- The variant genotype of the *TLR10* rs4129009 was associated with higher baseline and post-bronchodilator FEV1/FVC at 10-13 years of age after bronchiolitis at <6 months of age, confirmed with adjusted analyses.
- Altered TLR10 function may be protective for post-bronchiolitis lung function reduction.

2 | MATERIAL AND METHODS

2.1 | Study design

We have prospectively followed up 166 children who were hospitalised due to bronchiolitis at the age of <6 months in the Department of Pediatrics, Tampere University Hospital, Finland, in 2001-2004.[14] During hospitalisation, virus aetiology of bronchiolitis was studied in nasopharyngeal aspirates using immunofluorescence and polymerase chain reaction.[14] In 2014-2015, 138 (83.1%) children participated in the prospectively scheduled control visit at the age of 10-13 years.[15]

2.2 | Study subjects and examinations

At the age of 10-13 years, 138 children answered the structured questionnaire and 89 (64.6%) attended the clinical examination which included flow-volume spirometry with a bronchodilation test.[15] Data on virus aetiology were available in 88/89 (98.9%) cases.[16] Weight status was reported as body mass index for age *z*-scores (zBMIs) using Finnish growth references,[17] and one child was excluded from the final analyses because of underweight.[18] Data on the *TLR1* rs5743618, *TLR2* rs5743708 and *TLR6* rs5743810 genes were available from 82 and on the *TLR10* rs4129009 gene from 81 children.[10]

As published previously in detail,[15] we defined current asthma as a regular use of inhaled corticosteroid (ICS) medication during the previous 12 months, or alternatively, as the presence of asthma-presumptive symptoms (repeated wheezing, or night cough, or prolonged cough for four weeks or longer) during the previous 12 months and in addition, the rise of forced expiratory volume in one second (FEV1) of 12% and 0.2 L or more in the bronchodilation test. The structured questionnaire covered the presence of asthma-presumptive symptoms during the previous 12 months, as well as the history of maternal smoking when the children were infants.

2.3 | Flow-volume spirometry and bronchodilation test

Flow-volume spirometry was performed with a calibrated spirometer Vmax Carefusion (Becton, Dickinson and Company).[16] Measurements were repeated until three artefact-free spirograms with rapid rise to peak flow, and descending curve over 6 seconds was seen. After baseline measurements, all 89 children received 0.1 mg of salbutamol four times through a spacer (Ventolin Evohaler, GlaxoSmithKline), and spirometry was repeated 15 minutes after bronchodilator administration. ICS or other asthma controller medication was not interrupted before the lung function testing. The parameters obtained from flow-volume spirometry were FVC (forced vital capacity), FEV1, MEF50 (mid-expiratory flow at 50% of FVC) and FEV1/FVC.

2.4 | Genetics

SNPs of the *TLR1* rs5743618, *TLR2* rs5743708, *TLR6* rs5743810 and *TLR10* rs4129009 were selected to the present analyses because of their functional properties[6,8] and the earlier associations of the *TLR1* and *TLR10* with post-bronchiolitis asthma in the present cohort.[11-13] All four members of the TLR2 subfamily were included, since they form heterodimers and function as co-receptors for each other.[6,8]

As published previously,[10,11] the genotyping of the *TLR1* rs5743618, *TLR2* rs5743708 and *TLR6* rs5743810 was performed by pyrosequencing[11] and the genotyping of the *TLR10* rs4129009 was

performed by high-resolution melting analysis.[10] The genetic studies of the patients were done as coded in the National Institute of Health and Welfare, Turku (*TLR1*, *TLR2* and *TLR6*) and in the Department of Medical Microbiology and Immunology, University of Turku (*TLR10*), Finland.

2.5 | Statistics

SPSS for Windows version 25.0 (IBM Corp) was used for statistical analyses of the present data. The results of the continuous variables are presented as means and 95% confidence intervals (95% CIs) and the results of the categorised variables as numbers and frequencies. Analysis of variance (ANOVA) was used for comparisons of lung function parameters between the *TLR1*, *TLR2*, *TLR6* or *TLR10* wild vs variant genotypes. The two-tailed *P* values of <.05 were considered statistically significant. The genotypes that were homozygous for minor alleles, or heterozygous for minor alleles as well, were included in the group of variant genotype.

Current asthma, maternal smoking during infancy, RSV aetiology for bronchiolitis and zBMI were included as confounders in the adjusted analyses due to associations detected earlier in the present cohort study.[16,18] The association of passive smoking in infancy with reduced lung function later in childhood has been documented earlier in other studies.[19]

First, the *TLR1* rs5743618, *TLR2* rs5743708, *TLR6* rs5743810 and *TLR10* rs4129009 SNPs were analysed separately, and then as combinations constructed based on the available knowledge how TLR1, TLR2, TLR6 and TLR10 form heterodimers. Finally, all combinations that the four SNPs could form were investigated for their associations with spirometry.

2.6 | Ethics

The Ethics Committee of the Tampere University Hospital District has approved the study. An informed written parental consent was obtained from all study subjects before the hospitalisation in infancy and at the control visits. Personal information of the study subjects was not given to the laboratories where the genetic tests were done.

3 | RESULTS

The mean age of the 82 study subjects was 11.6 ± 0.9 years (range 10.3-13.2 years) and 41 (50.0%) were girls. During hospitalisation in infancy, RSV was detected in 58 (70.7%), rhinoviruses in 12.2% and other viruses in 13.4% of cases. In 8 (9.8%) cases more than one virus was found. Ten (12.2%) children were negative for viruses. Maternal smoking in infancy was present in 24 (29.3%) and asthma at the time of the current control visit in 10 (12.2%) of the children. The mean zBMI was 0.28 ± 0.97 (range −1.63 to 2.45), and 22 (26.8%) children were overweight or obese.

The genotypes of the *TLR1* rs5743618, *TLR2* rs5743708, *TLR6* rs5743810 and *TLR10* rs4129009, and the minor allele frequencies (MAFs) of these four SNPs and the respective MAFs in the Finnish population[20] are presented in Table 1.

3.1 | *TLR1*, *TLR2*, *TLR6* and *TLR10* polymorphisms

There were no significant associations between the wild and variant genotypes of the *TLR1* rs5743618, the *TLR2* rs5743708 and the *TLR6* rs5743810 in lung function by spirometry in baseline or post-bronchodilator measurements (Data not shown).

In the case of the *TLR10* rs4129009, 66 children had the wild (AA) genotype, 15 children had the heterozygous variant (AG) genotype, and none had the homozygous variant (GG) genotype. In adjusted analyses, baseline FEV1/FVC, baseline MEF50 and post-bronchodilator FEV1/FVC were significantly lower in children with the wild genotype of the *TLR10* rs4129009 than in children who had the variant genotype (Table 2). There were no other significant differences in baseline or post-bronchodilation spirometry between wild and variant genotypes of the *TLR10* rs4129009 (Table 2).

3.2 | Combinations of *TLR2/1*, *TLR2/6*, *TLR2/10* and *TLR10/1* polymorphisms

When we constructed the combinations of the *TLR2* rs5743708 and *TLR1* rs5743618 genotypes, only one child had the combination of the variant *TLR2* and the variant *TLR1*, and this case was

TABLE 1 Genotypes and minor allele frequencies of genes encoding Toll-like receptors (TLR) 1, 2, 6 and 10 in 82 former bronchiolitis patients of the present study and in the Finnish population

Gene	SNP	Wild (Major/Major)	Variant (Major/Minor)	Variant (Minor/Minor)	MAF	FIN
TLR1	rs5743618	GG 0.76	GT 0.20	TT 0.05	0.15	0.17
TLR2	rs5743708	CC 0.94	CT 0.06	TT 0.0	0.03	0.03
TLR6	rs5743810	CC 0.31	CT 0.46	TT 0.23	0.46	0.42
TLR10[a]	rs4129009	AA 0.82	AG 0.19	GG 0.0	0.09	0.08

Note: FIN, minor allele frequencies in the Finnish population as in Ref ([20]); MAF, minor allele frequencies in the study population; SNP, single nucleotide polymorphism.

[a]N = 81 for TLR10.

TABLE 2 Parameters of flow-volume spirometry in relation to *TLR10* rs4129009 genotypes in 81 former bronchiolitis patients

	Wild (AA) N = 66		Variant (AG)[b] N = 15		
Parameter in spirometry[a]	**Mean (SD)**	**95% CI**	**Mean (SD)**	**95% CI**	**Adjusted *P* value***
Baseline					
FVC	96.7 (11.5)	93.9-99.5	92.6 (11.8)	86.1-99.1	.102
FEV1	89.2 (11.2)	86.5-92.0	88.3 (11.8)	81.8-94.9	.832
FEV1/FVC	92.4 (7.6)	90.5-94.2	97.4 (8.0)	93.0-101.9	**.002**
MEF50	83.7 (20.7)	78.7-88.8	93.6 (21.4)	81.8-105.4	**.035**
Post-bronchodilator					
FVC	97.4 (12.1)	94.5-100.4	92.9 (11.0)	86.8-99.0	.089
FEV1	93.0 (11.7)	90.2-95.9	89.8 (12.9)	82.7-96.9	.361
FEV1/FVC	95.5 (6.6)	93.9-97.1	98.6 (7.1)	94.7-102.5	**.011**
MEF50	95.8 (22.5)	90.3-101.3	105.9 (24.7)	92.2-119.5	.065

Abbreviations: CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; MEF50, mid-expiratory flow at 50% of FVC; SD, standard deviation.

Statistically significant findings are marked with bold font.

[a]Expressed as percentages of predicted.

[b]None of the study subjects had the variant (GG) genotype.

*ANOVA, adjusted for current asthma, maternal smoking in infancy, RSV aetiology of bronchiolitis and zBMI (for definitions, see the text).

TABLE 3 Parameters in flow-volume spirometry in relation to combinations of *TLR2* rs5743708 and *TLR1* rs5743618 genotypes in 81 former bronchiolitis patients

	***TLR2* wild *TLR1* wild N = 58**	***TLR2* wild *TLR1* variant N = 19**	***TLR2* variant *TLR1* wild N = 4**	
Parameter in spirometry[a]	**Mean (SD) [95% CI]**	**Mean (SD) [95% CI]**	**Mean (SD) [95% CI]**	**Adjusted *P* value***
Baseline				
FVC	96.4 (11.6) [93.4-99.5]	94.5 (11.8) [88.8-100.1]	93.5 (14.5) [70.5-116.5]	.515
FEV1	89.2 (11.0) [86.3-92.1]	89.1 (12.0) [83.3-94.8]	91.3 (13.0) [70.5-112.0]	.942
FEV1/FVC	92.6 (7.3) [90.7-94.6]	95.9 (8.2) [91.9-99.8]	97.8 (6.7) [87.1-108.5]	**.034**
MEF50	84.6 (20.4) [79.2-89.9]	91.4 (22.3) [80.7-102.2]	89.0 (18.7) [59.3-118.7]	.257
Post-bronchodilator				
FVC	97.3 (11.9) [94.1-100.4]	94.9 (11.2) [89.5-100.3]	92.0 (17.5) [64.2-119.8]	.437
FEV1	93.2 (11.8) [90.1-96.3]	90.9 (12.2) [85.0-96.8]	91.5 (13.8) [69.5-113.5]	.736
FEV1/FVC	95.8 (6.4) [94.1-97.4]	97.3 (6.9) [94.0-100.7]	100.0 (7.7) [87.7-112.3]	.114
MEF50	97.4 (22.8) [91.4-103.4]	102.6 (24.1) [91.0-114.2]	92.0 (16.1) [66.3-117.7]	.418

Note: Only one study subject had combination of genotypes of the variant *TLR2* rs5743708 and variant *TLR1* rs5743618 and was excluded from the statistical analyses.

Abbreviations: CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; MEF50, mid-expiratory flow at 50% of FVC; SD, standard deviation.

Statistically significant findings are marked with bold font.

[a]Expressed as percentages of predicted.

*ANOVA between the three groups, adjusted for current asthma, maternal smoking in infancy, RSV aetiology of bronchiolitis and zBMI (for definitions, see the text).

excluded. Baseline FEV1/FVC was lowest in children with the wild *TLR2* genotype combined with the wild *TLR1* genotype. There were no statistically significant associations in post-bronchodilator parameters in relation to *TLR2* and *TLR1* genotype combinations (Table 3).

When the *TLR2* rs5743708 and the *TLR6* rs5743810 were analysed as combined, all four possible combinations were present, and there were no statistically significant differences in any baseline or post-bronchodilator spirometry parameter in relation to the*TLR2* and *TLR6* genotype combinations (Data not shown).

When the *TLR2* rs5743708 and the *TLR10* rs4129009 were analysed as combined, three combinations of the possible four were present. Baseline and post-bronchodilator FEV1/FVC were lowest in children with the wild *TLR2* genotype combined with the wild *TLR10* genotype and highest in children with the wild *TLR2* genotype combined with the variant *TLR10* genotype. However, there were no other significant associations in spirometry parameters in relation to the *TLR2* and *TLR10* genotype combinations (Table 4).

When the *TLR10* rs4129009 and the *TLR1* rs5743618 were analysed as combined, three combinations of the possible four were present. Baseline and post-bronchodilator FEV1/FVC were lowest in children with the wild *TLR10* genotype combined with the variant *TLR1* genotype and highest in children with the variant *TLR10* genotype combined with the variant *TLR1* genotype. There were no other significant associations in spirometry parameters in relation to the *TLR10* and *TLR1* genotype combinations (Table 5).

3.3 | Combinations including *TLR1*, *TLR2*, *TLR6* and *TLR10*

There were 16 possible combinations of the genotypes including all the four *TLR2* subfamily members, and in the present study, 9 combinations were found. Two combinations were present in only two children and they were excluded. Thus, the analyses were performed in 79 subjects for 7 combinations.

Eight children with the variant genotypes of the *TLR1* rs5743618 and *TLR10* rs4129009 combined with the wild genotypes of *TLR2* rs5743708 and *TLR6* rs5743810 had significantly higher post-bronchodilator FEV1/FVC compared to those 71 children with another genotype combination (99.5 vs 95.9, $P = .025$) (Supplementary Table S1). In addition, 7 children with the variant genotypes of the *TLR1*

TABLE 4 Parameters in flow-volume spirometry in relation to combinations of *TLR2* rs5743708 and *TLR10* rs4129009 in 81 former bronchiolitis patients

Parameter in spirometry[a]	*TLR2* wild *TLR10* wild N = 61 Mean (SD) [95% CI]	*TLR2* wild *TLR10* variant N = 15 Mean (SD) [95% CI]	*TLR2* variant *TLR10* wild N = 5 Mean (SD) [95% CI]	Adjusted *P* value*
Baseline				
FVC	96.9 (11.5) [93.9-99.8]	92.6 (11.8) [86.1-99.1]	94.8 (12.9) [78.8-110.8]	.243
FEV1	89.3 (11.1) [86.5-92.2]	88.3 (11.8) [81.8-94.9]	88.0 (13.4) [71.3-104.7]	.944
FEV1/FVC	92.3 (7.2) [90.5-94.2]	97.4 (8.0) [93.0-101.9]	93.0 (12.2) [77.9-108.1]	**.007**
MEF50	84.0 (20.5) [78.8-89.3]	93.6 (21.4) [81.8-105.4]	80.2 (25.5) [48.6-111.8]	.101
Post-bronchodilator				
FVC	97.7 (11.8) [94.7-100.8]	92.9 (11.0) [86.8-99.0]	93.6 (15.5) [74.3-112.9]	.192
FEV1	93.3 (11.7) [90.3-96.3]	89.8 (12.9) [82.7-96.9]	89.4 (12.9) [73.4-105.4]	.579
FEV1/FVC	95.4 (6.2) [93.8-97.0]	98.6 (7.1) [94.7-102.5]	96.1 (10.9) [82.6-109.7]	**.037**
MEF50	96.6 (22.5) [90.9-102.4]	105.9 (24.7) [92.2-119.5]	85.2 (20.6) [59.6-110.8]	.116

Note: None of the study subjects had combination of genotypes *TLR10* rs4129009 variant, *TLR2* rs5743708 variant.

Abbreviations: CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; MEF50, mid-expiratory flow at 50% of FVC; SD, standard deviation.

Statistically significant findings are marked with bold font.

[a]Expressed as percentages of predicted.

*ANOVA between the three groups, adjusted for current asthma, maternal smoking in infancy, RSV aetiology of bronchiolitis and zBMI (for definitions, see the text).

TABLE 5 Parameters in flow-volume spirometry in relation to combinations of *TLR10* rs4129009 and *TLR1* rs5743618 genotypes in 81 former bronchiolitis patients

Parameter in spirometry[a]	*TLR10* wild *TLR1* wild N = 61 Mean (SD) [95% CI]	*TLR10* wild *TLR1* variant N = 5 Mean (SD) [95% CI]	*TLR10* variant *TLR1* variant N = 15 Mean (SD) [95% CI]	Adjusted *P* value*
Baseline				
FVC	96.3 (11.7) [93.3-99.3]	101.2 (8.8) [90.3-112.1]	92.6 (11.8) [86.1-99.1]	.230
FEV1	89.3 (11.1) [86.5-92.1]	88.4 (14.1) [70.9-105.9]	88.3 (11.8) [81.8-94.9]	.916
FEV1/FVC	92.8 (7.3) [90.9-94.7]	86.9 (9.4) [75.3-98.5]	97.4 (8.0) [93.0-101.9]	**.003**
MEF50	84.4 (20.0) [79.3-89.5]	75.6 (29.3) [39.3-111.9]	93.6 (21.4) [81.8-105.4]	.081
Post-bronchodilator				
FVC	97.0 (12.3) [93.9-100.2]	102.0 (8.5) [91.4-112.6]	92.9 (11.0) [86.8-99.0]	.194
FEV1	93.1 (11.9) [90.1-96.2]	92.2 (10.4) [79.3-105.1]	89.8 (12.9) [82.7-96.9]	.645
FEV1/FVC	95.9 (6.4) [94.3-97.6]	90.3 (6.5) [82.2-98.3]	98.6 (7.1) [94.7-102.5]	**.012**
MEF50	96.8 (22.4) [91.0-102.5]	83.8 (22.3) [56.2-111.4]	105.9 (24.7) [92.2-119.5]	.110

Note: None study subjects had combination of genotypes *TLR10* rs412900 variant, *TLR1* rs5743618 wild.

Abbreviations: CI, confidence interval; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; MEF50, mid-expiratory flow at 50% of FVC; SD, standard deviation.

Statistically significant findings are marked with bold font.

[a]Expressed as percentages of predicted.

*ANOVA between the three groups, adjusted for current asthma, maternal smoking in infancy, RSV aetiology of bronchiolitis and zBMI (for definitions, see the text).

rs5743618, *TLR6* rs5743810 and *TLR10* rs4129009 combined with the wild genotype of the *TLR2* rs5743709 had significantly higher baseline FEV1/FVC (99.2 vs 93.1, *P* = .012) compared to those 72 children with another genotype combination (Supplementary Table S1).

4 | DISCUSSION

There are three main findings in the present cohort study at the age of 10-13 years after hospitalisation for bronchiolitis in early infancy. First, the wild genotype of the *TLR10* rs4129009 was associated with lower baseline and post-bronchodilator FEV1/FVC than the variant genotype. Second, in the combined analyses with the heterodimers consisting of TLR10 and TLR2, and of TLR10 and TLR1, respectively, both baseline and post-bronchodilator FEV1/FVC were lowest in children having the wild genotype of the *TLR10* rs4129009. Third, the analyses including the 9 identified combinations of the wild and variant genotypes of the four TLR2 subfamily encoding genes confirmed that just the variant genotype of the *TLR10* rs4129009 was protective for lung function reduction after severe bronchiolitis in early infancy.

Toll-like receptors are present in various cells of the immune system but also in epithelial cells in the airways.[5] TLRs recognise microbial structures and activate the innate immune system by initiating production of inflammatory cytokines, which further induce the responses of adaptive immunity.[5] Bacteria and viruses, including RSV and rhinoviruses, activate the heterodimers TLR2/TLR1 and TLR2/TLR6.[5,21,22] The specific activators of TLR10 are not known,[7] and TLR10 seems to use different signalling routes than other receptors of the TLR2 subfamily.[23] In addition, there is evidence that TLR10 is competitive for the heterodimers formed by TLR2 subfamily members and acts mainly as anti-inflammatory receptors.[7]

Only few previous studies with varying designs have evaluated the associations of the genes encoding the TLR2 subfamily members with lung function. In addition, the study subjects have been adults and the findings variable. A study from the Netherlands found an association between the *TLR2* gene polymorphisms and FEV1 in 110 adults with chronic obstructive pulmonary disease,[24] but there were no significant findings regarding the *TLR2* rs5743708, which

we determined in the present study. In a Canadian study, polymorphisms in the *TLR2*—including the *TLR2* rs5743708 we determined—were associated with better lung function in 275 swine operation workers.[25] A Belgian study found significant associations between polymorphisms of the *TLR1* or *TLR2* genes and lung function reduction in 89 cystic fibrosis patients.[26] In those SNPs, which were determined in this study, the significant association was found in *TLR2* rs5743708, but the *TLR1* rs5743618 was not investigated.[26] In the same Belgian study, there were no significant associations between *TLR6* or *TLR10* polymorphisms and lung function, including the *TLR6* rs5743810 and the *TLR10* rs4129009, which we determined in this study.[26]

In the present study, the variant genotype of the *TLR10* rs4129009 polymorphism was associated with lower risk of lung function reduction at the age of 10-13 years after hospitalisation for infant bronchiolitis. In contrast, the same *TLR10* polymorphism increased the risk of persistent asthma at the same age in this same post-bronchiolitis cohort.[13] Thus, the direction of the association was opposite when the outcome was asthma and when the outcome was lung function reduction. The reason might be an interaction between TLRs and the causative viruses of bronchiolitis. We have reported, earlier in this cohort, irreversible lung function changes especially after RSV bronchiolitis[16] and an increased asthma risk after bronchiolitis caused by other viruses, such as rhinoviruses.[27] The findings are in line with other earlier post-bronchiolitis studies,[4,28] in which increased asthma risk was associated especially to rhinovirus bronchiolitis.[28] Unlike RSV, which is known to cause epithelial cell necrosis in the airways, rhinovirus is a less invasive pathogen and do not cause significant cell damage.[29] TLRs not only have a key role in recognising pathogens, but they are also able to recognise endogenous molecules from dying and necrotic cells and might have a significant role in the regeneration of injured tissue.[30]

The main limitation of the present study was the small number of the study subjects for genetic analyses, which increased the risk of false-negative findings. On the other hand, exploring the associations between various combinations of polymorphisms and many measured parameters in spirometry means a risk for positive findings by random. However, the findings regarding the *TLR10* rs4129009 were constant in basic and combined analyses. In addition, the findings were robust to adjustments with current asthma, maternal smoking in infancy, RSV aetiology of bronchiolitis in infancy and weight status. The most remarkable strength of the present study was the long prospective follow-up time with repeated scheduled control examinations including lung function measurements. Finnish children who were hospitalised for bronchiolitis at <6 months of age represent clinical and ethnic homogeneity that is a clear advantage in genetic and cohort studies.

In conclusion, we found evidence of an association between *TLR10* rs4129009 gene polymorphism and post-bronchiolitis lung function. Our current finding suggests a role for TLR10 in regulating protective elements for lung function development in children who are predisposed to RSV bronchiolitis in infancy.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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REFERENCES

1. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet*. 2017;390:946-958.
2. Zomer-Kooijker K, Uiterwaal CSPM, van der Gugten AC, Wilbrink B, Bont LJ, van der Ent CK. Decreased lung function precedes severe respiratory syncytial virus infection and post-respiratory syncytial virus wheeze in term infants. *Eur Respir J*. 2014;44:666-674.
3. Backman K, Piippo-Savolainen E, Ollikainen H, Koskela H, Korppi M. Irreversible airway obstruction in adulthood after bronchiolitis in infancy: evidence from a 30-year follow-up study. *Respir Med*. 2014;108:218-223.
4. Sigurs N, Gustafsson PM, Bjarnason R, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med*. 2005;171:137-141.
5. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell*. 2006;124:783-801.
6. Tapping RI, Omueti KO, Johnson CM. Genetic polymorphisms within the human Toll-like receptor 2 subfamily. *Biochem Soc Trans*. 2007;35:1445-1448.
7. Oosting M, Cheng S, Bolscher JM, et al. Human TLR10 is an anti-inflammatory pattern-recognition receptor. *Proc Natl Acad Sci USA*. 2014;111:4478.
8. Hasan U, Chaffois C, Gaillard C, et al. Human TLR10 is a functional receptor, expressed by B cells and plasmacytoid dendritic cells, which activates gene transcription through MyD88. *J Immunol*. 2005;174:2942-2950.
9. Lauhkonen E, Koponen P, Nuolivirta K, Paassilta M, Toikka J, Korppi M. Lung function by impulse oscillometry at age 5–7 years after bronchiolitis at age 0–6 months. *Pediatr Pulmonol*. 2015;50:389-395.
10. Lauhkonen E, Koponen P, Vuononvirta J, et al. Gene polymorphism of toll-like receptors and lung function at five to seven years of age after infant bronchiolitis. *PLoS ONE*. 2016;11:e0146526.
11. Koponen P, Vuononvirta J, Nuolivirta K, Helminen M, He Q, Korppi M. The association of genetic variants in toll-like receptor 2 subfamily with allergy and asthma after hospitalization for bronchiolitis in infancy. *Pediatr Infect Dis J*. 2014;33:463-466.
12. Törmänen S, Korppi M, Teräsjärvi J, et al. Polymorphism in the gene encoding toll-like receptor 10 may be associated with asthma after bronchiolitis. *Sci Rep*. 2017;7:2956.
13. Törmänen S, Korppi M, Lauhkonen E, et al. Toll-like receptor 1 and 10 gene polymorphisms are linked to postbronchiolitis asthma in adolescence. *Acta Paediatr*. 2018;107:134-139.
14. Nuolivirta K, Koponen P, He Q, et al. Bordetella pertussis infection is common in nonvaccinated infants admitted for bronchiolitis. *Pediatr Infect Dis J*. 2010;29:1013-1015.
15. Törmänen S, Lauhkonen E, Riikonen R, et al. Risk factors for asthma after infant bronchiolitis. *Allergy*. 2017;73:916-922.
16. Riikonen R, Lauhkonen E, Törmänen S, et al. Prospective study confirms that bronchiolitis in early infancy increases the risk

of reduced lung function at 10–13 years of age. *Acta Paediatr*. 2019;108:124-130.
17. Saari A, Sankilampi U, Hannila M, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: length/height-for-age, weight-for-length/height, and body mass index-for-age. *Ann Med*. 2011;43:235-248.
18. Riikonen R, Törmänen S, Saari A, et al. Preliminary communication suggests overweight was associated with reduced lung function in adolescence after infant bronchiolitis. *Acta Paediatr*. 2019;108:1729-1730.
19. Jayes L, Haslam PL, Gratziou CG, et al. SmokeHaz: systematic reviews and meta-analyses of the effects of smoking on respiratory health. *Chest*. 2016;150:164-179.
20. Abecasis GR, Auton A, Brooks LD, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012;491:56-65.
21. Triantafilou K, Vakakis E, Richer EAJ, 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;2:22-29.
22. Murawski MR, Bowen GN, Cerny AM, et al. Respiratory syncytial virus activates innate immunity through toll-like receptor 2. *J Virol*. 2009;83:1492-1500.
23. Guan Y, Ranoa DRE, Jiang S, et al. Human TLRs 10 and 1 share common mechanisms of innate immune sensing but not signaling. *J. Immunol*. 2010;184:5094-5103.
24. Budulac SE, Boezen HM, Hiemstra PS, et al. Toll-like receptor (TLR2 and TLR4) polymorphisms and chronic obstructive pulmonary disease. *PLoS ONE*. 2012;7:e43124.
25. Gao Z, Dosman JA, Rennie DC, et al. Association of Toll-like receptor 2 gene polymorphisms with lung function in workers in swine operations. *Ann Allergy Asthma Immunol*. 2013;110:50.
26. Haerynck F, Mahachie John JM, Van Steen K, et al. Genetic variations in toll-like receptor pathway and lung function decline in Cystic fibrosis patients. *Hum Immunol*. 2013;74:1649-1655.
27. Koponen P, Helminen M, Paassilta M, Luukkaala T, Korppi M. Preschool asthma after bronchiolitis in infancy. *Eur Respir J*. 2012;39:76-80.
28. Kotaniemi-Syrjanen A, Vainionpaa 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.
29. Vandini S, Calamelli E, Faldella G, Lanari M. Immune and inflammatory response in bronchiolitis due to respiratory Syncytial Virus and Rhinovirus infections in infants. *Paediatr Respir Rev*. 2017;24:60-64.
30. Zhang Z, Schluesener HJ. Mammalian toll-like receptors: from endogenous ligands to tissue regeneration. *Cell Mol Life Sci*. 2006;63:2901-2907.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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PUBLICATION IV

Genetic variations in Toll-like receptors 4 or 7 were not linked to post-bronchiolitis lung function in adolescence.

Riikonen R, Korppi M, Törmänen S, Nuolivirta K, Helminen M, He Q, Lauhkonen E.

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BRIEF REPORT

ACTA PÆDIATRICA NURTURING THE CHILD WILEY

Genetic variations in Toll-like receptors 4 or 7 were not linked to post-bronchiolitis lung function in adolescence

Bronchiolitis is a common cause of hospitalisation in infancy and increases the risk for later asthma and lung function abnormalities. Toll-like receptors (TLR) are key proteins of innate immunity that recognise microbes and moderate immune reactions, and there is evidence of association between *TLR* gene polymorphisms and lung diseases.[1]

We have prospectively followed the post-bronchiolitis cohort enrolled in 2001-2004 in Tampere, Finland, and we recently reported that single nucleotide polymorphism (SNP) of the *TLR10* rs4129009 was associated with better lung function in 81 children at 10-13 years of age.[2] This SNP was also associated with asthma at 5-7 years of age and with persistent asthma from that age to 10-13 years.[3] The genes encoding TLR1, TLR2 and TLR6, the other three members of the TLR2 subfamily, showed no association with lung function in adolescence.[2]

We previously have reported lung function by impulse oscillometry in relation to polymorphisms of the genes encoding nine TLRs in this post-bronchiolitis cohort at 5-7 years of age.[4] The *TLR4* rs4986790 was associated with airway reactivity to exercise and the *TLR7* rs179008 with baseline lung function in girls.[4] The present study evaluated associations of those SNPs with lung function at 10-13 years of age studied with flow-volume spirometry.

Our original post-bronchiolitis cohort comprised 166 children, hospitalised for bronchiolitis at less than six months of age and followed until 5-7 years of age.[3] At 10-13 years of age, 89 of them underwent flow-volume spirometry (Vmax Carefusion, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) before and after bronchodilation.[2] Post-bronchodilator blows were performed 15 minutes after four doses of 0.1 mg salbutamol (Ventolin, GlaxoSmithKline, London, UK) inhaled through a spacer.[2] The parameters of the flow-volume spirometry are presented as percentages of predicted population-based, sex-specific, height-related references. Asthma controller medication was not interrupted before the clinical examination.

Data on *TLR4* rs4986790 (1194 A/G) and *TLR7* rs179008 (171 A/T) polymorphisms were available for 82 children (50% girls). *TLR4* rs4986790 was genotyped by pyrosequencing and *TLR7* rs179008 by PCR-based sequencing, as previously detailed.[4]

Statistical analyses were performed with SPSS for Windows, version 25.0 (IBM Corp, Armonk, NY, USA). Results are reported as means and standard deviations (SD). Differences in flow-volume spirometry parameters between children with wild and variant genotypes of the *TLR4* and *TLR7* were studied with analysis of variance (ANOVA). The genotypes that were homozygous or heterozygous for minor alleles were considered variant genotypes. Because the TLR7 encoding genes are located in X-chromosome, the lung function results for the *TLR7* rs179008 genotypes are reported by sex.[4] The confounders in the adjusted analyses were current asthma, exposure to maternal smoking in infancy, respiratory syncytial virus aetiology of infant bronchiolitis and current body mass index for age z scores. The collection and definitions of the confounders, and the definition of current asthma, have previously been described.[2]

The Ethics Committee of Tampere University Hospital District approved the study and the parents provided written, informed consent for the study, including the genetic studies.

In the *TLR4* rs4986790, 69 of the 82 (84%) children had the wild AA genotype, 13 (16%) the variant AG genotype and none the variant GG genotype. In the non-adjusted analyses, baseline forced expiratory volume in one second (FEV1) was 88.2 % of predicted in children with the wild genotype versus 94.1 % in those with the variant genotype ($P = .08$). In the adjusted analyses, there were no significant differences between children with wild and variant genotypes in any baseline or post-bronchodilator flow-volume spirometry parameters (Table 1).

In 41 girls with the *TLR7* rs179008, 23 (56%) had the wild AA genotype, 16 (39%) the variant AT genotype and two (5%) the variant TT genotype. In 41 boys, 33 (80.5%) had the wild AA genotype and 8 (19.5%) the variant TT genotype. There were no significant differences either in boys or girls between those with wild and variant genotypes in any baseline or post-bronchodilator flow-volume spirometry parameter (Data not shown).

We were unable to confirm the permanence of the association between *TLR4* rs4986790 or *TLR7* rs179008 polymorphisms and lung function, when children with a history of bronchiolitis were studied with impulse oscillometry and flow-volume spirometry five years apart. In our previous study of the same cohort, reduced lung function, measured by impulse oscillometry at the mean age of 6.3 years, predicted rather well reduced lung function by flow-volume spirometry at the mean age of 11.4 years.[5] The numbers of study subjects were rather small for separate analyses in relation to *TLR4* and *TLR7* genotypes, and this increased the risk of false-negative findings. That is why this negative result is a preliminary finding with minor clinical significance. However, studies on gene polymorphisms are needed, until large-scale databases are available and allow more comprehensive analyses, including genetics of lung function in childhood and the impact of infant bronchiolitis.

TABLE 1 TLR4 rs4986790 genotypes in relation to parameters of flow-volume spirometry in 82 former bronchiolitis patients

Parameter in spirometry[a]	Wild (AA) n = 69 Mean (SD)	Variant (AG) n = 13 Mean (SD)	*P* value	Multivariate *P* value (ANOVA)[b]
Baseline				
FVC	95.5 (11.7)	98.2 (11.0)	.442	.391
FEV1	88.2 (11.0)	94.1 (11.0)	.080	.117
FEV1/FVC	92.8 (8.1)	96.4 (5.8)	.137	.167
After bronchodilation				
FVC	96.2 (11.9)	98.1 (12.4)	.605	.505
FEV1	91.8 (11.7)	96.2 (12.4)	.222	.231
FEV1/FVC	95.7 (6.9)	98.5 (5.2)	.178	.169

Note: None of the children had the variant GG genotype.

Abbreviations: ANOVA, analysis of variance; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; SD, standard deviation.

[a]Expressed as percentage of predicted.

[b]Adjusted for current asthma, maternal smoking in infancy, respiratory syncytial virus aetiology of bronchiolitis and body mass index for age *z* scores.

CONFLICTS OF INTEREST

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REFERENCES

1. Arora S, Ahmad S, Irshad R, et al. TLRs in pulmonary diseases. *Life Sci*. 2019;233:116671.
2. Riikonen R, Korppi M, Törmänen S, et al. Toll-like receptor 10 rs4129009 gene polymorphism is associated with post-bronchiolitis lung function in adolescence. *Acta Paediatr*. 2020;109:1634-1641.
3. Korppi M, Törmänen S. Toll-like receptor 1 and 10 variations increase asthma risk and review highlights further research directions. *Acta Paediatr*. 2019;108:1406-1410.
4. Lauhkonen E, Koponen P, Vuononvirta J, et al. Gene polymorphism of toll-Like receptors and lung function at five to seven years of age after infant bronchiolitis. *Plos One*. 2016;11:e0146526.
5. Lauhkonen E, Riikonen R, Törmänen S, et al. Impulse oscillometry at preschool age is a strong predictor of lung function by flow-volume spirometry in adolescence. *Pediatr Pulmonol*. 2018;53:552-558.

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PUBLICATION V

Risk factors for irreversible airway obstruction after infant bronchiolitis.

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Risk factors for irreversible airway obstruction after infant bronchiolitis

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ABSTRACT

Background: Increasing evidence shows that environmental factors in childhood play a role in development of irreversible airway obstruction. We evaluated early-life and preschool-age risk factors for irreversible airway obstruction in adolescence after bronchiolitis in infancy.

Methods: This study is a secondary analysis of data collected during prospective long-term follow-up of our post-bronchiolitis cohort. Risk factor data were collected during hospitalisation and on follow-up visits at 5–7 and 10–13 years of ages. Lung function was measured from 103 participants with impulse oscillometry at 5–7 years of age and from 89 participants with flow-volume spirometry at 10–13 years of age.

Results: Asthma diagnosis at <12 months of age showed a significant association with irreversible airway obstruction at 10–13 years of age independently from current asthma. Irreversible airway obstruction was less frequent in children with variant than wild genotype of the *Toll-like receptor 4 (TLR4)* rs4986790, but the significance was lost in logistic regression adjusted for current asthma and weight status. Higher post-bronchodilator respiratory system resistance at 5 Hz and lower baseline and post-bronchodilator reactance at 5 Hz by impulse oscillometry at 5–7 years of age were associated with irreversible airway obstruction at 10–13 years of age.

Conclusion: Asthma diagnosis during the first living year and worse lung function at preschool age increased the risk for irreversible airway obstruction at 10–13 years of age after bronchiolitis. *TLR4* rs4986790 polymorphism may be protective for development of irreversible airway obstruction after bronchiolitis.

1. Introduction

Irreversible airway obstruction, defined usually by reduction in post-bronchodilator (post-BD) measurements in flow-volume spirometry, is considered as a marker of airway remodelling which may, typically in middle or old ages, lead to chronic obstructive pulmonary disease (COPD). Development of irreversible airway obstruction is a multifactorial process with roots in infancy and even at prenatal age [1]. Although smoking is a major culprit, also genetic, developmental and environmental factors such as infections play a role. These factors influence during growing years both by deteriorating the attainable maximal flows, such as forced expiratory volume in 1 s (FEV1), and further by accelerating the age-dependent FEV1 decline during adulthood [2]. Early-life bronchiolitis has been associated with lower post-BD lung function in comparisons with control subjects from school age until adulthood especially after respiratory syncytial virus (RSV) bronchiolitis [3–5]. One previous post-bronchiolitis study reported increased risk for irreversible airway obstruction, defined as post-BD FEV1/forced vital capacity (FVC) <5th percentile of the GLI 2012 guidelines in adults aged thirty years [6].

Abbreviations: Analysis of covariance, ANCOVA; body mass index for age z-score, zBMI; chronic obstructive pulmonary disease, COPD; confidence interval (CI) forced expiratory volume in 1 s, FEV1; forced vital capacity, FVC; impulse oscillometry, IOS; inhaled corticosteroids, ICS; odds ratio, OR; polymerase chain reaction, PCR; post-bronchodilator, post-BD; respiratory syncytial virus, RSV; respiratory system reactance at 5 Hz, Xrs5; respiratory system resistance at 5 Hz, Rrs5; single nucleotide polymorphism, SNP; standard deviation, SD; Toll-like receptor (receptor), TLR; *Toll-like receptor (gene)*, *TLR*.

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In the present post-bronchiolitis cohort study, we have followed-up 166 children hospitalised for bronchiolitis at younger than 6 months of age until 10–13 years of age. Lung function was studied before and after bronchodilator administration at 5–7 years of age by impulse oscillometry (IOS), and 20% of study subjects had abnormal baseline values in respiratory system resistance at 5 Hz (Rrs5) and/or in respiratory system reactance at 5 Hz (Xrs5), but only one child presented with irreversible obstruction [7]. At the age of 10–13 years, study subjects and age-matched controls performed flow-volume spirometry including FEV1 and FVC before and after bronchodilator administration. At that age, baseline FEV1 reduction was present in 25% and FEV1/FVC reduction also in 25% of study subjects, and the figures for post-BD FEV1 and FEV1/FVC reductions were 18% and 10%, respectively [8]. The differences in baseline and post-BD FEV1 and FEV1/FVC compared to controls were significant, in the case of irreversible FEV1 reduction even independently from current asthma [8]. Asthma was associated with reduced baseline FEV1 and FEV1/FVC, but instead, not with post-BD FEV1 or FEV1/FVC [8].

We have previously published our findings on risk factors for asthma at 10–13 years of age in the present cohort, and in adjusted analyses, maternal asthma was the only independently significant risk factor [9]. When we evaluated single nucleotide polymorphisms (SNP) of genes encoding nine Toll-like receptors (TLR) at 5–7 of age, we found preliminary evidence that the *TLR4* rs4986790, *TLR6* rs5743810 and *TLR7* rs179008 were associated with IOS results [10]. At the age of 10–13 years, the variant genotype of the *TLR10* rs4129009 was associated with better baseline and post-BD lung function [11].

The aim of the present study was to evaluate early-childhood and preschool-age risk factors for irreversible airway obstruction at 10–13 years of age after bronchiolitis in infancy. In addition, we evaluated whether baseline or post-BD Rrs5 or Xrs5 measured with IOS at preschool-age, were associated with irreversible airway obstruction in adolescence.

2. Materials and methods

2.1. Study design

The present study on risk factors for irreversible airway obstruction at 10–13 years of age after bronchiolitis in infancy is a secondary analysis of the data collected during the prospective long-term follow-up of our post-bronchiolitis cohort. We defined irreversible airway obstruction as post-BD FEV1 or post-BD FEV1/FVC below the 95% lower tolerance limits of population-based, sex-specific, height-related reference values with a reversibility of less than 12% in the bronchodilation test. Current asthma was defined as regular use of inhaled corticosteroids (ICS), or alternatively, as presence of asthma-presumptive symptoms and a diagnostic finding in the bronchodilation test [9]. Persistent asthma was defined as a presence of asthma diagnosis at 5–7 years of age and current asthma at 10–13 years of age.

Originally, the cohort consisted of healthy, full-term infants hospitalised for bronchiolitis in 2000–2004 at younger than 6 months of age [12]. Bronchiolitis was defined as lower respiratory infection with rhinitis, cough and tachypnoea, accompanied by wheezes or diffuse crackles on auscultation. RSV aetiology of bronchiolitis was studied by antigen detection or polymerase chain reaction (PCR) and rhinovirus aetiology by PCR in nasopharyngeal aspirates taken during hospitalisation [12].

In 2008–2009, 166 children participated in the control visit [13], and 103 under 7 years old children performed IOS, including Rrs5 and Xrs5 measurements at baseline, after exercise and after inhalation of 0.3 mg salbutamol through a spacer [7]. IOS was measured with impulse oscillometer (Master Screen IOS; Jaeger, Hochberg, Germany). Three graphically solid, artefact-free curves were obtained according to the ATS/ERS guidelines [14]. IOS results were reported as z-scores from national height-adjusted reference values [15].

In 2012–2013, 138 children participated in the control visit at 10–13 years of age [9], and 89 of them attended clinical examination with flow-volume spirometry including FEV1 and FEV1/FVC at baseline and after bronchodilator inhalation [8]. Flow-volume spirometry was measured with a calibrated spirometer Vmax Carefusion (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). First, three acceptable, artefact-free flow-volume curves were obtained. Then, children inhaled 0.4 mg salbutamol through a spacer, and post-BD FEV1 and FEV1/FVC were measured 15 min later. The results were analysed as percentages of population-based, sex-specific, height-related reference values, and the thresholds for abnormal values according to the 95% lower tolerance limits were $\leq$80% for boys and $\leq$82% for girls in FEV1 and $\leq$87% for boys and $\leq$88% for girls in FEV1/FVC, respectively [8].

Weight and height were measured during clinical examinations and body mass index for age z-score (zBMI) was calculated [16]. The cut-off values were determined for underweight, normal weight, overweight and obesity according to the Finnish growth reference data [16]. One girl of the 89 attendants was excluded due to underweight [17].

2.2. Risk factors

The recruited patients were younger than 6 months of age on admission to hospital, and for the current analyses, the infants were further divided into subgroups by the 4-week and 12-week cut-off limits. RSV and rhinovirus aetiologies of bronchiolitis, maternal and parental asthma, maternal smoking during pregnancy and infancy, atopic dermatitis in infancy, and asthma diagnosis at <12 months, at <3 years and at <5 years of ages were, in addition to age on admission, included as early-life risk factors. Risk factors at 5–7 years of age included atopic dermatitis, allergic rhinitis, asthma diagnosis and Prick test positivity at least to one of eight airborne allergens at 5–7 years of age [13].

Data on risk factors were based on findings made during hospitalisation or at control visits, or alternatively, on structured questionnaires filled by the families during hospitalisation and before both prospectively scheduled control visits at 5–7 years and 10–13 years of age [9, 13]. Questionnaires were checked and completed, if needed, together with the study physician. Asthma diagnoses at any age from birth to the 5–7 years control visit were required to be doctor-diagnosed. Atopic dermatitis and allergic rhinitis needed to be symptomatic during the last 12 months [13].

IOS results at 5–7 years of age were studied as risk factors for irreversible airway obstruction by spirometry at 10–13 years of age. These analyses were possible for 62 participants with data available for both IOS and spirometry measurements, and in addition, for presence of current asthma at 10–13 years of age. Baseline and post-BD Rrs5 and Xrs5, were included in the analyses as continuous variables.

2.3. Genetics

TLR4 rs4986790, *TLR7* rs179008 and *TLR10* rs4129009 SNPs were included in the present study because of their previously found associations with post-bronchiolitis lung function in the present cohort [10, 11]. TLR1, TLR2, TLR6 and TLR10 are called together as the TLR2 subfamily because they interact as co-receptors for each other. For that reason, also the genes encoding TLR1, TLR2 and TLR6, which are considered promoting the signalling of the receptors [18], were included in the analyses in addition to the gene of the inhibitory TLR10 [19]. Genetic data was available in 82 study subjects for the *TLR1*, *TLR2*, *TLR4*, *TLR6* and *TLR7* SNPs, and in 81 study subjects for the *TLR10* SNP. As published previously in detail [10,20], *TLR1* rs5743618, *TLR2* rs5743708, *TLR4* rs4986790 and *TLR6* rs5743810 were genotyped by pyrosequencing, the *TLR7* rs179008 by PCR-based sequencing and the *TLR10* rs4129009 by high-resolution melting analysis.

2.4. Statistics

Statistical analyses were performed using the SPSS Statistics for Windows version 25.0 (IBM Corp., Armonk, NY, USA). The results are reported as frequencies, means, standard deviations (SD) and 95% confidence intervals (95%CI), respectively. Chi-square and Fisher's exact tests, when appropriate, were used for categorised variables and the *t*-test for continuous variables in crude analyses. The p values < 0.05 or 95%CIs not overlapping were considered as statistically significant findings. Analysis of covariance (ANCOVA), providing adjusted p-values, and multivariate logistic regression, providing adjusted odds ratios (ORs) were used in multivariate analyses. In comparisons between children with irreversible airway obstruction and those with normal post-BD FEV1 and FEV1/FVC, the current asthma and current zBMI at 10–13 years of age were used as covariates due to their associations with post-bronchiolitis lung function observed previously in the present cohort [8,17]. Since no child with irreversible airway obstruction had the variant *TLR4* rs4986790 genotype, we changed the number 0 to the number 1, by adding one virtual case with irreversible airway obstruction and the *TLR4* variant genotype, but without current asthma and overweight, to make adjusted analyses technically possible.

2.5. Ethics

The study was approved by the Ethics Committee of the Tampere University Hospital district. Written consent was obtained from the parents during hospitalisation and at all control visits.

3. Results

Irreversible airway obstruction was present in 21/88 (23.9%) former bronchiolitis patients at the age 10–13 years. FEV1 reduction was present in 16/21 children and FEV1/FVC reduction in 9/21 children, as reported previously [8].

3.1. Baseline characteristics

As seen in Table 1, there were no significant differences between 21 former bronchiolitis patients with irreversible airway obstruction and 67 former bronchiolitis patients with normal post-BD FEV1 and FEV1/FVC in age, gender, height or zBMI, nor in the presence of current atopic dermatitis, current allergic rhinitis or current or persistent asthma. Five children (23.8%) with irreversible airway obstruction versus three (4.5%) of those with normal post-BD FEV1 and FEV1/FVC used ICSs ($p = 0.017$).

Table 1
Characteristics of 21 children with irreversible airway obstruction and 67 children with normal post-bd FEV1 and FEV1/FVC at the age of 10–13 years after hospitalisation for bronchiolitis at <6 months of age.

Characteristics at 10–13 years	Irreversible airway obstruction n = 21	Normal post-BD FEV1 and FEV1/FVC n = 67	
Continuous variables	**Mean (SD)**	**Mean (SD)**	**p value**[a]
Age (years)	11.7 (0.9)	11.6 (1.0)	0.925
Height (cm)	153.5 (7.7)	150.6 (9.1)	0.201
zBMI	0.56 (1.14)	0.16 (0.9)	0.103
Categorised variables	**n (%)**	**n (%)**	**p value**[b]
Girls	9 (42.9)	35 (52.2)	0.453
Current atopic dermatitis	8 (38.1)	19 (28.4)	0.399
Current allergic rhinitis	12 (57.1)	29 (43.3)	0.267
Current ICS use	5 (23.8)	3 (4.5)	**0.017**
Current asthma	5 (23.8)	7 (10.4)	0.148
Persistent asthma	3 (14.3)	3 (4.5)	0.145

SD, standard deviation. For definitions, see material and methods. Significant findings are highlighted with bold font.
[a] t-test test between the three groups.
[b] Chi-square test or Fisher's exact test between the three groups.

3.2. Symptoms and bronchodilator use

Wheezing was present during the last 12 months in 9/21 (42.9%) of former bronchiolitis patients with irreversible airway obstruction versus in 14/67 (20.9%) of those with normal post-BD FEV1 and FEV1/FVC ($p = 0.046$). Instead, the presence of prolonged cough or night cough did not differ between these two groups (Data not shown). Eight children (38.1%) with irreversible airway obstruction versus 12 (17.9%) of those with normal post-BD FEV1 and FEV1/FVC had used bronchodilators during the last 12 months ($p = 0.074$).

3.3. Risk factors

Age younger than four or 12 weeks on admission for bronchiolitis, or RSV or rhinovirus aetiology of bronchiolitis, showed no significant associations with irreversible airway obstruction at 10–13 years of age (Table 2). Likewise, maternal asthma, parental asthma, maternal smoking during pregnancy, maternal smoking during infancy, and presence of atopic dermatitis in infancy showed no significant associations with irreversible airway obstruction (Table 2). Asthma diagnosis at <12 months, but not at <3 years or at <5 years of age, was associated with airway obstruction at 10–13 years of age. Children with asthma diagnosis at <12 months had a 12.6-fold risk for irreversible airway obstruction in multivariate logistic regression adjusted for zBMI and current asthma (Table 2).

The studied risk factors at 5–7 years of age, such as asthma, atopic dermatitis, allergic rhinitis or skin prick test positivity, did not differ significantly between children with irreversible airway obstruction and those with normal post-BD FEV1 and FEV1/FVC at 10–13 years of age (Table 2).

3.4. TLR genotypes in relation to airway obstruction

Wild versus variant genotypes of *TLR1* rs5743618, *TLR2* rs5743708, *TLR6* rs5743810, *TLR7* rs179008 or *TLR10* rs4129009 were not associated with irreversible airway obstruction at the age of 10–13 years (Table 3). Irreversible airway obstruction was less frequent in 13 study subjects with the variant AG genotype than in those 69 with wild AA genotype of *TLR4* rs4986790 (Table 3). However, the significance was lost in logistic regression adjusted for zBMI and current asthma. None of the study subjects had the homozygous variant *TLR4* rs4986790 GG genotype.

3.5. IOS results in relation to airway obstruction

IOS data at 5–7 years of age was available in 17 children with irreversible airway obstruction and in 45 children with normal post-BD FEV1 and FEV1/FVC at the age of 10–13 years. Higher post-BD Rrs5 and lower baseline and post-BD Xrs5 showed significant associations with irreversible airway obstruction (Table 4).

4. Discussion

There are three main results in this prospective long-term post-bronchiolitis follow-up study on early-childhood and preschool-age risk factors for irreversible airway obstruction by spirometry in adolescence. First, asthma diagnosis made by a doctor before one year of age increased the risk for irreversible airway obstruction. Second, lower baseline and post-BD lung function, measured with IOS at the age of 5–7 years, predicted irreversible airway obstruction at the age of 10–13 years. These results were robust to adjustments with current asthma. Third, the variant genotype of the*TLR4* rs4986790 was protective

Table 2
Early-life and preschool-age risk factors in 21 former bronchiolitis patients with irreversible airway obstruction versus those 67 with normal post-BD FEV1 and FEV1/FVC at the age of 10–13 years.

Risk factors	Irreversible airway obstruction n = 21 n (%)	Normal post-BD FEV1 and FEV1/FVC n = 67 n (%)	p value[a]	Adjusted OR (95%CI)[b]
Hospitalisation				
Hospitalisation age ≤ 4 weeks	4 (19.0)	9 (13.4)	0.501	1.5 (0.4–6.2)
Hospitalisation age ≤ 12 weeks	11 (52.4)	43 (64.2)	0.333	0.7 (0.3–2.0)
RSV aetiology[c]	15 (71.4)	47 (71.2)	0.985	1.7 (0.5–5.7)
Rhinovirus aetiology[c]	2 (9.5)	8 (12.1)	1.0	0.6 (0.1–3.6)
Early life				
Atopic dermatitis at <12 months	9 (42.9)	16 (23.9)	0.092	2.1 (0.7–6.1)
Maternal asthma	1 (4.8)	9 (13.6)	0.440	0.2 (0.0–2.1)
Parental asthma	3 (14.3)	11 (16.4)	1.0	0.6 (0.1–2.7)
Maternal smoking during pregnancy	5 (23.8)	9 (13.4)	0.308	2.1 (0.6–7.6)
Maternal smoking at <12 months	7 (33.3)	17 (25.4)	0.475	1.5 (0.5–4.5)
Asthma diagnosis at <12 months	4 (19.0)	1 (1.5)	**0.011**	**12.6 (1.3–126.4)**
Asthma diagnosis at <3 years	6 (28.6)	10 (14.9)	0.196	1.6 (0.4–5.9)
Asthma diagnosis at <5 years	7 (33.3)	12 (17.9)	0.143	1.6 (0.4–5.6)
Follow-up at 5–7 years of age				
Current atopic dermatitis at 5–7 years	8 (38.1)	20 (29.9)	0.624	0.7 (0.3–2.1)
Current allergic rhinitis at 5–7 years	6 (28.6)	23 (34.3)	0.479	0.6 (0.2–2.0)
Asthma diagnosis at 5–7 years	5 (23.8)	7 (10.4)	0.148	1.8 (0.4–7.7)
Prick test positivity at 5–7 years[d]	6 (30.0)	20 (34.5)	0.714	0.6 (0.2–2.0)

OR, odds ratio. For definitions, see material and methods. Significant findings are highlighted with bold font.
[a] Chi-square test or Fisher's exact test between the groups.
[b] Logistic regression adjusted for current asthma and zBMI.
[c] n = 87 for children with viral aetiology available.
[d] n = 78 for children with skin prick test results available.

against irreversible airway obstruction in crude analyses, but the significance of this finding was lost in analyses adjusted for current asthma.

The origin of irreversible airway obstruction may be in the first years of life [1,2,21]. Available literature provides evidence for two separate pathways. First, Tucson birth cohort study demonstrated that lung function deficit already at birth led to lower attainable maximal FEV1 seen in young adulthood, and by ageing, these subjects reach earlier than others the criteria of COPD in spirometry [21]. Lung function of these children remains lowered through childhood even in absence of asthma or wheezing [22]. Second, large longitudinal follow-up studies have showed irreversible airway obstruction as a result of persistent asthma [23–24]. This pathway was seen also in the Tucson birth cohort in persistent wheezers [22]. An altered, genetically regulated immune system, which modulates responses to environmental exposures, such as tobacco smoke or infections, may modify both pathways [11,25,26].

In the Tucson birth cohort, children who had lower respiratory tract infection with wheeze before three years of age had worse lung function through school age compared to children who had never wheezed [22]. By measurements of lung function shortly after birth in 125 children, the authors were able to identify a subgroup presenting with lower forced expiratory flows before any infection [27]. In this subgroup, most children wheezed during the first year of life, only a third of them wheezed during the third year of life [28], and none wheezed anymore at 6 years of age [27]. This subgroup showed an association with maternal smoking but not with atopy [28]. The results do not demonstrate only mechanical bronchial obstruction because of narrower airways but may also suggest higher bronchial reactivity of infants.

In our present post-bronchiolitis cohort of children hospitalised at <6 months of age, worse lung function at preschool age increased the risk for irreversible obstruction in adolescence. The results were robust

Table 3
Gene polymorphisms of *TLR1*, *TLR2*, *TLR4*, *TLR6*, *TLR7* and *TLR10* in relation to irreversible airway obstruction in 82 former bronchiolitis patients.

TLR genotype		Irreversible airway obstruction n = 20 n (%)	Normal post-BD FEV1 and FEV1/FVC n = 62 n (%)	p value[a]	Adjusted OR (95%CI)[b]
TLR1 rs5743618	wild GG	14 (70.0)	48 (77.4)	0.554	1.2 (0.4–4.0)
	variant GT, TT	6 (30.0)	14 (22.6)		
TLR2 rs5743708	wild CC	18 (90.0)	59 (95.2)	0.591	2.3 (0.3–15.4)
	variant CT	2 (10.0)	3 (4.8)		
TLR4 rs4986790	wild AA	20 (100.0)	49 (79.0)	**0.031**	0.2 (0.0–1.6)[2]
	variant AG	0 (0)	13 (21.0)		
TLR6 rs5743810	wild CC	5 (25.0)	20 (32.3)	0.591	1.4 (0.4–4.5)
	variant CT, TT	15 (75.0)	42 (67.7)		
TLR7 rs179008 Girls	wild AA	5 (55.6)	18 (56.3)	1.0	0.8 (0.2–4.0)
	variant AT, TT	4 (44.4)	14 (43.8)		
TLR7 rs179008 Boys	wild AA	9 (81.8)	24 (80.0)	1.0	0.5 (0.1–4.2)
	variant TT	2 (18.2)	6 (20.0)		
TLR10 rs4129009	wild AA	16 (80.0)	50 (82.0)	1.0	1.0 (0.3–3.6)
	variant AG, GG	4 (20.0)	11 (18.0)		

TLR, Toll-like receptor. For definitions see material and methods. Significant findings are highlighted with bold font. None of the study subjects had *TLR2* rs5743708 variant CC, *TLR4* rs4986790 variant GG, *TLR10* rs3129009 GG and none boy had *TLR7* rs179008 variant AT. *TLR7* n = 41 for girls and n = 41 for boys. *TLR10* n = 81. Since no child with irreversible airway obstruction had the variant *TLR4* rs4986790 genotype, we added one virtual case to make adjusted analyses technically possible (see methods for details).
[a] Chi-square test or Fisher's exact test between the groups.
[b] Logistic regression adjusted for current asthma and zBMI.

Table 4
Results of impulse oscillometry in 62 children expressed as continuous variables in z scores at the age of 5–7 years. Children with irreversible airway obstruction in flow-volume spirometry at the age of 10–13 years were compared to those with normal post-BD FEV1 and FEV1/FVC.

IOS results	Irreversible airway obstruction n = 17		Normal post-BD FEV1 and FEV1/FVC n = 45		Adjusted p value[a]
	Mean (SD)	95%CI	Mean (SD)	95%CI	
Baseline Rrs5	0.18 (0.99)	−0.33 to 0.69	−0.22 (0.93)	−0.50 to 0.06	0.230
Post-BD Rrs5	−1.22 (0.84)	**−1.65 to -0.78**	−1.92 (0.75)	**−2.1 to -1.70**	**0.004**
Baseline Xrs5	−1.63 (1.53)	**−2.4 to -0.85**	−0.43 (0.99)	**−0.72 to -0.13**	**0.001**
Post-BD Xrs5	−0.21 (0.70)	**−0.57 to 0.15**	0.56 (0.56)	**0.40 to 0.73**	**<0.001**

SD, standard deviation; Rrs5, respiratory system resistance at 5 Hz; Xrs5, respiratory system reactance at 5 Hz. For definitions see material and methods. Significant findings are highlighted with bold font.
[a] ANCOVA between the groups, adjusted for current asthma and zBMI.

to adjustments with asthma. These findings suggest that there was a group of children with reduced growth of lung function independently from asthma, and this growth reduction started before school age. In addition, we were able to show that asthma diagnosis at <12 months of age, but not asthma diagnoses after that age, was an independently significant risk factor for irreversible airway obstruction in adolescence.

A post-bronchiolitis follow-up from Missouri, USA, demonstrated a steep decline of FEV1 between 10 and 16 years of age after a transient increase until 9 years of age in 122 children with RSV bronchiolitis at <12 months of age [29]. The declined lung function was associated with male gender, doctor-diagnosed asthma by age 6 and allergic sensitization by age 7 years [29]. Since 60% of study subject had doctor-diagnosed asthma and nearly half of them reported recent symptoms at latest follow-ups, the study in fact demonstrated lung function decline in asthmatic patients with persistent symptoms [29]. In line, the Tucson birth cohort study reported lowered baseline lung functions at 6 and 11 years of ages in persistent wheezers although these children did not show significant lung function deficits as newborns [22]. The earlier reduction of lung function in the Tucson birth cohort may come from their definition of persistent wheeze, which included only those who had persistent symptoms from early childhood [22]. Both the Tucson birth cohort and Missouri post-bronchiolitis cohort studies, in line with other longitudinal studies, suggest that airway remodelling resulting from persistent asthma leads to irreversible airway obstruction [22–24,29]. The power of our post-bronchiolitis study was not enough to prove or rule out irreversible airway obstruction in children with current or persistent asthma. Interestingly, current use of ICS was associated with irreversible airway obstruction in adolescence suggesting earlier lung function deficit in children with more severe post-bronchiolitis asthma.

In a previous Finnish post-bronchiolitis follow-up study, risk factors for irreversible airway obstruction were evaluated at 27–30 years of age in 47 participants hospitalised for bronchiolitis and 22 participants hospitalised for pneumonia at younger than two years of age, and in 138 controls [6]. In that study, former bronchiolitis and current asthma were identified as independently significant risk factors for irreversible airway obstruction at the age of 27–30 years, defined as post-BD FEV1/FVC <5th percentile of the GLI 2012 guidelines [6]. The results suggest separate roles for infant bronchiolitis and later asthma as risk factors for irreversible airways obstruction in adulthood.

Polymorphisms in genes encoding TLRs may change their recognising and signalling functions leading to altered regulation of immunological responses. *TLR4* rs4986790 variations have been associated with better lung function, especially in adults with COPD [25], and in agreement, the variant genotype protected from post-bronchiolitis irreversible airway obstruction in the present study. As published previously from this cohort, the risk for post-bronchiolitis asthma did not differ in relation to *TLR4* rs4986790 variation [30]. The *TLR10* rs4129009 was not associated with irreversible bronchial obstruction in the present study, though the variant genotype was associated with better baseline and post-BD lung function [11] and a higher risk for asthma in adolescence [26]. These findings appear to be conflicting, but they may also mean that different qualities of immune regulation predominate in the emergence of asthma and irreversible airway obstruction.

The strengths of the present study are the long-term follow-up of a unique cohort of children hospitalised for bronchiolitis at the age under 6 months and the careful collection of risk factor data at prospectively scheduled control visits. Assessment of airway obstruction was done with IOS at preschool-age, and spirometry was performed not earlier than at the age when children were capable for forced expiratory maneuvers. Baseline and post-BD lung function was studied at both ages. However, lung function was not studied before 5–7 years of age. Although 166 children were invited to the follow-up visits, only 103 and 89, respectively, participated in lung function studies. These small numbers are the main limitation of the study, which means that our study was under-powered to find all existing differences between the groups constructed on the basis of irreversible obstruction in spirometry.

5. Conclusion

In this prospective long-term post-bronchiolitis follow-up, current ICS use, suggesting severe post-bronchiolitis asthma, was associated with irreversible airway obstruction in adolescence. In addition, doctor-diagnosed asthma before one year of age and worse results in IOS at preschool age increased the risk for irreversible airway obstruction independently from asthma. We found preliminary evidence that *TLR4* rs4986790 variation may be protective against irreversible airway obstruction after bronchiolitis. Our findings in children hospitalised for bronchiolitis in early infancy reflects lung function outcomes in high-risk infants and suggest different origins for development of irreversible airway obstruction. Earlier lung function deficit may develop via pathways that differs from those typically seen in post-bronchiolitis asthma.

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Author contributions

Riikka Riikonen has contributed to conceiving and designing the study, analysing the data, interpreting the results and writing the manuscript.

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Sari Törmänen has contributed to collecting the data and writing the manuscript.

Petri Koponen has contributed to collecting the data and writing the manuscript.

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Merja Helminen has contributed to collecting the data and writing the manuscript.

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Eero Lauhkonen has contributed to conceiving and designing the study, collecting the data, interpreting the results and writing the manuscript.

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] A. Bush, COPD: a pediatric disease, J Chron Obstr Pulmon Dis 5 (2008) 53–67.

[2] F.D. Martinez, Early-life origins of chronic obstructive pulmonary disease, N. Engl. J. Med. 375 (2016) 871–878.

[3] H. Fjaerli, T. Farstad, G. Roed, G. Ufert, P. Guldbrandsen, P. Nakstad, et al., Acute bronchiolitis in infancy as risk factor for wheezing and reduced pulmonary function by seven years in Akershus County, Norway, BMC Pediatr. 5 (2005) 31.

[4] N. Sigurs, F. Aljassim, B. Kjellman, P. Robinson, F. Sigurbergsson, R. Bjarnason, et al., Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life, Thorax 65 (2010) 1045–1052.

[5] E. Goksör, P.M. Gustafsson, B. Alm, M. Åmark, G Wennergren, Reduced airway function in early adulthood among subjects with wheezing disorder before two years of age, Pediatr. Pulmonol. 43 (2008) 396–403.

[6] K. Backman, E. Piippo-Savolainen, H. Ollikainen, H. Koskela, M. Korppi, Irreversible airway obstruction in adulthood after bronchiolitis in infancy: evidence from a 30-year follow-up study, Respir. Med. 108 (2014) 218–223.

[7] E. Lauhkonen, P. Koponen, K. Nuolivirta, M. Paassilta, J. Toikka, M. Korppi, Lung function by impulse oscillometry at age 5-7 years after bronchiolitis at age 0-6 months, Pediatr. Pulmonol. 50 (2015) 389–395.

[8] R. Riikonen, E. Lauhkonen, S. Törmänen, K. Backman, P. Koponen, M. Helminen, et al., Prospective study confirms that bronchiolitis in early infancy increases the risk of reduced lung function at 10-13 years of age, Acta Paediatr. 108 (2019) 124–130.

[9] S. Törmänen, E. Lauhkonen, R. Riikonen, P. Koponen, H. Huhtala, M. Helminen, et al., Risk factors for asthma after infant bronchiolitis, Allergy 73 (2017) 916–922.

[10] E. Lauhkonen, P. Koponen, J. Vuononvirta, J. Teräsjärvi, K. Nuolivirta, J. Toikka, et al., Gene polymorphism of toll-Like receptors and lung function at five to seven years of age after infant bronchiolitis, PloS One 11 (2016), e0146526.

[11] R. Riikonen, M. Korppi, S. Törmänen, K. Nuolivirta, M. Helminen, Q. He, et al., Toll-like receptor 10 rs4129009 gene polymorphism is associated with post-bronchiolitis lung function in adolescence, Acta Paediatr. 109 (2020) 1634–1641.

[12] K. Nuolivirta, P. Koponen, Q. He, A. Halkosalo, M. Korppi, T. Vesikari, et al., Bordetella pertussis infection is common in nonvaccinated infants admitted for bronchiolitis, Pediatr. Infect. Dis. J. 29 (2010) 1013–1015.

[13] P. Koponen, M. Helminen, M. Paassilta, T. Luukkaala, M. Korppi, Preschool asthma after bronchiolitis in infancy, Eur. Respir. J. 39 (2012) 76–80.

[14] N. Beydon, S. Davis, E. Lombardi, J. Allen, H. Arets, P. Aurora, et al., An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children, Am. J. Respir. Crit. Care Med. 175 (2007) 1304–1345.

[15] L.P. Malmberg, A. Pelkonen, T. Poussa, A. Pohjanpalo, T. Haahtela, M. Turpeinen, Determinants of respiratory system input impedance and bronchodilator response in healthy Finnish preschool children, Clin. Physiol. Funct. Imag. 22 (2002) 64–71.

[16] A. Saari, U. Sankilampi, M. Hannila, V. Kiviniemi, K. Kesseli, L. Dunkel, New Finnish growth references for children and adolescents aged 0 to 20 years: length/height-for-age, weight-for-length/height, and body mass index-for-age, Ann. Med. 43 (2011) 235–248.

[17] R. Riikonen, S. Törmänen, A. Saari, P. Koponen, M. Helminen, K. Nuolivirta, et al., Preliminary communication suggests overweight was associated with reduced lung function in adolescence after infant bronchiolitis, Acta Paediatr. 108 (2019) 1729–1730.

[18] R.I. Tapping, K.O. Omueti, C.M. Johnson, Genetic polymorphisms within the human Toll-like receptor 2 subfamily, Biochem. Soc. Trans. 35 (2007) 1445–1448.

[19] M. Oosting, S. Cheng, J.M. Bolscher, R. Vestering-Stenger, T.S. Plantinga, I. C. Verschueren, et al., Human TLR10 is an anti-inflammatory pattern-recognition receptor, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 4478.

[20] P. Koponen, J. Vuononvirta, K. Nuolivirta, M. Helminen, Q. He, M. Korppi, The association of genetic variants in toll-like receptor 2 subfamily with allergy and asthma after hospitalization for bronchiolitis in infancy, Pediatr. Infect. Dis. J. 33 (2014) 463–466.

[21] D. Stern, W. Morgan, A. Wright, S. Guerra, F. Martinez, Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study, Lancet 370 (2007) 758–764.

[22] W. Morgan, D. Stern, D. Sherril, S. Guerra, Outcome of asthma and wheezing in the first 6 years of life: follow-up through adolescence, Am. J. Respir. Crit. Care Med. 172 (2005) 1253–1258.

[23] A. Tai, H. Tran, M. Roberts, N. Clarke, J. Wilson, C. Robertson, The association between childhood asthma and adult chronic obstructive pulmonary disease, Thorax 69 (2014) 805–810.

[24] M.J. McGeachie, K.P. Yates, X. Zhou, F. Guo, A. Stenberg, M. Van Natta, et al., Patterns of growth and decline in lung function in persistent childhood asthma, N. Engl. J. Med. 374 (2016) 1842–1852.

[25] S. Arora, S. Ahmad, R. Irshad, Y. Goyal, S. Rafat, N. Siddiqui, et al., TLRs in pulmonary diseases, Life Sci. 233 (2019) 116671.

[26] S. Törmänen, M. Korppi, E. Lauhkonen, P. Koponen, J. Teräsjärvi, J. Vuononvirta, et al., Toll-like receptor 1 and 10 gene polymorphisms are linked to postbronchiolitis asthma in adolescence, Acta Paediatr. 107 (2018) 134–139.

[27] F. Martinez, A.L. Wright, L. Taussig, C.J. Holmberg, M. Halonen, W. Morgan, et al., Asthma and wheezing in the first 6 years of life, N. Engl. J. Med. 332 (1995) 133–138.

[28] L. Taussig, A.L. Wright, C.J. Holberg, M. Halonen, W. Morgan, F. Martinez, Tucson Children's respiratory study: 1980 to present, J. Allergy Clin. Immunol. 111 (2003) 661–675.

[29] M. Kitcharoensakkul, L. Bacharier, T. Schweiger, B. Wilson, C. Goss, D. Lew, et al., Lung function trajectories and bronchial hyperresponsiveness during childhood following severe RSV bronchiolitis in infancy, Pediatr. Allergy Immunol. 32 (2021) 457–464.

[30] S. Törmänen, M. Korppi, J. Teräsjärvi, J. Vuononvirta, P. Koponen, M. Helminen, et al., Polymorphism in the gene encoding toll-like receptor 10 may be associated with asthma after bronchiolitis, Sci. Rep. 7 (2017) 2956.