Tampere University

ANNUKKA HOLSTER

Prospective Bronchiolitis Cohort Study

Interleukin 17 gene polymorphisms in bronchiolitis, post-bronchiolitis asthma and lung function

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ACADEMIC DISSERTATION
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PunaMusta Oy – Yliopistopaino
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To my family

ABSTRACT

Background: Bronchiolitis, a lower respiratory tract infection that occurs during infancy, is mainly caused by respiratory syncytial virus (RSV) and is the leading cause of infant hospitalisation. Susceptibility to severe bronchiolitis and post-bronchiolitis asthma has been established as a multifactorial entity, with genetic and viral factors being particularly prominent. Interleukin (IL)-17A and IL-17F, encoded by the *IL17A* and *IL17F* genes, respectively, are mainly proinflammatory cytokines involved in inflammation and asthma.

Aims: The aims of this long-term prospective cohort study were (1) to evaluate the clinical course and the viral aetiology of bronchiolitis in infancy at less than 6 months of age in relation to the *IL17A* rs2275913, rs4711998 and rs8193036 single nucleotide polymorphisms (SNPs) and the *IL17F* rs763780, rs11465553 and rs7741835 SNPs, and (2) to evaluate the associations between these *IL17A* and *IL17F* SNPs and post-bronchiolitis asthma and lung function in children aged 5–7 years and 11–13 years. Minor allele frequencies (MAFs) of the *IL17A* rs2275913, rs4711998 and rs8193036 SNPs and the *IL17F* rs763780, rs11465553 and rs7741835 SNPs in bronchiolitis patients were compared to the MAFs in the Finnish population obtained from two publicly available databases.

Materials and methods: Originally, 166 previously healthy, full-term infants aged less than 6 months who were hospitalised for bronchiolitis were enrolled in the study. The viral aetiology of the bronchiolitis cases was determined by virus antigen and genome detection in nasopharyngeal aspirates, and RSV was the predominant causative agent. During hospitalisation, each patient's disease severity markers, such as the length of hospital stay and the need for and duration of oxygen administration and/or feeding support, were registered, and blood samples were collected for later genetic studies. Follow-up visits took place when the patients were 5–7 and 11–13 years of age. Asthma and allergy diagnoses, asthma-presumptive symptoms and use of inhaled bronchodilators or inhaled corticosteroids (ICSs) were registered. Lung function was measured with impulse oscillometry (IOS) at 5–7 years of age and with flow-volume spirometry (FVS) at 11–13 years of age.

Results: The MAFs of the *IL17A* rs2275913 (A), rs4711998 (A) or rs8193036 (C) and the *IL17F* rs763780 (C), rs11465553 (T) or rs7741835 (T) SNPs did not differ between the cases and the controls, which consisted of 99 Finnish samples from the 1000 Genomes Project and the Finnish data (from 1734 to 12558, depending on the SNP) of the Genome Aggregation Database. There were no significant differences in the severity or RSV aetiology of bronchiolitis during hospitalisation or in the asthma outcomes between children with the wild or variant genotypes of the analysed *IL17A* or *IL17F* SNPs until the age of 5–7 years. At 11–13 years of age, children with the variant GA or AA genotype of *IL17A* rs2275913 had a significantly lower prevalence of asthma, use of ICSs during the last 12 months or prevalence of allergic rhinitis than those with the wild genotype GG. In adjusted analyses, the difference in the use of ICSs during the last 12 months remained statistically significant (aOR 0.25). In the IOS results obtained at 5–7 years of age, the *IL17A* rs2275913 wild genotype GG was associated with exercise-induced airway resistance; however, in the FVS results obtained at 11–13 years of age, no abnormalities were apparent. Children with the variant genotype TC or CC of *IL17F* rs763780 had used ICSs more often between the follow-up visits from 5–7 to 11–13 years (aOR 3.58) than those with the wild genotype TT. The results also showed that the *IL17A* rs4711998 and rs8193036 SNPs, or the *IL17F* rs11465553 and rs7741835 SNPs had no obvious impact on post-bronchiolitis asthma.

Conclusions: In this post-bronchiolitis cohort study, some evidence was found that suggests that the variant genotype of *IL17A* rs2275913 may be protective against post-bronchiolitis asthma at school age; moreover, the same variant genotype was found to already present with less bronchial hyperresponsiveness at preschool age. In addition, the variant genotype of *IL17F* rs763780 may increase the risk for post-bronchiolitis asthma persisting from 5 to 13 years of age.

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Tausta: Viruksen aiheuttama bronkioliitti eli ilmatiehyttulehdus on imeväisikäisen alempien hengitysteiden tulehdus, ja se on myös yleisin syy imeväisikäisen sairaalahoitoon. Toistuvat hengenahdistukset ovat yleisiä bronkioliitin jälkeisinä vuosina. Sairaalahoitoisen bronkioliitin jälkeen on kuvattu keuhkojen toimintahäiriöitä, ja pitkäaikaisseurantatutkimusten perusteella bronkioliitin sairastaneilla lapsilla on suurentunut riski sairastua myöhemmin astmaan. Yksilön perimällä on suuri vaikutus sekä bronkioliitin vaikeusasteeseen, että bronkioliitin jälkeiseen astmasairastavuuteen. Interleukiini (IL)-17A ja -17F ovat tulehdusta pääsääntöisesti voimistavia sytokiineja, jotka on aiempien tutkimusten perusteella yhdistetty tulehduksiin ja astmaan. Variaatiot geeneissä, jotka koodittavat IL-17A (*IL17A*) ja IL-17F (*IL17F*) tuotantoa, saattavat olla yhteyksissä bronkioliitin vaikeusasteeseen ja bronkioliitin jälkeiseen astmasairastavuuteen.

Tavoitteet: Tämän väitöskirjatutkimuksen tarkoituksena oli tutkia *IL17* geenien (*IL17A* rs2275913, *IL17A* rs4711998, *IL17A* rs8193036, *IL17F* rs763780, *IL17F* rs11465553 ja *IL17F* rs7741835) yksittäisten nukleotidien polymorfismien yhteyttä alle puolivuotiaana sairastetun bronkioliitin vaikeusasteeseen tai bronkioliitin aiheuttajavirukseen, sekä yhteyttä sairastetun bronkioliitin jälkeiseen astman esiintyvyyteen esikoulu- ja kouluiässä. Lisäksi tutkimuksessa tarkasteltiin *IL17A* ja *IL17F* geenipolymorfismien vaikutusta keuhkofunktioon sairastetun bronkioliitin jälkeen. Tavoitteena oli myös selvittää *IL17A* ja *IL17F* geenipolymorfismin esiintymistä bronkioliittiin sairastuneilla lapsilla verrattuna yleisesti suomalaiseen väestöön käyttäen verrokkeina saatavilla olevia geenitietokantoja.

Menetelmät: Tutkimukseen otettiin mukaan 166 aiemmin tervettä, täysiaikaisena syntynyttä alle kuuden kuukauden ikäistä imeväistä, jotka joutuivat bronkioliitin vuoksi sairaalahoitoon. Bronkioliitin vaikeusastetta kuvaavat tekijät, kuten sairaalahoidon tarve tai sen kesto, tai mahdollinen lisähapen tai -ravitsemuksen tarve tai sen kesto, kirjattiin ylös sairaalahoidon aikana. Bronkioliitin virusetiologia määritettiin nenänielun imulimanäytteistä. Bronkioliitin jatkoseurantakäynnit järjestettiin 5-7- ja 11-13 vuoden ikäisenä, jolloin selvitettiin mahdolliset astma- ja/tai allergiadiagnoosit, inhaloitavien hengitysteitä avaavien lääkkeiden tai inhaloitavien

kortisonilääkkeiden tarve tai mahdollisten astmaan viittaavien oireiden esiintyminen. Keuhkojen toimintaa testattiin impulssioskillometrialla 5-7 vuoden käynnillä ja spirometrialla 11-13 vuoden käynnillä. Verinäytteet geneettisiä tutkimuksia varten kerättiin sekä sairaalahoidon aikana että myöhemmillä kontrollikäynneillä.

Tulokset: Bronkioliittikohortin lapsilla ei esiintynyt tutkittujen *IL17* geenien polymorfismeja enempää kuin suomalaisella kontrolliväestöllä. Tutkittujen *IL17A* tai *IL17F* geenien polymorfismeilla ei ollut yhteyttä bronkioliitin vaikeusasteeseen, bronkioliitin aiheuttajavirukseen, eikä myöskään astman esiintyvyyteen 5-7 vuoden iässä. Sen sijaan myöhemmin 11-13-vuotiaana bronkioliittikohortin *IL17A* rs2275913 valtagenotyyppillä havaittiin tilastollisesti merkitsevä, hieman yli 3-kertainen riski sairastua astmaan verrattuna saman *IL17A* geenipolymorfismin varianttigenotyyppiin. Tämän lisäksi sama *IL17A* rs2275913 valtagenotyyppi assosioitui rasituksen provoimaan hengitysteiden vastuksen lisääntymiseen 5-7 vuoden iässä. Muita poikkeavia keuhkofunktiolöydöksiä ei havaittu esikouluiässä tai kouluiässä millään *IL17A* tai *IL17F* geenipolymorfismilla. 11-13 vuoden iässä *IL17F* rs763780 varianttigenotyyppiin yhdistyi inhaloitavien kortikosteroidien lisääntynyt käyttö kontrollikäyntien välissä 5-13-vuotiaana.

Yhteenveto: Tutkimus antoi alustavia havaintoja siitä, että *IL17A* rs2275913 ja *IL17F* rs763780 geenipolymorfismit voivat assosioitua bronkioliitin jälkeiseen astmaan kouluiässä, mutta ei aikaisemmin. Tämän lisäksi *IL17A* rs2275913 geenipolymorfismi näytti liittyvän bronkioliitin jälkeisen keuhkofunktion alenemaan esikouluiässä, mutta ei enää myöhäisemmällä iällä.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by numerals I–V:

I Holster A, Teräsjärvi J, Lauhkonen E, Törmänen S, Helminen M, Koponen P, Korppi M, Peltola V, He Q, Nuolivirta K. *IL-17A* gene polymorphism rs2275913 is associated with the development of asthma after bronchiolitis in infancy. Allergology International 2018; 67: 109-113.

II Nuolivirta K, Holster A, Teräsjärvi J, Lauhkonen E, Törmänen S, Helminen M, Koponen P, Korppi M, He Q. *IL17A* gene polymorphism rs4711998 and rs8193036 are not associated with postbronchiolitis asthma in Finnish children. Acta Paediatrica 2018; 107: 1290-1291.

III Holster A, Teräsjärvi J, Barkoff A-M, Lauhkonen E, Törmänen S, Helminen M, Korppi M, He Q, Nuolivirta K. *IL17F* rs763780 single nucleotide polymorphism is associated with asthma after bronchiolitis in infancy. Acta Paediatrica 2021; 110: 222-227.

IV Holster A, Riikonen R, Korppi M, Nuolivirta K, He Q, Lauhkonen E. *Interleukin 17A* gene variations and lung function at school age after bronchiolitis in infancy. Acta Paediatrica 2022; 111: 640-641.

V Holster A, Riikonen R, Korppi M, Nuolivirta K, He Q, Lauhkonen E. *Interleukin 17F* polymorphisms showed no association with lung function at school age after infant bronchiolitis. Acta Paediatrica 2021; 110: 219-221.

This thesis contains unpublished publicly available data on the allele frequencies of *IL17A* rs2275913, *IL17A* rs4711998, *IL17A* rs8193036, *IL17F* rs763780, *IL17F* rs11465553 and *IL17F* rs7741835 in the Finnish samples of the 1000 Genomes Project (https://www.internationalgenome.org/) and the Genome Aggregation Database (https://gnomad.broadinstitute.org/).

AUTHOR'S CONTRIBUTIONS

The author of this dissertation contributed to all five original publications, four as the main author and one as the second author. This long-term prospective follow-up study was originally designed by professor (emeritus) Matti Korppi. The data collection during hospitalisation for bronchiolitis was performed by docent Kirsi Nuolivirta, and docent Merja Helminen. At the 5–7-year follow-up, the data collection was performed by Petri Koponen, MD, PhD, and at the 11–13-year follow-up, the data collection was performed by docent Eero Lauhkonen, and Sari Törmänen, MD, PhD. The laboratory analyses were performed by professor Quishui He, Johanna Teräsjärvi, MSc and Alex-Mikael Barkoff, MSc. None of the original publications have been a part of any other academic dissertation.

The author of this dissertation contributed to the original publications as follows:

I The author participated in the data analyses and had the main responsibility for writing the manuscript.

II The author participated in the data analyses and co-wrote the manuscript.

III The author performed the data analyses, had the main responsibility for writing the manuscript and was responsible for the publication process.

IV The author participated in the data analyses and had the main responsibility for writing the manuscript.

V The author participated in the data analyses and had the main responsibility for writing the manuscript.

ABBREVIATIONS

ANOVA	Analysis of variance
aOR	Adjusted odds ratio
BD	Bronchodilation
CD	Cluster differentiation
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
DNA	Deoxyribonucleid acid
ECT	Exercise challenge test
FEV1	Forced expiratory volume in one second
FVC	Forced vital capacity
FVS	Flow-volume spirometry
HRMA	High-resolution melting analysis
ICSs	Inhaled corticosteroids
IL17A	Interleukin-17A (gene)
IL-17A	Interleukin-17A (cytokine)
IL17F	Interleukin-17F (gene)
IL-17F	Interleukin-17F (cytokine)
IOS	Impulse oscillometry
LOS	Length of hospital stay
LRTI	Lower respiratory tract infection
MAF	Minor allele frequency
NK	Natural killer
OR	Odds ratio
PCR	Polymerase chain reaction
Post-BD	Post-bronchodilator
RNA	Ribonucleic acid
RR	Risk ratio
Rrs5	Respiratory system resistance at 5 Hz
RSV	Respiratory syncytial virus
SD	Standard deviation
SNP	Single nucleotide polymorphism
SPSS	Statistic Package of Social Science

STEPS	Steps to the Healthy Development and Well-being of Children (study)
Th1	T-helper 1
Th2	T-helper 2
Th17	T-helper 17
Xrs5	Respiratory system reactance at 5 Hz
zBMI	Body mass index z-score
Zrs5	Respiratory system mechanical impedance at 5 Hz
95%CI	95% confidence interval

1 INTRODUCTION

Bronchiolitis is a virus-induced inflammation of the small airways that affects infants and young children worldwide and causes most of the infant hospitalisation in high-income countries (1). The upper age limit for diagnosis of bronchiolitis is 12 months in most European countries and 24 months in the USA (2). The respiratory syncytial virus (RSV) is the most common causative agent of bronchiolitis, especially in infants less than 6 months of age (3). Among all bronchiolitis patients, 1–3% require hospitalisation as a result of the disease (4), particularly those aged less than 6 months (5). In recent years, several entities have been identified under the clinical diagnosis of bronchiolitis, depending on the age of onset, the viral aetiology of the bronchiolitis and/or a history of atopic eczema (2, 6).

The development of lung function is a continuous process that starts in the prenatal period in utero and continues throughout the first years of life and into early adolescence (7). This process is genetically regulated and modified by environmental factors throughout life (8). Longitudinal cohort studies have suggested that lung function trajectories may vary between individuals and fluctuate over time and that abnormal lung function trajectories may originate early in life (9-11).

Epidemiological studies have determined that 20–40% of infants hospitalised for bronchiolitis will subsequently develop childhood asthma (12-15). Hence, this raises the question of whether bronchiolitis in early infancy results in injury that alters normal lung development and predisposes the child to subsequent asthma or whether certain infants have a pre-existing aberration in their immune response or airway function that predisposes them to both severe bronchiolitis and later asthma.

Susceptibility to severe bronchiolitis and later asthma is evidently multifactorial, with underlying genetic mechanisms, especially those regulating an individual's immune response to environmental agents, such as viruses (16). Interleukin (IL)-17A and IL-17F are members of the IL-17 family and share high protein sequence homology (17). IL-17A and IL-17F are proinflammatory cytokines predominantly secreted by T helper 17 (Th17) cells (18). IL-17A and IL-17F production is encoded by the *IL17A* and *IL17F* genes, respectively (19, 20). *IL17A* and *IL17F* are both located in the genomic region that has been linked to asthma-related phenotypes in

multiple genomic scans (21, 22). Single nucleotide polymorphisms (SNPs) in the *IL17A* and *IL17F* genes may impact gene function and result in abnormal cytokine production; therefore, they are the subject of intensive study.

Previous studies have documented an association between SNPs in *IL17A* and *IL17F* and susceptibility to asthma in different populations (23-27). Thus, the aim of this thesis was to study the impact of the *IL17A* rs2275913, rs4711998 and rs8193036 variants and the *IL17F* rs763780, rs11465553 and rs7741835 variants on the viral aetiology and clinical course of bronchiolitis in infants aged less than 6 months and on subsequent asthma and lung function at 5–7 and 11–13 years of age.

2 REVIEW OF THE LITERATURE

2.1 Epidemiology of bronchiolitis

2.1.1 Definition and clinical characteristics

Bronchiolitis is defined as the first lower respiratory tract infection (LRTI) in infants with viral-induced inflammation of the small airways, bronchioles and surrounding tissue (28, 29). Bronchiolitis is a clinical diagnosis that is based on the following typical symptoms: low grade fever, rhinorrhoea, nasal congestion, tachypnoea, cough, increased respiratory effort and thoracal retractions (29, 30).

The upper age limit of bronchiolitis varies between 12 and 24 months, depending on the region; 12 months is the limit in European countries, and 24 months is the limit in the USA (31). In Finland, bronchiolitis is generally defined as the first expiratory breathing difficulty or wheezing episode in infants younger than 12 months of age (28, 30). The course of the disease is usually mild; however, certain infants, such as those with known risk factors, may require hospitalisation for bronchiolitis and even treatment in an intensive care unit (1).

Age is associated with the severity of bronchiolitis, and infants who need intensive monitoring or treatment are typically those under 6 months of age (5). These infants, especially those under 3 months, are, in fact, less likely to present with wheezing as a bronchiolitis symptom; instead, the clinical picture resembles viral pneumonia with audible crackles (32). In addition, especially in infants aged less than 2 months, apnoea may be one of the first symptoms of bronchiolitis (33).

There are several well-established risk factors for bronchiolitis, namely day-care attendance, the presence of older siblings and tobacco exposure—particularly maternal smoking during pregnancy (34). The known risk factors for severe bronchiolitis requiring hospitalisation are prematurity, congenital heart disease or chronic lung disease (31, 34). Nevertheless, most infants hospitalised for bronchiolitis are born full-term with no known risk factors (30). The current treatment for bronchiolitis is limited to symptomatic treatment (29); therefore,

bronchiolitis treatment consists mainly of supportive therapies, such as supplementary oxygen and fluid support and ventilatory support if needed (1).

2.1.2 Incidence and seasonality

In terms of its incidence, bronchiolitis occurs in 20–30% of children in the first year of life and in 10–20% of children in the second year of life (4). Birth cohort studies have confirmed that the incidence of bronchiolitis is 18–32% during the first year of life (34). Approximately, 1–3% of infants with bronchiolitis require hospitalisation, and in most cases, these are infants aged less than 6 months (4).

The incidence of bronchiolitis displays a seasonal pattern. It tends to occur during the cold winter months in regions with temperate climates and peaks during the spring and autumn in Asia and during the winter in North America and Europe (35, 36). In Finland, the incidence of bronchiolitis has followed a biennial pattern, with major epidemics alternating with minor outbreaks every other winter, followed by varying spring outbreaks (37). The majority of bronchiolitis cases are mild and self-limiting and do not require visits to health professionals. The mortality rate for bronchiolitis is low, and those most at risk of death from bronchiolitis are children with underlying illnesses or other risk factors (38, 31).

2.2 Viral aetiology of bronchiolitis

Modern molecular-level detection techniques have enabled the identification of a diverse group of viruses capable of causing bronchiolitis. Respiratory viruses spread in aerosols, infect hosts via direct contact and then replicate in the epithelial cells of the airways (31).

The most common aetiology of bronchiolitis is RSV, which causes up to 80% of hospitalised bronchiolitis cases (2). Other viruses that cause bronchiolitis include rhinovirus, metapneumovirus, influenza virus, adenovirus, coronavirus, bocavirus and parainfluenza virus (29). Mixed infections are also common; they are found in 5–38% of bronchiolitis cases (39-41). Since RSV is the most prevalent cause of bronchiolitis, many previous studies on virus bronchiolitis have used the classification of RSV bronchiolitis versus non-RSV bronchiolitis (42-44).

2.2.1 Respiratory syncytial virus (RSV)

RSV is a single-stranded enveloped ribonucleic acid (RNA) virus (45). RSV is the leading cause of severe cases of LRTI in infants and young children, resulting in 33 million infections, over 3 million hospitalisations and over 100,000 deaths in children aged 0–60 months annually worldwide (46).

Bronchiolitis in infants aged less than 12 months is usually caused by RSV, and the youngest infants are at higher risk: RSV is the causative virus in 50–80% of bronchiolitis cases in infants less than 3 months of age (31). Interestingly, a recent Israeli retrospective cohort study showed that the risk of hospitalisation for RSV bronchiolitis was greater for slightly older infants, with infants aged 0–5 months having a 7.66-fold higher risk and infants aged 6–11 months having a 12.88-fold higher risk when compared to infants aged 12–24 months (47). This finding may be partially explained by ethnicity and by the association between calendar month of birth and RSV bronchiolitis (47).

RSV is a highly contagious pathogen that affects the airway epithelium, which is an important component of the antiviral response—acting as a physical and immunological barrier (48). RSV infection can result in the production of proteases and oxygen radicals that may cause major airway damage and lead to abnormal development of the structure and function of the airway and lung tissue (49).

The RSV incubation period is 4–6 days. This is followed by viral replication in the nasal epithelium that results in congestion, rhinorrhoea and poor feeding (due to nasal obstruction) (30). RSV bronchiolitis is characterised by acute inflammation of the small airways that leads to oedema of the airway wall, mucus hyper-production and necrosis of the airway epithelium (29). This is followed by neutrophil recruitment and a massive release of proinflammatory mediators, such as cytokines and chemokines, which finally leads to bronchoconstriction (50). In the lungs, the release of proinflammatory mediators and the recruitment of inflammatory cells to the infected and injured tissue and their migration across the endothelium are the crucial early immune defence events, and these are mediated by the innate immune system (51). As in other less-studied viral infections, the outcome of an RSV infection depends on the host's genetic diversity and its ability to strike a balance between controlling viral replication and tissue damage (52). Innate and adaptive immune responses are involved in viral clearance, and the regeneration of the bronchiolar epithelium begins within 3–4 days of the resolution of symptoms (30).

RSV epidemics occur annually, and nearly all children (98%) have been infected with RSV by the age of 2 years (2, 52). Recurrent RSV infections are common, and

secondary infections are milder than primary ones (53). In invasive infections, lung pathology is known to persist even after the virus has been efficiently cleared (54), and RSV infection early in life may cause later bronchial obstructive symptoms by damaging the growing lung or by altering the host's immune response (55). In addition, infants born with narrower intrathoracic airways seem to be more vulnerable to obstructive conditions and may even be prone to lower respiratory involvement during viral infection (56).

2.2.2 Other viruses (non-RSV)

Rhinoviruses are the second most common viral agent of bronchiolitis during infancy and are responsible for 20–40% of severe bronchiolitis cases among children aged less than 2 years (31). In older children (12–24 months), rhinoviruses are the most common viruses found in bronchiolitis and wheezing bronchitis (28). Rhinoviruses are nonenveloped, positive-strand RNA viruses and are classified into three species: rhinovirus-A, rhinovirus-B and rhinovirus-C. Within these species, there are more than 160 distinct rhinovirus genotypes (57). While rhinoviruses cause less cellular damage than RSV (31), in vitro studies have indicated that they have cytotoxic activity against epithelial cells, especially in respiratory epithelium, and may consequently contribute to the initiation of airway remodelling (58).

In contrast to RSV, rhinovirus infections do not occur in epidemics but are recorded year-round. In temperate climates, the incidence peaks in the early autumn and late spring (59). As many as 35% of asymptomatic children have been shown to return positive results for rhinovirus in mucus samples, although the virus does not cause chronic infection or colonise healthy subjects (60, 61).

Viruses other than RSV and rhinoviruses account for minor proportions of bronchiolitis cases. A systematic review and meta-analysis ranked respiratory viruses found in bronchiolitis in children aged 0–24 months in the pre-coronavirus disease 2019 (COVID-19) pandemic era (62). After RSV (59.2%) and rhinoviruses (19.3%), the most dominant viruses were bocavirus (6.1%) and adenovirus (6.1%), followed by metapneumovirus (5.4%), parainfluenza virus 1, 2 or 3 (5.4%), influenza viruses A or B (3.2%), coronavirus (2.9%) and enterovirus (2.9%) (62).

2.3 Long-term outcome of bronchiolitis

Classically, there are three main phenotypes of early childhood wheezers (63). Transient wheezers make up 60% of cases, and they have symptoms only during respiratory infections acquired before 3 years of age. Among this group, lung function can be subnormal before any infection or wheezing and improves partially over time. The remaining 40% are persistent wheezers, who continue to wheeze after 3 years of age. About 50% of them become sensitised to inhaled allergens before school age, and hence are called atopic wheezers. Persistent wheezers without allergic sensitisation start to wheeze in the first year of life, and wheeze also after infancy, but only during respiratory infection. The risk of asthma is not high in this group—it is close to the risk in the general population—however, lung function often remains subnormal through childhood, even in non-symptomatic children (63). It has not yet been thoroughly elucidated whether viral bronchiolitis causes later wheezing and asthma or is only an indicator of a child prone to asthma.

Numerous prospective and retrospective birth cohort studies and long-term follow-ups in various populations have confirmed an association between severe bronchiolitis and the risk of subsequent asthma at preschool age (12, 43, 49, 64-68), at school age (69-72) and in adulthood (42, 73, 74).

Comparing the findings of post-bronchiolitis studies is difficult for one principal reason: the upper age limit for bronchiolitis has varied from 6 months to 2 years. This age range means that heterogeneous groups of patients with diverse bronchiolitis symptoms have been included in studies. Children older than 12 months present with different wheezing phenotypes that consist of bronchiolitis with wheezing only once, repeated wheezing or even asthma, whereas in younger children, clinical bronchiolitis may present even without wheezing (32) as described previously in this thesis. In addition, the impact of the causative virus in bronchiolitis, such as RSV, a rhinovirus or non-RSV, can play a significant role in the post-bronchiolitis long-term outcome (75).

Two recently published systematic reviews and meta-analyses assessed the association between early-life bronchiolitis and the development of asthma, and both used the upper age limit of less than 2 years for bronchiolitis (4, 14). Overall, the risk ratio (RR) for post-bronchiolitis asthma was found to be 2.46. When the risk was assessed with age taken into account, the RR for asthma was 2.40 in children aged less than 10 years and 2.54 for children aged 10 years or older (4). The other meta-analysis assessed the magnitude of the virus aetiology in the association of bronchiolitis with asthma at 5-7 years of age (14). In this meta-analysis, four cohort

studies were included, where the upper age limit for diagnosis of bronchiolitis was 6 months (76) or 12 months (49, 55, 77). RSV bronchiolitis increased the risk of developing asthma later by 7.21-fold compared to healthy controls. Further analyses that compared RSV bronchiolitis with rhinovirus bronchiolitis revealed that the asthma risk was 2.72-fold higher after rhinovirus bronchiolitis than after RSV bronchiolitis (14).

The overall prevalence of doctor-diagnosed asthma at school age is about 5% (78-80). To date, the present prospective post-bronchiolitis cohort study is the only one assessing the risk of asthma after severe bronchiolitis in early infancy (at less 6 months of age). Nearly 70% of the cases in the present cohort had RSV bronchiolitis (81), 12.7% had current asthma at preschool age and 13.0% had asthma at school age (81, 82). The prevalence of asthma after RSV and non-RSV bronchiolitis was 8.2% and 24%, respectively, at preschool age (81), and 11.0% and 17.0%, respectively, at school age (82).

Atopic dermatitis is a genetically determined skin disease and is often observed as the first step of the atopic march (83). The development of atopy is interesting, since the persistent form of wheezing and asthma are closely associated with atopy (63). In early life, atopy typically manifests as atopic dermatitis (84). In turn, atopic dermatitis has been the most constantly observed risk factor for post-bronchiolitis asthma development (12, 75, 85, 86). Although most studies have highlighted an association between atopy, bronchiolitis and asthma (4, 75), different findings have also been presented (36, 87). For example, the effect of early bronchiolitis on later asthma was more pronounced within a subgroup of infants without atopic dermatitis (36). In a nested cohort study that included 125 infants hospitalised for bronchiolitis at less than 12 months of age, atopic dermatitis occurred more often in the healthy controls, with an odds ratio of 2.72 (12). A probable explanation for these findings is that atopic versus non-atopic asthma development is strongly associated with the virus aetiology of bronchiolitis: RSV bronchiolitis is more likely associated with later non-atopic asthma, whereas non-RSV bronchiolitis (especially rhinovirus bronchiolitis) is more likely associated with later atopic asthma (88).

The association between asthma and respiratory allergies is well known and termed ‘united airway disease’ (89). In bronchiolitis patients, the later risk of sensitisation to an aeroallergen at the age of 7 years was correlated with a family history of atopy (70). In the present bronchiolitis cohort, asthma and prolonged rhinitis at 5–7 years of age were significantly associated with a positive skin prick test result to inhaled allergens (90). Moreover, severe RSV bronchiolitis at less than 6 months of age was associated with an increased risk of allergic rhinitis at 13 years of

age (91). This brings up the question of whether RSV bronchiolitis induces the development of asthma and allergy or whether asthma and atopy may share common genetic mechanisms and have the same risk variants. Certain polymorphisms in a critical region of the DNA may lead to dysregulation of the expression of immune-related genes, further predisposing the individual to severe bronchiolitis, atopic dermatitis, subsequent wheezing and, finally, asthma.

Post-bronchiolitis studies conducted to assess the effect of gender have shown that there is an increased risk for asthma associated with the male gender before the teenage years (12, 77, 92). In adulthood, the balance shifts to the opposite condition, with the female gender associated more closely with a risk for asthma than the male gender (73, 93).

2.4 Bronchiolitis and lung function

Several studies have demonstrated that severe bronchiolitis in infancy is associated with lung function reductions at preschool age (44, 94, 95) and at school age (91, 92, 96-98). The development of the airways and lungs begins during the first gestational weeks and continues throughout the entire gestational period, and lung development also continues after birth. Postnatally, the focus is on alveolarisation, which may continue until early adulthood (99). Viral infections, especially during the first years of life, may reduce lung growth and hence lead to altered lung function.

The effects of prenatal and postnatal maternal smoking on lung function have been analysed in post-bronchiolitis studies (100, 101). There is evidence that exposure to maternal smoking in utero has a negative effect on infant lung function (100, 101). However, findings on the association between tobacco smoke exposure and lung function in later childhood are not concordant (102). Another study showed that obesity has a negative effect on lung function; participants with obesity that continued from childhood to adulthood were found to have worse lung function than participants who were normal-weight children and normal-weight adults (103). In the present post-bronchiolitis cohort, there was no correlation between prenatal or postnatal maternal smoking and later abnormal lung function in children aged 5–7 or 11–13 years. Instead, in the same cohort, a correlation between overweight and impulse oscillometry (IOS) parameters showing obstructive changes was found (104), and this trend continued in parameters measured by flow-volume spirometry (FVS) at 11–13 years of age (105).

Inflammatory lesions and impairment of lung function are specific features of respiratory viral infections, which primarily result in higher airway-specific resistance and bronchoconstriction. RSV and rhinoviruses both alter tissue damping, reflecting increased parenchymal elastic stiffness, which decreases reactance and further increases resistance (106). In RSV bronchiolitis, the accumulation of mucus and cell debris significantly contributes to intraluminal obstruction in the small airways. This results in hyperinflated lungs, where air gets trapped in distal alveoli, and this further leads to localised atelectasis after the air trapped in the alveoli is absorbed (Figure 1) (30). Hyperinflation is a typical short-term pulmonary abnormality that is seen a year later in 17% of bronchiolitis cases (107). Bronchiolitis that causes hospitalisation may have an effect on long-term lung growth in children (108).

There may be a congenital pulmonary predisposition for bronchiolitis. Studies showed that pulmonary function was already reduced at birth in newborns who developed acute bronchiolitis later in infancy compared to those who did not develop bronchiolitis (109, 110). In addition, a prospective birth cohort study of unselected infants showed that infants with decreased lung function at birth were predisposed to severe RSV infection and further post-RSV wheeze (49). Still, an unresolved question is whether developing bronchiolitis in early life alters normal lung development and predisposes the child to subsequent wheezing and reduced lung function or whether certain infants have a pre-existing abnormality in their airway function that predisposes them to both severe bronchiolitis and post-bronchiolitis wheezing, and to further reduction in lung function (30).

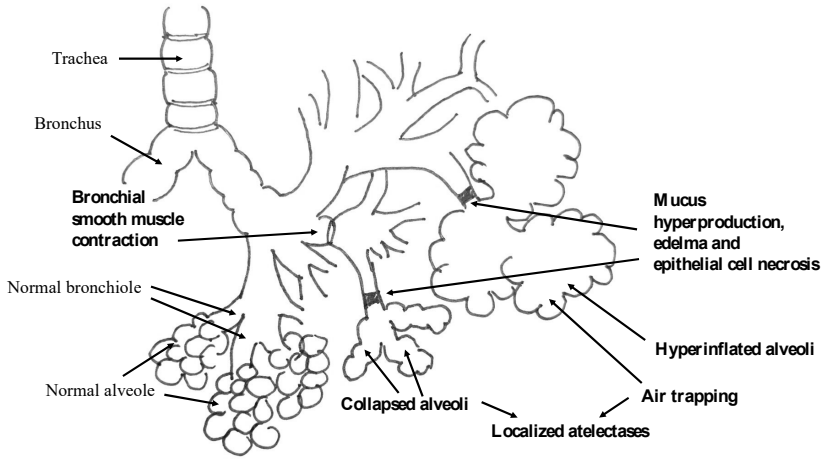


Figure 1. A schematic illustration of the pathophysiology of RSV bronchiolitis.

Modified from (30).

2.4.1 Post-bronchiolitis lung function at preschool age

IOS is an application of the forced oscillation technique in which measurements are taken during tidal breathing without the need for any extra maneuvers (111). IOS has been successfully used in individuals from the age of 2–3 years onwards (112). IOS is used to measure the respiratory system mechanical impedance (Zrs), which represents the resistive and reactive forces that must be overcome to drive an oscillating flow signal into the respiratory system. The parameters affecting these forces are the respiratory system resistance (Rrs) in the airways, and the elastance of the lung parenchyma and chest wall measured by the respiratory system reactance (Xrs) (113). Low frequencies (< 15 Hz) are considered to reflect the small, peripheral airways, and higher frequencies (> 20 Hz) the larger, more central airways (114). Rrs increases when there is proximal or peripheral obstruction, and low-frequency increases tend to occur when there is peripheral obstruction, such as in asthma (115). Reactance decreases as a result of peripheral restriction caused by fibrosis or hyperinflation (115). Airway resistance is an important parameter of lung function, reflecting the level of airway construction and the ability to breathe (106). In clinical use, the most important parameters are Rrs at 5 Hz (Rrs5) and reactance at 5 Hz (Xrs5) (116). Rrs5 increases and Xrs5 decreases in cases of obstruction of distal airways (115). Population-based reference values for Rrs5 and Xrs5 are available for Finnish children aged 2–7 years old (117).

Bronchial hyperreactivity is common in young children following severe RSV bronchiolitis; however, this reactivity tends to decrease during the school years (63, 98). In addition, reduced lung function after bronchiolitis can occur even without the development of asthma (118). Severe RSV LRTI at less than 2 years of age has been associated with restrictive features of lung function through childhood (97), whereas severe rhinovirus LRTI at less than 2 years of age has been associated with bronchial reactivity (94). Also, the need for hospitalisation is a common indirect marker of severe bronchiolitis, and a case-control study that included 159 infants hospitalised for RSV bronchiolitis provided further supporting evidence for this by showing that they had impaired lung function at the age 6 years compared to non-hospitalised patients (119).

2.4.2 Post-bronchiolitis lung function at school age

FVS is a standard method for examining lung function in children aged over 6–7 years. It is used to measure air volume during forced inspiration and expiration in relation to time (120). The main clinical variables in FVS are forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and the ratio between FEV1 and FVC (FEV1/FVC) (120). FVC is reduced in restrictive lung diseases. In obstructive diseases, where airways are typically narrowed, FEV1 and/or FEV1/FVC are reduced. When the obstruction is peripheral, FEV1 can be normal; however, when the obstruction is more centrally located, FEV1 decreases in relation to FVC (121).

An LRTI caused by RSV is a risk factor for a persistently low lung function trajectory that extends from infancy into young adulthood (63, 122). Severe bronchiolitis in infancy—especially that caused by RSV—is associated with lower pre- and post-bronchodilator lung function that lasts into adolescence and adulthood, and many children have presented with persistent bronchial hyperresponsiveness (44, 49, 77, 98, 123).

Although asthma-presumptive symptoms decrease with age, the degree of lung function has been shown to track over life (124). When longitudinal trajectories were studied, a significant change was seen in the pre- and post-bronchodilator lung function from the age of 5–6 years to a mean of 12 years of age among infants with a history of severe infant bronchiolitis (98). Further analyses showed a transient increase in FEV1 and FVC from under 7 to 7–9 years of age, which was followed by a subsequent decline at the age of 10–16 years (98). Persistent lung function decline in children could be of crucial importance since reduced lung function in young adults is one of the strongest predictors of chronic obstructive pulmonary disease (COPD) in older years (125). A few studies have reported a sustained increased risk of asthma and lower lung function after severe bronchiolitis in infancy, even at 30 years of age (108). This may suggest permanent structural alterations in the airways and elicit the hypothesis that bronchiolitis predisposes the development of COPD (108). In line with this hypothesis, wheezing with RSV at less than 2 years of age was found to be associated with reduced FVC, suggesting a restrictive pattern of lung function (97).

A recent systematic review assessed the association between RSV LRTI at less than 3 years of age and pulmonary function measured at a mean age of 10 years (95). In 13 studies, there was no association found between RSV LRTI during the first three years of life and abnormal pulmonary function; however, 16 studies reported

such an association. Abnormal measurements varied across the studies, showing restrictive, obstructive and mixed restrictive–obstructive outcomes, and the most common abnormality was obstruction of airways with or without bronchodilator reversibility (95).

2.5 Immunology in early childhood viral infections

Infancy is a time of rapid immunological development (4). Yet, little is known about how viral infections influence the maturation of the postnatal immune system. This is an important point because the development of innate and adaptive immune responses affects the clinical expression of respiratory viral infections or, vice versa, may predispose the infant to severe bronchiolitis. The responses of the host, rather than the virus itself, may be responsible for the manifestation and outcome of viral infections, such as bronchiolitis. Thus, these early responses to the virus may influence the later outcomes of the infection and, finally, the post-bronchiolitis respiratory health. Failure to remove an inflammatory stimulus can lead to chronic inflammation and result in further tissue damage.

Several biological and immunological mechanisms underlie the association between bronchiolitis and later asthma development (28, 52, 126). In particular, the first two years of life are considered a window for later health (127). The immune system of newborns and in early life is not immature; rather, it is distinct from that of older children, mainly because it lacks immunological memory (128). In addition, shortly after birth, newborns are exposed to and colonised by microbes. The formation of the respiratory microbial flora in early infancy is affected by host and environmental factors, such as the use of antibiotics and probiotics, dietary and hygiene aspects and house pets. These factors may be associated with early onset respiratory diseases, such as respiratory infections and asthma (129-131).

2.5.1 Innate immunity

Innate immunity is a genetically determined, first-line, non-programmed defence mechanism that plays a key role in orchestrating early responses to viral infections (16). The ciliated epithelial cells in the airways are the main target for viruses and form the primary site for viral replication (16, 75). Hence, the respiratory epithelium is the first protective barrier of the innate immune system: it prevents viral invasion

by different mechanisms. The epithelial cells also participate in the recruitment of inflammatory cells (132). In addition, the nasal and pharyngeal microbiota may modulate the mucosal and systemic host responses to a viral infection (133).

The innate immune defence mounted during inflammation involves several cell types and interactions, and it has a specific role in microbe detection and the initiation of the further response to an infection (134). The key cells involved are leukocytes, such as granulocytes (neutrophils and eosinophils) and agranulocytes (monocytes and macrophages), mast cells and dendritic cells (135). Natural killer (NK) cells are the first-line lymphocytes that act against viral infections affecting cytokine production (136).

Neutrophils, which account for 70% of circulating human blood leukocytes (134), rapidly migrate to the foci of infection and are often the first cells recruited to defend the host. During acute respiratory infections, both blood and nasal neutrophil populations increase rapidly within the first 72 hours (137). They limit viral replication, but at the same time, they release enzymes that may damage the surrounding tissues (135). Neutrophils are the most abundant cell type in the airways of infants with bronchiolitis caused by RSV or rhinoviruses (137, 138), and RSV bronchiolitis has specifically been associated with a strong local neutrophil response (49). In addition, mucosal neutrophil activation, even before exposure, has been found to be highly predictive of symptomatic disease (139). Neutrophils also clear extracellular pathogens, such as bacteria (140).

Eosinophils make up less than 5% of the circulating human blood leukocyte population. They can survive for up to 12 hours, but have the ability to prolong their life for days, if necessary (135). Eosinophilia in infants with RSV infection has been associated with airway obstruction (141). However, *in vitro* studies have demonstrated that eosinophils enhanced RSV clearance and thus reduced infectivity (142). Elevated blood eosinophil counts have been associated with bronchiolitis caused by rhinoviruses in previous studies (6, 40).

During viral infections, macrophages in the alveoli may have both immunoregulatory and antigen-presenting capabilities (16). Upon microbe invasion, monocytes migrate to the inflamed tissue and can differentiate into large, tissue-resident, phagocytic macrophages. Monocytes can further differentiate into different phenotypes, depending on the microenvironment (135), and subsequently participate in tissue repair or cytokine production (143). During an acute RSV infection, the number of monocytes increases in the peripheral blood, regardless of the disease severity (144).

Circulating NK cells and local dendritic cells are also involved in lung inflammation, and both have a significant role in controlling RSV replication (145). The number of dendritic cells and NK cells increases in the respiratory tract during an RSV infection, and a decrease in the number of cells or their function has been associated with worse clinical outcomes (146). In addition, lung dendritic cells recruit eosinophils to sites of airway inflammation and thus may play a role in the pathogenesis of asthma (147). Dendritic cells act as the messengers between the innate and adaptive immune systems (135).

2.5.2 Adaptive immunity

An adaptive immune response follows an innate immune response and is characterised by immunological memory. This is the ability to immediately and robustly respond to previously encountered pathogens in an antigen-specific manner (148). Adaptive immune responses that are directed towards viruses have two arms: one mediated by T lymphocytes and one mediated by B lymphocytes. Hence, the adaptive response is further separated into the cellular T cell-driven response and the humoral B cell-driven response (149).

Humoral responses target viral surface proteins, facilitating the neutralisation of extracellular viruses (150). Memory B cells and the antibodies produced by B cells are particularly important for protection against viral infections (151). Infants have decreased antibody responses compared to adults due to their relatively under-developed immune system having a limited B-cell repertoire (16). In addition, passively acquired maternal antibodies in the blood of young infants may have an immunosuppressive effect on the development of the infant's own immune response (152). It is suggested that during acute RSV infection, maternal antibodies may interfere with the humoral system of the infant, and this may be the reason for increased RSV disease severity in infants aged 1–3 months (16).

The cluster differentiation (CD) designation refers to the proteins found on the surface of T cells. CD4+ T lymphocytes are T-helper (Th) cells, and CD8+ T lymphocytes are cytotoxic T-killer cells. Memory T cells can be either CD4+ or CD8+. T lymphocytes recognise protein antigens (153). NK cells recognise ancestral glycolipid antigens and therefore function as a part of the innate immune system (154). CD4+ and CD8+ T cells play critical roles in achieving protective immunity after infection (153).

T cells participate in controlling RSV and rhinovirus infections by recognising viral antigens and facilitating both cytotoxic and antibody-mediated immune responses (16, 150). CD8+ T cells effectively participate in viral clearance. CD4+ T cells orchestrate the immune response against respiratory viruses and further differentiate into specific Th-cell subsets, including Th1, Th2 and Th17 cells (16, 126).

2.5.2.1 Type 1 and 2 T-helper cells

Both Th1 cells and Th2 cells are produced from a non-committed population of precursor cells called naïve T cells (155). Classically, Th1-cell responses are critical during acute infection, especially against bacterial and viral infections (155). Th1 cells enhance cell-mediated immunity and the production of antibodies. In contrast, Th2-cell responses are involved in anti-parasite immunity and allergic responses (126, 156). Th1 and Th2 cells are known to downregulate each other's activation (157). Th2 cells release interleukins to augment allergy-type responses and enhance B-cell activation (156). Eosinophils are also associated with Th2-cell responses, producing Th2 cell-inducing cytokines (158).

2.5.2.2 Type 17 T-helper cells and interleukin-17

Almost two decades ago, a distinct lineage of CD4+ T cells, named Th17 cells, was identified that fills the gap between Th1 and Th2 cells (126). Th17 cells have an important role in managing inflammation, contribute to immunity against bacteria and fungi and are typically linked to the defence of mucosal surfaces (159). Large quantities of Th17 cells have also been associated with autoimmune diseases (160, 161).

Th17 cells have been shown to possess more functional plasticity and instability than, for example, Th2 cells (162), leading to alternative T-cell states and differing functions (159). Evidence of this plasticity has been found in mice, with Th17 cells exhibiting the ability to differentiate into Th1-like cells in certain conditions (163). It has also been debated whether there is a distinct Th17-cell population that can become pathogenic by inducing autoimmunity and, thus, may contribute to the development of autoimmune tissue inflammation (159).

The main role of Th17 cells is to recruit and activate granulocytes at the site of infection, which is necessary for the clearance of microorganisms (164, 165). Th17

cells, alongside other inflammatory cells, release interleukin (IL)-17 cytokines, which are key inflammatory mediators in numerous diseases, such as autoimmune diseases, allergies and infections (18, 166). Bacteria, fungi, viruses and parasites share the capacity to induce the production of considerable levels of IL-17 during the early innate immune response (165).

The IL-17 cytokine family includes six members, namely IL-17A to IL-17F (165), of which IL-17A and IL-17F show 50% homology and bind to the same receptor: IL-17 receptor A (IL-17RA) (161, 167). IL-17A was the first and is the best characterised among the IL-17 cytokine family members. However, recently, both IL-17A and IL-17F have been widely studied, since they are both proinflammatory cytokines and have been associated with human diseases (167), such as asthma (168-172) and allergies (173).

2.6 T-helper cells and interleukin-17 in bronchiolitis and asthma

The pivotal role of Th cells in amplifying immune responsiveness is well established. Th-cell populations are classified based on the cytokines they secrete (155). There is evidence that both genetic and environmental factors influence the differentiation of Th-cell subsets, and it is also likely that this differentiation starts early in childhood (174). The role of different Th cells may be crucial in the pathogenesis of respiratory viral infections and asthma at different ages, highlighting also the importance of timing.

2.6.1 Type 1 and 2 T-helper cells in bronchiolitis and asthma

Animal studies have shown that a microbe-free environment locks the immune system in a Th2-type state (126), whereas microbiological events seem to be crucial in determining a potential switch from Th2-type to Th1-type immunity (175). It has been hypothesised that Th-cell priming occurs during the perinatal period, and these early immune responses can be categorised as Th1- or Th2-polarised memory during early childhood (176). Viral infections can contribute to Th2-type inflammation, which increases the risk of later asthma and allergy (177-179).

The role that adaptive cellular immunity plays in reducing the RSV burden is clear in children with defective T-cell responses: RSV infection is often more severe and correlates with a decreased frequency of T cells in the lungs (150). Activation of the

appropriate Th cells can have a major impact on the outcome of the infection (156). A viral respiratory infection in infancy may also alter the Th1/Th2 balance of an immune response and cause a dysregulated antiviral Th1-cell response and exaggerated inflammation, especially during RSV infection in infancy (180). It has also been hypothesised that the impaired primary Th1-type immune response may occur in early life as a consequence of shifting towards Th2-oriented immunity, which offers an opportunity for RSV or rhinovirus-induced LRTI (126, 181). Furthermore, impaired Th1-type immune responses favour viral replication during early acute infections, which can lead to a subsequent exaggerated Th1-type response (126). In a recent study, it was found that the majority of a group of rhinovirus-infected infants displayed a strong Th2-type response, while the majority of a group of RSV-infected infants exhibited a Th1-type response (182). In another study conducted with nasal samples from over 800 RSV-infected children under 5 years of age, it was found that the samples contained increased levels of Th1-associated cytokines and no detectable levels of Th2-associated cytokines (183).

The theory of an imbalance between Th1- and Th2-type responses, and specifically the presence of a Th2-skewed immune response, has been used to explain the pathogenesis of airway inflammation in asthma (184). Increased levels of Th2-type cytokines lead to higher eosinophil recruitment and bronchial hyperresponsiveness (185). In addition, most adults with asthma exhibit elevated eosinophil levels in sputum and peripheral blood samples and increased levels of Th2-type cytokines (18). Studies have indicated that severe asthmatic patients with Th1-dominated inflammation are infected with microbial organisms or viruses, indicating that respiratory pathogens may be potent triggers driving Th1-type immune response in these patients (186, 187). In addition, elevated levels of virus-specific Th1 cells have been found to correlate with worse lung function in adult asthmatics (126).

The Th1/Th2 theory cannot fully explain the wide spectrum of asthma endotypes. Therefore, other cells may play pivotal roles in the inflammatory network.

2.6.2 Type 17 T-helper cells and interleukin-17 in bronchiolitis and asthma

The role of Th17 cell-mediated immunity or IL-17 cytokines in infant bronchiolitis and childhood asthma has been poorly studied in humans. However, multiple studies have been performed in mice to assess the role of Th17 cells in viral respiratory infections, especially in RSV infection (126, 162). Respiratory tract colonisation by bacteria or viruses occurs in some patients with severe asthma, which facilitates the differentiation of Th17 cells, and this is especially the case in the context of allergic airway inflammation (126). Th17 cells have been found in the lungs of RSV-infected mice (161, 188).

In a study of IL-17A levels during the acute and recovery phases of disease conducted with a cohort of infants hospitalised for RSV bronchiolitis, it was found that local IL-17A production was increased during the recovery phase (189). Interestingly, IL-17A has also been associated with a reduction in the clinical symptoms of respiratory stress in RSV-infected infants (190). These results suggest a protective role for IL-17A against severe RSV bronchiolitis. However, when T-cell responses in airway and peripheral blood samples from healthy and RSV-infected infants were studied, acute RSV infection was associated with elevated IL-17A in the lungs (191). While Th17 cells were not detected in the peripheral blood samples from the healthy one-month-old infants, Th17 cell levels were significantly increased in the peripheral blood of the RSV-infected infants (191). These findings suggest that the Th17 cell response requires early on immune-related events to be activated.

Studies on the role of IL-17A in asthma or allergies have provided conflicting results so far. However, it seems that the levels of both IL-17A and IL-17F are increased in asthma, highlighting the proinflammatory feature of these cytokines. Children and adults with allergic asthma have been found to have increased levels of Th17 cells compared to healthy controls (192, 193). In addition, IL-17A production in adult asthmatics has been reported to positively correlate with airway hyperresponsiveness and the clinical severity of asthma (194, 195). In another study, teenage asthmatics with high IL-17A levels had significantly lower lung function parameters than control subjects (196). However, when the levels of IL-17A in adult patients with asthma or comorbid allergic rhinitis were examined, no significant levels of IL-17A were found (197). Other studies have shown that IL-17F is increased in asthma patients and correlates with both airway neutrophil levels and more severe disease (18, 26, 171, 198).

Evidence suggests that elevated Th17-cell immune responses tend to be more prominent in adult patients with severe and corticosteroid-insensitive asthma (198).

IL-17-producing Th2 cells secrete both Th2 and Th17 cytokines that have profound synergistic effects during inflammation. In addition, adult asthmatics with elevated levels of both eosinophils and neutrophils in their airways were found to correlate with lowest lung function, worse asthma control and more exacerbations than controls (199, 200).

2.7 *Interleukin 17* gene polymorphisms

Both innate and adaptive immune responses are considered important for the course of RSV and other viral infections in early childhood. (Figure 2) (126, 134, 166). Therefore, the genes that regulate innate and adaptive immunity, such as *IL17A* and *IL17F* among many others, can be assumed to be candidate genes for severe bronchiolitis. In addition, hospitalisation for bronchiolitis may increase the risk of asthma, as described previously. This suggests, that hospitalisation for bronchiolitis can be of genetic origin, and is further merely an indicator of a genetic predisposition to asthma rather than the cause of asthma.

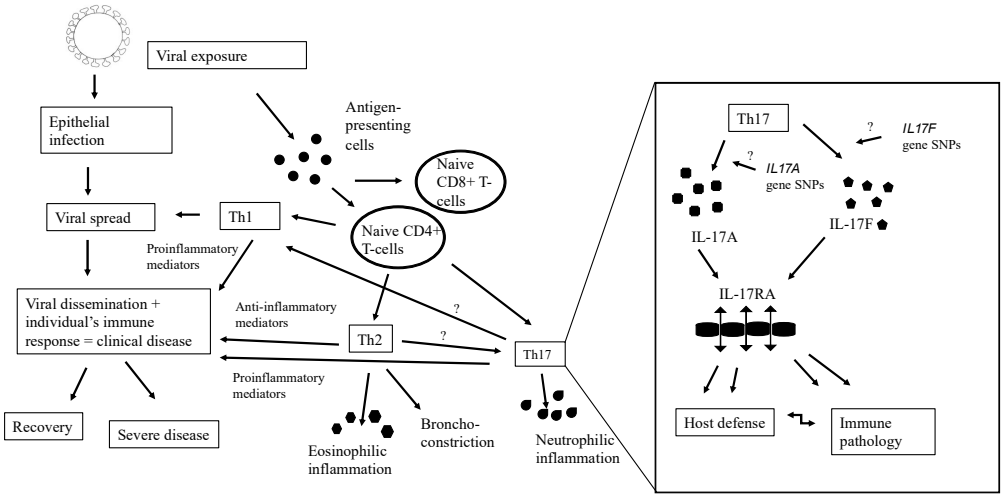


Figure 2. A schematic and simplified illustration of the Th1/Th2/Th17 –cell mediated immunity. Modified from (126, 134, 166).

Genetic information is saved in base sequences of DNA or RNA molecules (201). A specific DNA sequence is polymorphic if it varies between individuals, and the different sequence variants are alleles. A single nucleotide polymorphism (SNP) is a substitution of one single nucleotide (202). The average human genome contains 4-5 million SNPs that occur on average every 1000-2000 nucleotides (203).

In gene expression, DNA is first transcribed to RNA, and then translated to proteins. Three subsequent base pairs form a codon, which in the protein synthesis selects the amino acid that is connected to the peptide chain. SNP is a mutation in one base pair. SNPs may change the encoded amino acids, or can be silent, or simply occur in the noncoding regions (204). SNPs may alter the structure and function of the protein, or may change the structure without influencing the function, or may not change even the structure (204).

SNPs are considered functional if they induce amino acid changes, since measurements of changes in function of proteins are challenging and often impossible. In the context of this thesis, SNPs that may lead to variations in the inflammatory or immune response are of particular interest (22). The functionalities of SNPs in the *IL17A* and *IL17F* genes are only partially known. In this thesis, the focus is on three SNPs in the *IL17A* gene (rs2275913G/A, rs4711998G/A and rs8193036T/C) and three SNPs in the *IL17F* gene (rs763780T/C, rs11465553C/T and rs7741835C/T).

The *IL17A* rs2275913 SNP is located in the promoter region of the *IL17A* gene, and it may modify the transcription factor binding site and further influence the transcription rate of the gene (25). The influence of the *IL17A* rs2275913 SNP on IL-17A production in serum samples of healthy infants has been documented: the *IL17A* rs2275913 variant AA and AG genotypes were found to be associated with lower levels of IL-17A (205). The *IL17A* rs8193036 SNP was found to promote DNA methylation and mRNA and protein expression in white blood cells obtained from adults with inflammatory bowel disease (206). No data are available on the functionality of the *IL17A* rs4711998 SNP.

The *IL17A* rs2275913 SNP has been associated with the severity of bronchiolitis (207) and asthma (208) in children. In addition, many studies have been conducted on *IL17A* SNPs in adults. The *IL17A* rs8193036 SNP was found to be associated with asthma in adults (209), and the *IL17A* rs4711998 SNP was reported to be associated with asthma in Taiwanese children (210).

The *IL17F* rs763780 SNP lies in the coding region of the *IL17F* gene. It causes an A to G substitution in the DNA sequence and leads to a change in the amino acid

sequence of IL-17F (211). The functional impact of the *IL17F* rs763780 SNP on IL-17F production has been documented in previous studies (212, 213). The rs7741835 SNP was found to correlate with IL-17F levels and affect gene expression after stimulation with different allergens (214).

The *IL17F* rs763780 SNP has been associated with asthma in adults (212, 215-217). However, this association has not been detected in other studies (211, 218), including two recent meta-analyses (25, 26). In those studies, which also included children, all evidence of the association between the *IL17F* rs763780 SNP and asthma was clearly absent (17, 219). The *IL17F* rs7741835 SNP has also been associated with asthma in adults (214). The rs11465553 SNP has been included in genomic studies, but no association has been found with asthma to date (26, 211).

In the literature review conducted for this thesis, no earlier studies were found that examined the effect of the *IL17A* rs2275913, rs4711998 and rs8193036 SNPs or the *IL17F* rs763780, rs7741835 and rs11465553 SNPs on post-bronchiolitis asthma or lung function. The earlier studies that reported on associations between the studied *IL17A* or *IL17F* SNPs and asthma in children and adults are listed in Tables 1 and 2, respectively.

Table 1. Previous studies conducted in children that reported on associations between asthma and the IL17A single nucleotide polymorphisms (SNPs) rs2275913, rs4711998 and rs8193036 and the IL17F SNPs rs763780, rs11465553 and rs7741835. Major and minor alleles are defined as they are presented in the published study.

Study	***IL17* SNP major / minor allele**	**Study population**	**Ethnicity and age group**	**Asthma outcome**
Wang 2009 (220)	*IL17A* rs8193036 C / T *IL17A* rs4711998 A / G *IL17A* rs2275913 G / A	1027 cases (aged 5-12 years) vs. 931 controls	Asian children	*IL17A* rs8193036 wild allele CC increased the risk of asthma
Chen 2010 (208)	*IL17A* rs2275913 G / A	168 cases (mean age 5.23 years) 144 bronchiolitis cases (mean age 4.86 months) vs. 205 controls	Asian children	*IL17A* rs2275913 variant genotype AA increased the risk of more severe bronchiolitis, and risk of asthma and abnormal lung function
Maalmi 2014 (17)	*IL17A* rs2275913 G / A *IL17F* rs763780 T / C	171 cases (mean age 9.5 years) vs. 171 controls	African children	*IL17A* rs2275913 wild allele G increased the risk of asthma
Schieck 2014 (221)	*IL17A* rs2275913 G / A *IL17A* rs4711998 G / A *IL17A* rs8193036 T / C *IL17F* rs763780 T / C *IL17F* rs7741835 C / T	651 cases vs. 652 controls (age unreported)	Caucasian children	*IL17F* rs7741835 major allele C decreased the risk of asthma *IL17A* rs2275813 major allele G increased the risk of non-atopic asthma
Narbutt 2015 (222)	*IL17A* rs2275913 G / A	166 cases with atopic dermatitis (mean age 11.6 years) vs. 166 controls	Caucasian children and adults	*IL17A* rs2275913 variant genotype AA increased the risk of atopic asthma
El-Shal 2022 (223)	*IL17A* rs8193036 C / T	216 cases (mean age 8.33 years) vs. 216 controls	African children	*IL17A* rs8193036 minor allele T decreased the risk of asthma

Table 2. Previous studies conducted in adults that reported on associations between asthma and the *IL17A* single nucleotide polymorphisms (SNPs) rs2275913, rs4711998 and rs8193036 and the *IL17F* SNPs rs763780, rs11465553 and rs7741835. Major and minor alleles are defined as they are presented in the published study.

Study	***IL17* SNP major / minor allele**	**Study population**	**Ethnicity and age group**	**Asthma outcome**
Resende 2006 (224)	*IL17A* rs2275913 G / A	149 cases (mean age 39.32 years) 83 rhinitis patients (mean age 48.5 years) vs. 192 controls	African adults	*IL17A* rs2275913 variant genotype AA increased the risk of asthma vs. the risk of rhinitis
Hizawa 2006 (225)	*IL17F* rs763780 T / C	432 cases (median age 36 years) vs. 435 controls	Asian adults	*IL17F* rs763780 variant genotype CC decreased the risk of asthma
Bazzi 2011 (211)	*IL17F* rs763780 T / C *IL17F* rs11465553 C / T	100 cases (age unreported) vs. 102 controls	Arab adults	No associations
Qian 2012 (215)	*IL17F* rs763780 T / C	318 cases (mean age 39 years) vs. 352 controls	Asian adults	*IL17F* rs763780 minor allele C increased the risk of asthma exclusively in males
Ota 2014 (216)	*IL17F* rs763780 T / C	867 cases (age unreported)	Asian adults	*IL17F* rs763780 variant genotype CC decreased the risk of asthma
Du 2016 (218)	*IL17A* rs2275913 G / A *IL17A* rs8193036 C / T *IL17F* rs763780 T / C	125 cases (mean age 39 years) vs. 132 controls	Asian adults	*IL17A* rs2275913 variant genotype GA increased the risk of asthma *IL17A* rs8193036 variant genotype TT increased the risk of asthma
Liang 2018 (217)	*IL17F* rs763780 T / C	221 cases (mean age 36 years) vs. 223 controls	Asian adults	*IL17F* rs763780 variant genotype CC / CT increased the risk of asthma
Ganta 2023 (226)	*IL17A* rs2275913 G / A *IL17A* rs8193036 T / C	150 cases (mean age 40 years) vs. 150 controls	Asian adults	*IL17A* rs2275913 variant genotype AA / AG decreased the risk of asthma

The earlier meta-analyses that reported on associations between the studied *IL17A* or *IL17F* SNPs and asthma in children and adults are listed in Table 3.

Table 3. Previous meta-analyses in children and adults that reported on associations between asthma and the *IL17A* single nucleotide polymorphisms (SNPs) rs2275913, rs4711998 and rs8193036 and the *IL17F* SNPs rs763780 and rs11465553. Major and minor alleles are defined as they are presented in the published study.

Study	***IL17* SNP major / minor allele**	**Study population**	**Asthma outcome**
Jin 2015 (23)	*IL17A* rs4711998 G / A	9 studies 3650 cases vs. 3370 controls	*IL17A* rs4711998 variant genotype AA / AG decreased the risk of asthma
Wang 2015 (27)	*IL17F* rs763780 T / C	5 studies (1 of children) 1445 cases vs 1608 controls	*IL17F* rs763780 variant genotype CC decreased the risk of asthma
Ke 2015 (219)	*IL17F* rs763780 T / C	7 studies (2 of children) 2016 cases vs. 2184 controls	No associations
Zhu 2016 (209)	*IL17A* rs2275913 G / A *IL17A* rs8193036 T / C	7 studies (5 of children) 2882 cases vs. 2093 controls	*IL17A* rs8193036 variant genotype TT / TC decreased the risk of asthma The correlation still appeared in children when stratified by age.
Zhai 2018 (24)	*IL17A* rs2275913 G / A	10 studies (7 of children) 2510 cases vs. 2506 controls	*IL17A* rs2275913 major allele G decreased the risk of asthma
Liu 2022 (25)	*IL17A* rs2275913 G / A *IL17A* rs4711998 A / G *IL17A* rs8193036 C / T *IL17F* rs763780 T / C	6 studies (1 of children) 1087 cases vs. 1224 controls	No associations
Lee 2023 (26)	*IL17A* rs2275913 G / A *IL17A* rs4711998 A / G *IL17A* rs8193036 C / T *IL17F* rs763780 T / C *IL17F* rs11465553 C / A	20 studies (6 of children)	*IL17A* rs8193036 wild allele C increased the risk of asthma

3 AIMS OF THE STUDY

The principal aims of this thesis were to study the associations between single nucleotide polymorphisms (SNPs) in the genes encoding the cytokines interleukin (IL)-17A (*IL17A* gene) and IL-17F (*IL17F* gene) and 1) virus aetiology and clinical severity of bronchiolitis in early infancy (at less than 6 months of age), and 2) the prevalence of post-bronchiolitis asthma and lung function in school-aged children. The specific aims were:

1. To compare the frequencies of three *IL17A* SNPs (rs2275913, rs4711998 and rs8193036) and three *IL17F* SNPs (rs763780, rs11465553 and rs7741835) in children with infant bronchiolitis to those in reference populations from public registers (Articles I-III).

2. To study three *IL17A* SNPs (rs2275913, rs4711998 and rs8193036) and three *IL17F* SNPs (rs763780, rs11465553 and rs7741835) in relation to virus aetiology and the severity of bronchiolitis in infancy (at less than 6 months of age) (Articles I-III).

3. To evaluate the associations between the *IL17A* rs2275913, rs4711998 and rs8193036 SNPs and post-bronchiolitis asthma and asthma medications in children aged 5–7 and 11–13 years (Articles I-II).

4. To evaluate the associations between the *IL17F* rs763780, rs11465553 and rs7741835 SNPs and post-bronchiolitis asthma and asthma medications in children aged 5–7 and 11–13 years (Article III)

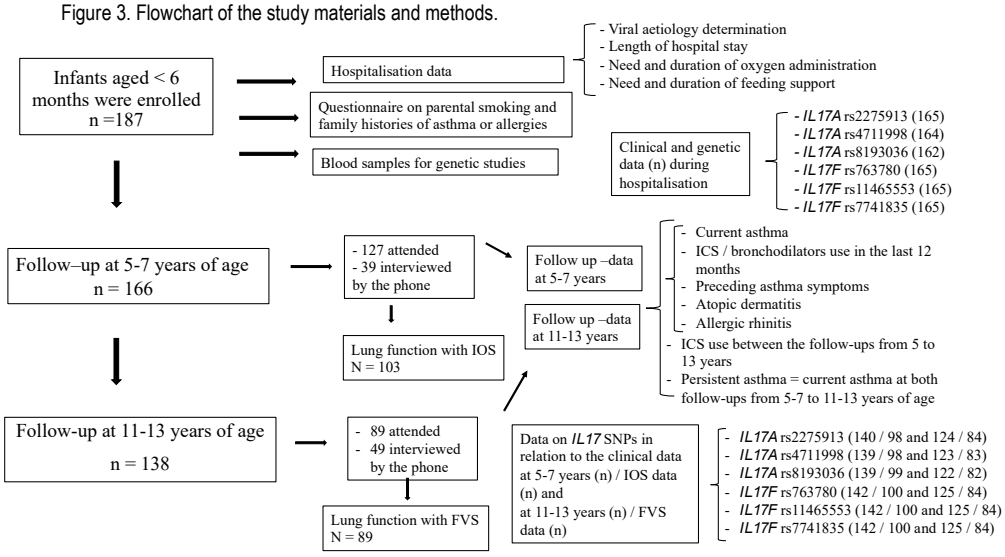
5. To study lung function in children aged 5–7 years using impulse oscillometry (IOS) and in children aged 11–13 years using flow-volume spirometry (FVS) in relation to the *IL17A* rs2275913, rs4711998 and rs8193036 SNPs and the *IL17F* rs763780, rs11465553 and rs7741835 SNPs (Articles IV-V).

4 MATERIALS AND METHODS

4.1 Patient enrolment and hospitalisation data

In the Department of Paediatrics at Tampere University Hospital (Finland), 187 previously healthy, full-term infants aged less than 6 months who were hospitalised for bronchiolitis were recruited for the study. The study periods were from December 1st, 2001 to May 31st, 2002, and from October 28th, 2002 to May 31st, 2004, covering three winters and two RSV outbreaks. Bronchiolitis was diagnosed on the basis of the patient's medical history and physical examination and was defined as the first episode of an acute LRTI associated with rhinorrhoea, cough, tachypnoea, feeding problems and diffuse wheezes and/or crackles on auscultation.

Figure 3. Flowchart of the study materials and methods.



The viral aetiology of bronchiolitis was determined from nasopharyngeal aspirates analysed by antigen detection using indirect immunofluorescence and by genome detection using reverse-transcriptase polymerase chain reaction (PCR) for

RSV, influenza viruses A and B, adenovirus and parainfluenza viruses 1, 2 and 3. Reverse-transcriptase PCR was also used to detect rhinovirus, metapneumovirus and bocavirus, since there are no antigen detection tests in use for these viruses. Altogether, the methodology allowed for the detection of ten different respiratory viruses as causative agents.

The disease severity markers, such as the need for and duration of oxygen administration, the need for and duration of feeding support or the length of hospital stay (LOS), were registered during the patient's hospitalisation. Supplementary oxygen was given when oxygen saturation, measured with pulse-oximetry, was less than 94%. Feeding support was defined as a need for intravenous fluids or feeding using a nasogastric tube. None of the study subjects required intensive care.

Each patient's parents were interviewed using a structured questionnaire, and data on parental smoking during pregnancy or infancy and family histories of asthma or allergies were registered during the patient's hospitalisation.

4.2 Follow-up at 5-7 years of age

The first follow-up was arranged to occur when the children were 5–7 years old and outside the main pollen seasons. The follow-up occurred in October 2008, January to March 2009 and October 2009. Prior to the first follow-up, the parents completed a structured questionnaire comprised of questions about previous or current use of asthma medication and symptoms presumptive for asthma, such as wheezing episodes, prolonged cough (for 4 weeks or longer) or coughing during the night without having an infection. In addition, data on atopic eczema, allergic rhinitis, doctor-diagnosed asthma and the age of asthma onset were registered. A paediatrician reviewed the completed questionnaire together with the child and parents and performed a clinical examination of the child.

A total of 166 children attended the follow-up: 127 participated in the clinical visit, and 39 parents/guardians completed the questionnaire and were interviewed by telephone. In cases where there was a respiratory infection in the preceding two weeks, the follow-up was rescheduled.

Lung function was tested using IOS (Master Screen IOS; Jaeger, Höchberg, Germany), followed by the exercise challenge test (ECT) and post-bronchodilator (post-BD) testing, including measurements of respiratory system resistance at 5 Hz (Rrs5) and reactance at 5 Hz (Xrs5), as published previously (227). To summarise, three acceptable measurements were required: one taken at baseline, one taken after

an ECT outdoors and one taken after an inhalation of 300 μg salbutamol (Ventolin, GSK, London, UK) through a spacer (Babyhaler, GSK, London, UK).

Bronchial hyperreactivity (BHR) was considered if the child had a 35% rise in response to exercise or a 35% decrease in response to bronchodilation in Rrs5, calculated as a change from the baseline Rrs5 (228). IOS data on lung function were available for 103 children.

4.3 Follow-up at 11-13 years of age

The second follow-up was arranged to occur between June 1st, 2014 and January 31st, 2015, when the children were 11–13 years old. Prior to the follow-up, the families were asked to complete a structured questionnaire that included questions about atopic eczema, allergic rhinitis, doctor-diagnosed asthma, previous or current use of asthma medication and symptoms presumptive for asthma, such as wheezing episodes, prolonged cough (for 4 weeks or longer) or coughing during the night without having an infection. The questionnaire completed prior to the second follow-up was designed to collect information on the period between the first follow-up at 5–7 years of age and the second follow-up at 11–13 years of age.

Among the 166 children invited to the follow-up, 138 (83.1%) attended: 89 participated in the clinical follow-up visit, and 49 parents/guardians completed the questionnaire and were interviewed by telephone. At the follow-up visit, the parents and children were interviewed to check the questionnaire data, the children were examined by a paediatrician and lung function was measured using FVS, including FEV1 and FVC, as published previously (105). Measurements were performed before and 15 min after the inhalation of 400 μg salbutamol (Ventolin, GSK, London, UK), and three acceptable measurements were required at both times. The best pre- and post-BD values for FEV1 were analysed and presented as age- and gender-specific and height-adjusted values (predicted FEV1). If the predicted FEV1 increased by 12% and 0.2 L or more after the administration of salbutamol, the bronchodilation test result was interpreted as positive to show a reversible bronchial obstruction. FVS data on lung function were available for 89 children.

4.4 Definitions

At the age of 5–7 years, the outcome variables were current asthma, current atopic eczema and current allergic rhinitis. Current asthma was defined as continuous or intermittent ICS use for asthma during the preceding 12 months or, alternatively, as reporting of doctor-diagnosed episodes of wheezing, prolonged cough or night cough during the preceding 12 months and a diagnostic finding in the exercise challenge test. Parent-reported use of ICSs or bronchodilators needed to occur during the past 12 months, and doctor-diagnosed allergic rhinitis and atopic dermatitis needed to be symptomatic during the past 12 months.

At the age of 11–13 years, the outcome variables were persistent asthma, current asthma and continuous use of ICSs. Current asthma was considered if the child had used ICSs continuously during the last 12 months or, alternatively, if the child had suffered from repeated wheezing or prolonged cough or night cough for 4 weeks or longer during the last 12 months and also had a diagnostic increase in FEV1 in the bronchodilation test. Persistent asthma was defined as the presence of asthma at both the 5–7-year and 11–13-year follow-up visits. In addition, we included parent-reported use of ICSs between the follow-ups at 5–7 years and 11–13 years, parent-reported use of bronchodilators in the past 12 months and doctor-diagnosed allergic rhinitis in the past 12 months.

The lung function analyses were secondary analyses; published IOS data obtained at 5–7 years of age and published FVS data obtained at 11–13 years of age were studied in relation to the *IL17A* rs2275913, rs4711998 and rs8193036 variations and the *IL17F* rs763780, rs11465553 and rs7741835 variations.

4.5 Genetics

Whole blood samples were collected during hospitalisation and frozen for further DNA analyses in genetic studies. Supplementary samples were collected during the follow-ups. Altogether, 165 blood samples were available for genotyping of the three *IL17A* and three *IL17F* SNPs.

The appropriate clinical and genetic data were available in 165 cases for the *IL17A* rs2275913 SNP, in 164 cases for the *IL17A* rs4711998 SNP and in 162 cases for the *IL17A* rs8193036 SNP. At the 5–7-year follow-up, data were available in 140 cases for the *IL17A* rs2275913 SNP and in 139 cases for both the *IL17A* rs4711998 and *IL17A* rs8193036 SNPs. At the 11–13-year follow-up, data were available in 124

for the *IL17A* rs2275913 SNP, in 123 cases for the *IL17A* rs4711998 SNP and in 122 cases for the *IL17A* rs8193036 SNP.

The appropriate clinical and genetic data were available in 165 cases for the *IL17F* rs763780, rs11465553 and rs7741835 SNPs. At the 5–7-year follow-up and 11–13-year follow-up, data were available in 142 cases and 125 cases, respectively, for the *IL17F* rs763780, rs11465553 and rs7741835 SNPs.

At the follow-up visit at 5–7 years of age, the data on lung function obtained by IOS and the genetic data were available in 98 cases for the *IL17A* rs2275913 and *IL17A* rs4711998 SNPs and in 99 cases for the *IL17A* rs8193036 SNP. Both the IOS and genetic data on the *IL17F* rs763780, *IL17F* rs11465553 and *IL17F* rs7741835 SNPs were available in 100 cases.

At the follow-up visit at 11–13 years of age, the data on lung function obtained by FVS and the genetic data were available in 84 cases for the *IL17A* rs2275913 SNP, in 83 cases for the *IL17A* rs4711998 SNP and in 82 cases for the *IL17A* rs8193036 SNP. Both the FVS and genetic data on the *IL17F* rs763780, *IL17F* rs11465553 and *IL17F* rs7741835 SNPs were available in 84 cases.

4.5.1 Genotyping of the *IL17A* gene polymorphisms

Genotyping of extracted DNA for the *IL17A* rs2275913 (-197 G>A), rs4711998 (-877 A>G) and rs8193036 (-737 C>T) variants was performed by high resolution melting analysis (HRMA) (Roche Diagnostics Light Cycler 480, Basel, Switzerland). For *IL17A* rs2275913, the primers (forward 5'-TCTGCCCTTCCCATTTTCCTTC-3' and reverse 5'-GGTTAAAATTTCCGCCCCCAATT-3') were designed using the Primer-Blast design tool. The high performance liquid chromatography (HPLC)-quality primers used in the HRMA analysis were as follows: the *IL17A* rs4711998 forward 5'-TCTTGTCCTAGTCCTCTGTATTC-3' and reverse 5'-GTAAG ATGAACTTGGACTCAGGTC-3', and the *IL17A* rs8193036 forward 5'-CTCCT TTCTAGTTCTCATCACTCTC-3' and reverse 5'-GGGGATAGAGACTGGACA AA-3' (229).

For *IL17A* rs2275913, HRMA PCR reactions were run at 95 °C for 10 min, followed by 45 amplification cycles at 95 °C for 10 s, at 59 °C for 10 s and at 72 °C for 15 s. For *IL17A* rs4711998 and rs8193036, HRMA PCR reactions were started with an initial denaturation for 3 min at 95 °C, and this was followed by 39 amplification cycles at 95 °C for 5 s, annealing for 10 s at 60 °C (*IL17A* rs4711998) or at 61 °C (*IL17A* rs8193036) and extension at 72 °C for 15 s. For all three *IL17A*

SNPs, after the PCR process, the final melting cycle conditions were as follows and as outlined by Roche: heating to 95 °C and holding for 1 min, and then cooling to the pre-hold temperature (40 °C) to make sure that all PCR products re-associated (229, 230).

4.5.2 Genotyping of the *IL17F* gene polymorphisms

For the detection of the *IL17F* rs763780 and rs11465553 alleles, capillary sequencing was used. The primers designed to amplify the 156-bp area of interest and used in the PCR prior sequencing were: (forward) 5'-TTGCAGAGCACTGGGTAAGG-3' and (reverse) 5' ACCAAGGCTGCTCTGTTTCT-3'. The primers were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Sequencing of the PCR products was performed at the Institute for Molecular Medicine Finland laboratories, Helsinki, Finland.

For the detection of *IL17F* rs7741835 alleles, HRMA was performed using a LightCycler480 version 5.1 (Roche, Basel, Switzerland) with a SensiFAST HRMA melting master kit (Bioline, London, UK). Primers were designed using the Primer-Blast design tool and purchased from Sigma-Aldrich Company (Saint Louis, MI, USA). HPLC-quality primers used in the HRMA analyses were as follows: forward 5'-ATGCAGCCTGATTGAGTAGGTT-3' and reverse 5'-ATGCAGCCTGATTG AGTAGGTT-3' (231).

For *IL17F* rs763780 and rs11465553, PCR mixtures were pre-incubated at 94 °C for 10 min, followed by 40 amplification cycles at 94 °C for 60 s, at 55 °C for 40 s and at 72 °C for 60 s. For *IL17F* rs7741835, HRMA PCRs were started with an initial denaturation for 3 min at 95 °C, and this was followed by 38 amplification cycles at 95 °C for 5 s, annealing for 10 s at 59 °C and extension at 72 °C for 15 s. After the PCR, HRMA was used to separate homozygote and heterozygote variants. The shape of the melting curves was used to define the SNPs (229).

4.6 Controls

The minor allele frequencies (MAFs) of all six studied *IL17A* and *IL17F* SNPs in the original cohort of 165 bronchiolitis patients were compared to the MAFs of those 99 Finnish population-based controls from the 1000 Genomes Project (232) and MAFs in the Finnish data of the Genome Aggregation Database

(https://gnomad.broadinstitute.org/). In that database, the number of controls varied from 1734 to 12558, depending on the SNP in question (Table 3).

In addition, both the allele frequencies and genotypes of *IL17A* rs2275913 were compared with those of 405 healthy full-term infants from South-West Finland who participated in the STEPS (Steps to the Healthy Development and Well-being of Children) birth cohort study (205).

4.7 Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Science (SPSS versions 23.0 and 25.0, IBM Corp., NY, USA).

The MAFs of the three *IL17A* and three *IL17F* SNPs of the study group (cases) were compared with those in the Finnish data of the 1000 Genomes Project and in the Finnish cases from the Genome Aggregation Database.

The hospitalisation parameters, and the 5–7-year or 11–13-year follow-up outcome parameters, were analysed in relation to the genotypes (wild vs. variant) of the three *IL17A* and three *IL17F* SNPs within the study group.

Chi-square and Fisher's exact tests were used for categorised variables when appropriate. A p value < 0.05 was considered statistically significant. Student's t test was used for normally distributed continuous variables, and the Mann–Whitney test was used for non-normally distributed continuous variables. The results are expressed as frequencies, proportional frequencies, means, medians, standard deviations (SD) and ranges, as appropriate. Logistic regression was used in analyses adjusted for RSV aetiology of bronchiolitis, current age and sex. The results are expressed as odds ratios (ORs), adjusted odds ratios (aORs) and 95% confidence intervals (95%CIs).

The IOS and FVS data were both studied as continuous variables. Analysis of variance (ANOVA) was used in the analyses of continuous data, and analyses were adjusted, when appropriate, for maternal smoking in infancy, RSV aetiology (oscillometry and spirometry data), current asthma and sex-specific, height-related body mass index-for-age z-scores (spirometry data). The results are presented as means, SDs and 95%CIs.

4.8 Ethics

The study was conducted in accordance with the World Medical Association Declaration of Helsinki. We obtained informed parental consent, including for the use of samples for genetic studies on bronchiolitis and asthma risk both during hospitalisation and at the follow-ups. The study protocol was approved by the Ethics Committee of the Tampere University Hospital district, Tampere, Finland. The personal data of the study subjects were not provided to the laboratories that performed the genetic studies. The families were also offered the opportunity to participate in the study by answering the questions in an interview via telephone.

5 RESULTS

5.1 The *IL17A* and *IL17F* polymorphisms in relation to the basic data on hospitalisation and follow-ups (Articles I-III)

Clinical data, which were collected during hospitalisation or at the follow-up visits, were available for 166 cases, and blood samples for genetic studies were available from 165 patients.

The mean age of the 165 bronchiolitis patients was 10.71 weeks (SD 6.82) during hospitalisation, and 84 (50.6%) were boys. RSV was the causative agent in 113 (68.5%) cases. As published previously, the mean age was 6.5 years at the first follow-up (81) and 11.7 years at the second follow-up (82).

The MAFs of the *IL17A* rs2275913 (A), rs4711998 (A) and rs8193036(C), and *IL17F* rs763780 (C), rs11465553 (T) and rs7741835 (T) did not differ between the cases and the 99 controls from the 1000 Genomes Project or the Finnish data of the Genome Aggregation Database (Table 4).

Neither the *IL17A* rs2275913 genotypes nor allele frequencies differed between the cases and the 405 controls recruited from the STEPS study (Article I).

Table 4. Comparison of the minor allele frequencies (MAFs) of the six *IL17A* and *IL17F* polymorphisms in the 165 infants with bronchiolitis with those in the Finnish data from the 1000 Genomes project[1] and the Genome Aggregation Database[2] (Article I, II and III).

Gene variation	Major / minor allele	MAF bronchiolitis group	MAF 1000 Genomes Project FIN data	MAF Genome Aggregation Database Finnish data	Statistical difference
IL17A rs2275913	G / A	0.45 147 / 330	0.429 85 / 198	0.4816 1672 / 3472	p1 = 0.717 p2 = 0.209
IL17A rs4711998	G / A	0.280 92 / 328	0.298 59 / 198	0.3017 1048 / 3474	p1 = 0.667 p2 = 0.754
IL17A rs8193036	T / C	0.377 122 / 324	0.394 78 / 198	0.4132 1433 / 3468	p1 = 0.692 p2 = 0.199
IL17F rs763780	T / C	0.112 37 / 330	0.076 15 / 198	0.1100 2762 / 25112	p1 = 0.175 p2 = 0.902
IL17F rs11465553	C / T	0.033 11 / 330	0.040 8 / 198	0.05072 1274 / 25116	p1 = 0.672 p2 = 0.152
IL17F rs7741835	C / T	0.245 81 / 330	0.182 36 / 198	0.2353 816 / 3468	p1 = 0.088 p2 = 0.722

MAF, minor allele frequency; p1, Bronchiolitis group vs. 1000 Genomes Project FIN data; p2, Bronchiolitis group vs. Genome Aggregation Database Finnish data; [1]https://www.ensembl.org; [2]https://gnomad.broadinstitute.org.

5.2 The *IL17A* and *IL17F* polymorphisms in relation to the severity of bronchiolitis (Articles I-III)

The wild genotype GG of *IL17A* rs2275913 was present in 48 (29.1%) children, the wild genotype GG of *IL17A* rs4711998 was present in 81 (49.4%) children and the wild genotype TT of *IL17A* rs8193036 was present in 62 (38.3%) children. The respective incidences of the variant genotypes were 70.9% (rs2275913, GA in 87 and

AA in 30), 50.6% (rs4711998, GA in 74 and AA in 9) and 61.7% (rs8193036, TC in 78 and CC in 22).

The wild genotype TT of *IL17F* rs763780 was present in 128 (77.6%) children, the wild genotype CC of *IL17F* rs11465553 was present in 153 (92.7%) children and the wild genotype CC of *IL17F* rs7741835 was present in 90 (50.5%) children. The respective incidences of the variant genotypes were 22.4% (rs763780, TC in 36 and CC in 1), 7.3% (rs11465553, CT in 12 and TT in 0) and 45.5% (rs7741835, CT in 69 and TT in 6).

There were no significant differences in either the virus aetiology of bronchiolitis or the severity markers of bronchiolitis, including the need for and duration of oxygen administration, the need for and duration of feeding support or the LOS, between the children with wild genotypes and those with variant genotypes of any of the six studied *IL17A* or the *IL17F* gene polymorphisms (Articles I, II and III).

5.3 The *IL17A* polymorphisms in relation to the clinical outcome at 5-7 years of age (Articles I-II)

The wild genotype GG of *IL17A* rs2275913 was present in 40 (28.6%) children, and the variant genotype was present in 100 (71.4%, GA in 75 and AA in 25) children. For *IL17A* rs4711998, the wild genotype GG was present in 71 (51.1%) cases and the variant genotypes GA and AA were present in 59 and 9 (48.9%) cases, respectively. For *IL17A* rs8193036, the wild genotype TT was present in 52 (37.4%) cases and the variant genotypes TC and CC were present in 68 and 19 cases (62.6%), respectively.

There were no significant differences in any of the wheezing, asthma or allergy parameters between the children with the *IL17A* rs2275913 wild genotype GG and those with the variant GA or AA genotypes, or between the children with the *IL17A* rs4711998 GG (wild), GA (variant) and AA (variant) genotypes, or between those with the *IL17A* rs8193036 TT (wild), TC (variant) and CC (variant) genotypes (Table 5). None of the nine children with the homozygous variant genotype AA of *IL17A* rs4711998 had asthma, whereas 14.6% of those with the heterozygous variant or wild genotype ($p = 0.26$) had asthma (Articles I and II).

5.4 The *IL17F* polymorphisms in relation to the clinical outcome at 5-7 years of age (Article III)

The wild genotype TT of *IL17F* rs763780 was present in 110 (77.5%) children, and the variant genotype was present in 32 (22.5%) children: TC in 31 cases and CC in one case. For *IL17F* rs11465553, the wild genotype CC was present in 131 (92.3%) cases and the variant genotype CT was present in 11 (7.7%) cases: none of the children had the homozygous variant TT genotype. The *IL17F* rs7741835 wild genotype CC was present in 82 (57.7%) cases, and the variant genotype was present in 60 (42.3%) cases: CT in 57 cases and TT in three cases. There were no significant differences in any of the wheezing, asthma or atopy parameters between the children with the wild genotypes and those with the variant genotypes of the three *IL17F* SNPs (Table 5).

Table 5. Asthma and use of bronchodilators and inhaled corticosteroids in relation to the presence of variant genotypes of three *IL17A* gene polymorphisms (rs2275913, rs4711998 and rs8193036) and three *IL17F* gene polymorphisms (rs763780, rs11465553 and rs7741835) at 5–7 years of age (Articles I, II and III).

Variant polymorphism	Variant / wild genotypes	Current asthma variant / wild	Use of bronchodilators variant / wild	Use of inhaled steroids variant / wild	Allergic rhinitis variant / wild
IL17A rs2275913 (AG, AA)	40 / 100	11 / 8 p = 0.16	15 / 11 p = 0.09	10 / 8 p = 0.12	28 / 12 p = 0.81
IL17A rs4711998 (GA, AA)	68 / 71	8 / 11 p = 0.48	10 / 16 p = 0.21	9 / 9 p = 0.97	19 / 20 p = 0.89
IL17A rs8193036 (TC, CC)	87 / 52	9 / 10 p = 0.14	16 / 10 p = 0.90	11 / 7 p = 0.89	24 / 16 p = 0.69
IL17F rs763780 (TC, CC)	32 / 110	7 / 12 p = 0.10	6 / 20 p = 0.57	6 / 12 p = 0.19	10 / 30 p = 0.42
IL17F rs11465553 (CT, TT)	11 / 131	0 / 19 p = 0.19	1 / 25 p = 0.36	0 / 18 p = 0.21	3 / 37 p = 0.62
IL17F rs7741835 (CT, TT)	51 / 74	9 / 10 p = 0.42	9 / 17 p = 0.25	6 / 12 p = 0.28	15 / 25 p = 0.28

Note: p values between subjects were calculated with variant versus wild genotype.

5.5 The *IL17A* polymorphisms in relation to the clinical outcome at 11-13 years of age (Articles I-II)

The *IL17A* rs2275913 genotypes were present as follows: the wild GG was in 34 (27.4%) children, the variant GA was in 67 (54.0%) children and the variant AA was in 23 (18.6%) children. The *IL17A* rs4711998 genotypes were present as follows: the wild GG was in 64 (52.0%) children, the variant GA was in 51 (41.5%) children and the variant AA was in 8 (6.5%) children. The *IL17A* rs8193036 genotypes were present as follows: the wild TT was in 45 (36.9%) children, the variant TC was in 59 (48.4%) children and the variant CC was in 18 (14.8%) children.

Children with the variant GA or AA genotype of *IL17A* rs2275913 had significantly lower reports of current asthma (7.8% vs. 23.5%, p = 0.02), and they had less frequent use of ICSs (5.6% vs. 17.6%, p = 0.04) and inhaled bronchodilators (17.8% vs. 35.3%, p = 0.04) during the last 12 months than children with the wild genotype GG (Table 6). In addition, children with the variant GA or AA genotype had significantly lower reports of current allergic rhinitis than children with the wild AA genotype (11.1% vs. 26.5%, p = 0.03) (Table 6).

Table 6. Asthma, allergic rhinitis and use of bronchodilators and inhaled corticosteroids in relation to the presence of the variant genotypes of three *IL17A* gene polymorphisms (rs2275913, rs4711998 and rs8193036) and three *IL17F* gene polymorphisms (rs763780, rs1146553 and rs7741835) at 11–13 years of age (Articles I, II and III).

Variant polymorphism	Variant / wild genotypes	Current asthma variant / wild	Use of broncho-dilators variant / wild	Use of inhaled steroids variant / wild	Persistent asthma variant / wild	Allergic rhinitis variant / wild
IL17A rs2275913 (GA, AA)	34 / 90	7 / 8 **p = 0.02**	16 / 12 **p = 0.04**	5 / 6 **p = 0.04**	4 / 5 p = 0.05	10 / 9 **p = 0.03**
IL17A rs4711998 (GA, AA)	59 / 64	10 / 5 p = 0.14	13 / 14 p = 0.56	7 / 4 p = 0.24	5 / 4 p = 0.24	13 / 6 p = 0.05
IL17A rs8193036 (TC, CC)	45 / 77	7 / 8 p = 0.16	15 / 13 p = 0.23	5 / 6 p = 0.20	4 / 5 p = 0.23	12 / 7 p = 1.00
IL17F rs763780 (TC, CC)	27 / 98	6 / 9 P = 0.07	8 / 20 P = 0.22	5 / 6 P = 0.06	4 / 5 P = 0.10	6 / 13 P = 0.39
IL17F rs11465553 (CT, TT)	9 / 116	0 / 15 p = 0.30	1 / 27 p = 0.36	0 / 11 p = 0.42	0 / 9 p = 0.50	2 / 17 p = 0.41
IL17F rs7741835 (CT, TT)	51 / 74	5 / 10 p = 0.37	9 / 19 p = 0.20	3 / 8 p = 0.27	7 / 2 p = 0.21	8 / 11 p = 0.55

Note: p values between subjects were calculated with variant versus wild genotype.

Statistically significant findings are marked with bold font.

Persistent asthma means the presence of asthma at both the 5–7-year and 11–13-year follow-up.

In multivariate analyses adjusted for age, sex, RSV aetiology of bronchiolitis and atopy at age < 12 months, use of ICSs during the last 12 months retained its statistical significance. In children with the variant genotype GA or AA, the aOR was 0.25 (95%CI 0.06–0.97). In this case, differences in current asthma, current allergic rhinitis and use of bronchodilators during the last 12 months marginally lost their significance (Table 7).

Table 7. Results of logistic regression performed with data on post-bronchiolitis asthma, allergy and asthma medications at 11–13 years of age in relation to the *IL17A* rs2275913 variant (GA, AA) polymorphism (Article I).

Diagnoses and asthma medications	Unadjusted OR (95%CI)	Adjusted OR* (95%CI)
Current asthma	**0.27 (0.09–0.83)**	0.32 (0.10–1.04)
Bronchodilators	**0.40 (0.16–0.96)**	0.44 (0.18–1.11)
Inhaled steroids	**0.28 (0.08–0.97)**	**0.25 (0.06–0.97)**
Persistent asthma	0.27 (0.07–1.07)	0.21 (0.04–1.02)
Allergic rhinitis	**0.35 (0.13–0.95)**	0.40 (0.13–1.22)

OR, odds ratio vs. presence of wild genotypes; CI, confidence interval.

*Adjusted for age, sex, RSV aetiology of bronchiolitis and atopic dermatitis in infancy

Statistically significant findings are marked with bold font.

The *IL17A* SNP rs4711998 was found to be associated with allergic rhinitis: children with the wild genotype GG reported allergic rhinitis less often than children with the variant GA or AA genotype (9.5% vs. 22.0%). The proportions were 9.4% of those with the homozygous wild GG genotype, 17.9% of those with the heterozygous variant GA genotype and 37.5% of those with the homozygous variant AA genotype ($p = 0.024$). There were no significant associations found between the *IL17A* SNP rs8193036 and any asthma or allergy outcome at the follow-up at 11–13 years of age (Table 6).

5.6 The *IL17F* polymorphisms in relation to the clinical outcome at 11-13 years of age (Article III)

The *IL17F* rs763780 genotypes were present as follows: the wild TT was in 98 (78.4%) children and the variant TC was in 26 (20.8%) children. Only one child had the variant CC genotype. The *IL17F* rs11465553 genotypes were present as follows: the wild CC was in 116 (92.8%) children and the variant CT was in nine (7.2%) children. None of the children had the variant TT genotype. The *IL17F* rs7741835 genotypes were present as follows: the wild CC was in 74 (59.2%) children, the variant CT was in 49 (39.2%) children and the variant TT was in two (1.6%) children.

Children with the *IL17F* rs763780 variant TC or CC genotype used ICSs more often between the first and second follow-up visits than those with the wild TT genotype (25.9% vs. 9.2%, p = 0.02). This finding retained its statistical significance in multivariate analyses adjusted for age, sex and RSV aetiology of bronchiolitis, when the aOR was 3.58 (95%CI 1.11–11.5) for the variant TC or CC genotype of *IL17F* rs763780. The children with the variant TC or TT genotype used ICSs during the last 12 months in 18.5% of cases, whereas 6.1% of those with the wild genotype TT used ICSs in the same period (6.1%, p = 0.06). The incidence of current asthma in the same groups was 22.2% and 9.2% (p = 0.07), respectively (Table 5). In multivariate analyses, the aOR reached 3.49, although the finding was not statistically significant (Table 8).

There were no significant differences in the asthma or allergy parameters between children with the wild genotypes of *IL17F* rs11465553 or rs7741835 and those with the variant genotypes (Table 6).

Table 8. Results of logistic regression performed with data on post-bronchiolitis asthma, allergy and asthma medications at 11–13 years of age in relation to the IL17F rs763780 variant (TC, CC) polymorphism (Article III).

Diagnoses and asthma medications	Unadjusted OR (95%CI)	*Adjusted OR (95%CI)
Current asthma	2.83 (0.91–8.81)	2.70 (0.81–8.90)
Bronchodilators	1.64 (0.63–4.29)	1.52 (0.56–4.13)
Inhaled steroids	3.49 (0.97–12.47)	3.49 (0.86–14.2)
Persistent asthma	3.24 (0.80–13.0)	3.77 (0.76–18.61)
Allergic rhinitis	1.29 (0.53–2.91)	1.88 (0.61–5.84)
Use of ICSs at 5–13 years of age	**3.46 (1.15–10.4)**	**3.58 (1.11–11.5)**

OR, odds ratio vs. presence of wild genotypes; CI, confidence interval.

*Adjusted for age, sex and RSV aetiology of bronchiolitis.

Statistically significant findings are marked with bold font.

5.7 The *IL17A* and *IL17F* polymorphisms in relation to the lung function data at 5-7 years of age (Articles IV-V)

There were no significant differences in either the baseline or post-BD Rrs5 or Xrs5 results between children with the wild genotypes of the *IL17A* rs4711998, rs8193036 and rs2275913 SNPs or the *IL17F* rs763780, rs11465553 or rs7741835 SNPs and those with the variant genotypes (Articles IV and V).

In the children with the wild genotype GG of *IL17A* rs2275913 (n = 24), exercise induced a greater increase in mean Rrs5 compared to that in the 74 cases with the

variant AG or AA genotype (1.528 vs. 0.202, p = 0.002). For the *IL17A* rs4711998 and rs8193036 SNPs, no such significant differences were observed. Seven children with the variant genotype CT of *IL17F* rs11465553 showed a higher mean Rrs5 (-0.822 vs. -1.776, p = 0.01) and a lower mean Xrs5 (-0.156 vs. 0.381, p = 0.04) than the 93 children with the wild CC genotype, meaning that mild peripheral obstruction was present. No child had the variant homozygous TT genotype. There were no significant differences in responses to BD between children with wild genotypes and those with variant genotypes in any of the three *IL17A* or three *IL17F* SNPs (Article IV).

5.8 The *IL17A* and *IL17F* polymorphisms in relation to the lung function data at 11-13 years of age (Articles IV-V)

In non-adjusted analyses, both the baseline FEV1/FVC and post-BD FEV1/FVC values were lower in the 39 children with the variant GA or AA genotype than in the 44 children with the wild GG genotype of *IL17A* rs4711998. The mean of the baseline FEV1/FVC was 91.4 in those with the variant genotype and 95.1 in those with the wild genotype (p = 0.03), and the means of the post-BD FEV1/FVC were 94.4 and 97.7, respectively (p = 0.02). The adjusted 95%CIs marginally overlapped, which means that statistical significance was lost. There were no significant differences in the baseline or post-BD FEV1 or FEV1/FVC values between children with wild the genotypes and those with the variant genotypes of the *IL17A* rs2275913 and rs8193036 SNPs (Table 9).

The mean baseline FEV1/FVC value was 93.2 (95%CI 91.4–94.9) in the 79 children with the wild *IL17F* rs11465553 CC genotype, and it was 98.4 (95%CI 95.1–101.8, p = 0.02) in the five children with the variant CT genotype. No such difference was observed in the post-BD FEV1/FVC values. No child had the variant homozygous TT genotype. When the baseline and post-BD FEV1 and FEV1/FVC values were analysed in relation to the *IL17F* rs11465553, rs763780 and rs7741835 SNPs, no significant associations were identified (Table 10).

Table 9. The baseline and post-bronchodilator parameters measured using flow-volume spirometry (FVS) in former bronchiolitis patients at 11–13 years of age expressed as continuous percent-of-predicted values in relation to the wild and variant genotypes of the *IL17A* rs2275913, rs4711998 and rs8193036 SNPs (Article IV).

***IL17A* genotypes**	**Baseline FEV1 Mean (SD) [95%CI]**	**Post-BD FEV1 Mean (SD) [95%CI]**	**Baseline FEV1/FVC Mean (SD) [95%CI]**	**Post-BD FEV1/FVC Mean (SD) [95%CI]**
IL17A rs2275913 Wild GG (n = 23)	88.7 (10.0) [84.4–93.0]	91.8 (10.9) [87.1–96.5]	91.8 (9.4) [87.8–95.9]	94.2 (6.8) [91.3–97.2]
IL17A rs2275913 Variant GA or AA (n = 61)	89.5 (11.8) [86.5–92.5]	93.0 (12.3) [89.9–96.2]	94.1 (7.1) [92.3–95.9]	97.0 (6.6) [95.3–98.7]
IL17A rs4711998 Wild GG (n = 44)	90.4 (11.2) [87.0–93.8]	93.7 (12.2) [90.0–97.4]	95.1 (7.8) [92.7–97.4]	97.7 (6.5) [95.7–99.7]
IL17A rs4711998 Variant GA or AA (n = 39)	88.6 (11.0) [85.0–92.2]	91.9 (11.4) [88.3–95.6]	91.4 (7.4) [89.0–93.8]	94.4 (6.4) [92.3–96.5]
IL17A rs8193036 Wild TT (n = 28)	88.4 (12.3) [83.6–93.2]	92.3 (13.6) [87.0–97.6]	92.4 (8.8) [89.0–95.8]	95.3 (6.8) [92.7–97.9]
IL17A rs8193036 Variant TC or CC (n = 54)	89.1 (10.4) [86.3–92.0]	92.6 (11.1) [89.6–95.6]	93.9 (7.4) [91.9–95.9]	96.8 (6.8) [94.9–98.6]

Note: Analysis of variance adjusted for maternal smoking when the child was < 12 months old, RSV/non-RSV aetiology of bronchiolitis during hospitalisation, current asthma and current sex-specific, height-related body mass index-for-age-z-scores at the study visit.

Table 10. The baseline and post-bronchodilator parameters measured using flow-volume spirometry (FVS) in former bronchiolitis patients at 11–13 years of age expressed as continuous percent-of-predicted values in relation to the wild and variant genotypes of the *IL17F* rs763780, rs11465553 and rs7741835 SNPs (Article V).

***IL17F* genotypes**	**Baseline FEV1 Mean (SD) [95%CI]**	**Post-BD FEV1 Mean (SD) [95%CI]**	**Baseline FEV1/FVC Mean (SD) [95%CI]**	**Post-BD FEV1/FVC Mean (SD) [95%CI]**
IL17F rs763780 Wild TT (n = 65)	89.2 (11.9) [86.3–92.1]	92.4 (12.2) [89.4–95.5]	93.1 (7.9) [91.1–95.0]	95.8 (6.8) [94.1–97.5]
IL17F rs763780 Variant TC or CC (n = 19)	89.2 (9.1) [85.2–94.0]	93.6 (11.0) [88.3–98.9]	94.8 (7.5) [91.2–98.4]	97.9 (6.3) [94.8–100.9]
IL17F rs11465553 Wild CC (n = 79)	89.4 (11.3) [86.9–91.9]	93.0 (11.9) [90.4–95.7]	93.2 (7.9)* [91.4–94.9]	96.0 (6.8) [94.5–97.6]
IL17F rs11465553 Variant TC (n = 5)	87.8 (12.0) [72.9–102.7]	87.4 (10.6) [74.2–100.6]	98.4 (2.7)* [95.1–101.8]	99.6 (4.7) [93.7–105.4]
IL17F rs7741835 Wild CC (n = 52)	89.0 (11.0) [85.9–92.0]	91.7 (11.8) [88.4–94.9]	94.7 (7.3) [92.7–96.8]	97.0 (6.3) [95.2–98.7]
IL17F rs7741835 Variant TC or TT (n = 32)	89.8 (11.8) [85.5–94.0]	94.4 (12.1) [90.0–98.7]	91.4 (8.3) [88.4–94.4]	95.1 (7.2) [92.5–97.7]

Note: Analysis of variance adjusted for maternal smoking when the child was < 12 months old, RSV/non-RSV aetiology of bronchiolitis during hospitalisation, current asthma and current sex-specific, height-related body mass index-for-age-z-scores at the study visit.

The numbers of homozygous variant genotypes were n = 1 for *IL17F* rs7741835 (TT), n = 0 for *IL17F* rs11465553 (CC) and n = 1 for *IL17F* rs763780 (CC).

*p = 0.02 between the wild and variant genotypes

6 DISCUSSION

In this thesis, the *IL17A* rs2275913, rs4711998 and rs8193036 SNPs and the *IL17F* rs763780, rs11465553 and rs7741835 SNPs have been evaluated in relation to 1) the clinical course and viral aetiology of bronchiolitis at less than 6 months of age, 2) the prevalence of post-bronchiolitis asthma and 3) post-bronchiolitis lung function at preschool and school age. The study was designed in a long-term prospective setting, and 187 infants were originally enrolled. Of the former bronchiolitis patients, 166 attended the follow-up at 5–7 years of age and 138 attended the follow-up at 11–13 years of age.

The hypothesis was that variations in *IL17A* and *IL17F* located in the genomic region linked to asthma-related phenotypes may influence the production or structure of the cytokines IL-17A and IL-17F, respectively, and thus impact the severity of bronchiolitis and post-bronchiolitis asthma or lung function

6.1 Severity and viral aetiology of bronchiolitis in relation to the *IL17* polymorphisms

The results clearly showed that there was no association between the three studied *IL17A* or three studied *IL17F* SNPs and virus aetiology or bronchiolitis severity at less than 6 months of age. Disease severity was determined by assessing the need for and duration of oxygen administration and the need for and duration of feeding support.

In the present bronchiolitis cohort, 66% of the infants had RSV as a causative virus of bronchiolitis. Studies have suggested that RSV infection may be associated with increased IL-17A production, and this cytokine may be prominent especially in neonates, contributing to more severe bronchiolitis (233, 234). In animal studies, the involvement of IL-17A has been explored in RSV infections concomitant with experimental asthma (235). For example, an increase in mucus production was found to be associated with increased levels of IL-17A in the lungs (235). Intriguingly, it has been reported that IL-17F is not upregulated in an RSV mouse model (233).

Our study is the first to assess the role that the *IL17A* rs4711998 and rs8193036 SNPs and the *IL17F* rs763780, rs11465553 and rs7741835 SNPs play in the clinical course of bronchiolitis. Two previous studies have been published that focused on the *IL17A* gene variation rs2275913 and the severity of bronchiolitis (207, 208). In one of the studies, a case-control study conducted with 121 Brazilian infants hospitalised for bronchiolitis at the mean age of 3.4 months, the *IL17A* rs2275913 variant genotype AG/GG was associated with more severe disease than the wild genotype AA (207). In the other study, which included 144 Chinese bronchiolitis patients with a mean age of 4.9 months, the hospital stay was longer—suggesting more severe disease—in children homozygous for the minor A allele of *IL17A* rs2275913 than in those with AG or GG genotypes (208).

Previous studies on the impact of IL-17A on the severity of RSV bronchiolitis have shown that milder disease is associated with higher levels of IL-17A (189, 236, 237). Higher levels of IL-17A in nasal wash specimens have been associated with a decreased risk for hospitalisation for bronchiolitis at less than 24 months of age (238). Furthermore, plasma IL-17A levels were found to be higher in infants with mild bronchiolitis than in those with severe bronchiolitis (236). Increased levels of IL-17A were also found in nasopharyngeal aspirates from non-ventilated infants with RSV bronchiolitis compared to ventilated infants, suggesting a possible protective role for IL-17A in bronchiolitis (189). In addition, when nasal and blood cytokine levels were measured in 40 infants under one year of age infected with RSV, elevated levels of IL-17A were associated with less clinical symptoms; IL-17F was undetectable in both the infected infants and controls (190), highlighting the potential protective role that IL-17A may play during RSV infection.

IL-17A is vital in neutrophil recruitment and activation (237). In one study, mice infected with RSV were found to have elevated numbers of IL-17A-producing cells, a finding that was further associated with increased neutrophilic infiltration in the lungs (191). Another study revealed that RSV infection induced the production IL-17A but not IL-17F in the lungs of allergen-sensitised RSV-infected mice, indicating a role of IL-17A in the response to RSV infection in allergic Th2-type immune environments (233).

In recent studies conducted in humans, IL-17A has been associated with RSV infection and post-bronchiolitis wheeze (239, 240). A case-control study assessed the longitudinal plasma cytokine levels during episodes of RSV bronchiolitis in children under one year of age and at subsequent follow-ups (239). There were no differences in the cytokine concentrations in the bronchiolitis phase; however, a significant positive correlation was found between the number of post-bronchiolitis wheezing

episodes and the level of IL-17A measured at the one-month and 12-month follow-ups (239), suggesting a possible role for IL-17A in post-bronchiolitis wheezing. In the most recently conducted study, which involved 46 RSV-infected Chinese children with a mean age of 4 months, increased levels of IL-17A in nasopharyngeal aspirates were associated with more severe pneumonia caused by RSV, but not with the LOS or the severity of bronchiolitis symptoms, such as wheezing or the need for oxygen supply (240). In the same study, IL-17A was found to contribute to persistent airway inflammation and airway hyperresponsiveness in an RSV infection murine model (240).

6.2 Post-bronchiolitis asthma in relation to the *IL17* polymorphisms

In this study, there were no significant associations found between the three studied *IL17A* variations or the three studied *IL17F* variations and post-bronchiolitis asthma outcomes at 5–7 years of age. Instead, a significant association was found between *IL17A* rs2275913 and asthma at 11–13 years of age: children with the variant GA or AA genotype had significantly lower occurrences of current asthma and had used both ICSs and inhaled bronchodilators significantly less often than those with the wild genotype GG. In line with this finding, children with the *IL17A* rs2275913 variant GA or AA genotype had significantly lower occurrences of current allergic rhinitis than children with the wild GG genotype. These results suggest an association between this *IL17A* SNP and a genetic predisposition to asthma, especially in co-existence with allergic rhinitis. Similar findings were reported in an African study (224) No associations were found between post-bronchiolitis asthma outcomes and the *IL17A* rs4711998 and rs8193036 SNPs.

Children with the *IL17F* rs763780 variant TC or CC genotype used more ICSs from 5 to 13 years of age than those with the wild TT genotype. The incidence of persistent asthma, meaning the presence of asthma between the 5–7-year and 11–13-year follow-ups, was more than 3-fold higher in children with the variant TC or CC genotype of *IL17F*; however, the finding was not statistically significant. There were no significant differences in any of the post-bronchiolitis asthma outcomes in relation to the *IL17F* rs1165553 or rs7741835 SNP.

The overall prevalence of asthma is 7–9% in school-aged children in Finland (241). In hospital-based cohorts, the prevalence of post-bronchiolitis asthma reportedly varies from 10% to 40% in both preschool- and school-aged children (28). In the present bronchiolitis cohort, current asthma was present at the 5–7-year

follow-up in 12.7% of the children (81), and at the 11–13-year follow-up, it was present in 13.0% of the children (82) Notably, the prevalence of current asthma was 23.5% in children with the wild GG genotype of *IL17A* rs2275913, and this was nearly four times higher than the asthma prevalence in Finnish children. In children with the variant genotypes AG or AA of *IL17A* rs2275913, the risk of asthma was similar to the overall risk in the Finnish child population. The protection against post-bronchiolitis asthma associated with the *IL17A* rs2275913 variation, which was one of the main findings of the study, was also observed in the comparisons between the study data and population data. It was noteworthy that the variation did not protect against bronchiolitis, as demonstrated in the comparative analyses conducted with data from three different control populations, including 404 infants from South-West Finland (205).

Five previous studies have assessed the relationship between the three studied *IL17A* SNPs and the three studied *IL17F* SNPs and asthma in children (17, 208, 220-222), but conflicting results have been generated. In 168 Chinese asthmatic children, the *IL17A* rs2275913 homozygous variant genotype AA was associated with increased asthma risk and abnormal lung function at the mean age of 5.23 years (208). A similar result was reported in a study that included 166 atopic dermatitis patients of Eastern European descent with a mean age of 11.6 years: the variant AA genotype of *IL17A* rs2275913 was associated with an increased risk of asthma (222). The findings of the present thesis do not align with these previously reported findings. However, in other studies, the risk of asthma was increased in 651 European children (age unreported) (221) and in 171 African children (mean age of 9.5 years) (17) when the major allele G of *IL17A* rs2275913 was present, and these findings are in agreement with the present results. Furthermore, in 1027 Asian children, there was no association found between the *IL17A* rs2275913 SNP and asthma risk at the age of 5–12 years (220). The *IL17A* rs8193036 and rs4711998 SNPs were included in the same study, and the wild genotype CC of rs8193036 was associated with increased asthma risk (220). In line with this finding, another study showed that the variant *IL17A* rs8193036 T allele was associated with decreased asthma risk in Egyptian children (223). These discrepancies in the results on the associations between *IL17A* variations and asthma risk may simply reflect the ethnic differences among the study groups.

In a study that included 150 Asian asthmatic adults, the *IL17A* rs2275913 variant genotype AA or AG was found to be more common in asthmatic patients than in healthy subjects (226). This finding contrasts with the results of our study. In another study conducted with Asian adults, the *IL17A* rs8193036 homozygous variant TT

genotype occurred more frequently in a group of 125 patients with asthma than in a group of control subjects (218).

Two previous studies compared the *IL17F* rs763780 SNP and asthma risk in children; however, neither study found any association between the two entities (17, 221). Similarly, meta-analyses that have included both Asian and Caucasian patients, and child and adult patients, have not found any association between *IL17F* rs763780 and asthma (25, 26, 219). A further seven studies, which included over 2100 adults, have evaluated the risk of asthma in relation to the *IL17F* rs763780 SNP, but they have provided discordant results (215-218, 222, 225). The *IL17F* rs763780 minor allele C has been associated with increased asthma risk (215, 217) and also with decreased asthma risk, as the homozygous variant genotype CC (216, 225), including in one meta-analysis that contained child patients (27). The variant allele C in *IL17F* rs763780 may have small effects on gene expression, and it has also been linked to other possibly functional polymorphisms within *IL17F* or other genes involved in the inflammatory response. In the abovementioned study conducted with 651 asthmatic children, it was found that the *IL17F* rs7741835 major allele C was under-represented in asthma patients, suggesting a protective role for the allele against asthma (221). IL-17F can be detected in a wider range of tissues than IL-17A, which suggests that IL-17F has more diverse biological functions than IL-17A (216).

6.3 Post-bronchiolitis lung function in relation to the *IL17* polymorphisms

In the present study, a greater increase in the mean Rrs5 values after exercise was observed in the IOS results of children with the wild GG genotype of *IL17A* rs2275913 compared to those with the variant AG or AA genotype. This result aligns with the finding that asthma is associated with the same genotype. At school age, however, no abnormal values were observed for the parameters measured using FVS in relation to the *IL17A* rs2275913 SNP.

Children with the variant genotype CT of *IL17F* rs11465553 showed higher mean Rrs5 values and lower mean Xrs5 values in their IOS results at preschool age compared to children with the wild genotype CC. In contrast, the opposite was observed at school age: the mean baseline FEV1/FVC value was lower in children with the wild CC genotype of *IL17F* rs11465553 than in those with the variant

genotype. Thus, no evidence of persistent lung function reduction was found in relation to the *IL17F* rs11465553 genotypes.

It should be noted at this point that no previous studies have been conducted and published on the association between the *IL17A* rs2275913, rs4711998 and rs8193036 SNPs or the *IL17F* rs763780, rs11465553 and rs7741835 SNPs and post-bronchiolitis lung function.

6.4 Genetic distribution of the *IL17* polymorphisms compared to the Finnish population

The 1000 Genomes Project has pioneered the provision of publicly available population-based gene variance data (232). The Finnish data consists of 99 cases, which is a relatively small cohort for genetic association studies. The Genome Aggregation Database (https://gnomad.broadinstitute.org/) is the largest publicly available dataset for gene variations (242); however, the data are provided by individual researchers, and there is no population-based collecting programme in place. At the moment, the Finnish data consists of many thousands of cases, and the number of cases is constantly increasing.

The vast majority of gene variance has benign outcomes, and if a variant is common in one population, it is generally benign across all populations (242). Cytokine genes are highly polymorphic, and polymorphisms in coding or non-coding regions can affect gene expression and function (243).

When the MAFs of the *IL17A* rs2275913, rs4711998 and rs8193036 SNPs and the *IL17F* rs763780, rs11465553 and rs7741835 SNPs were compared between the bronchiolitis cohort cases and the subjects in the two genome databases (the controls), no differences were found. This means that the minor alleles were not over-represented in the bronchiolitis cohort and, thus, that all the outcomes detected within the bronchiolitis cohort and the identified associations with the *IL17A* and *IL17F* SNPs reflect real associations.

6.5 Methodological aspects: strengths and limitations

6.5.1 Study design

The main strengths of our cohort study are the prospective design, long-term follow-up (until 13 years of age), and systematic data collection performed during both bronchiolitis hospitalisation and at the follow-up visits. The use of novel techniques between 2000 and 2004 made it possible to 1) identify the causative virus in 92% of the bronchiolitis cases, 2) confirm the predominance of RSV and 3) recognise that the observed outcomes were mainly the result of RSV bronchiolitis. Previous long-term post-bronchiolitis studies have used 12 or 24 months as the upper age cut-off. Using an age limit that is above 12 months for the recruitment of study subjects increases the heterogeneity of the study population, as participants in whom bronchiolitis may represent the first episode of asthma may be included. The youngest infants tend to have a higher risk for developing more severe bronchiolitis; however, they seem to experience better long-term outcomes. In this cohort study, the inclusion criterion was hospitalisation for bronchiolitis during the first 6 months of life, and this novel aspect of our study allowed us to report on the long-term outcomes of pure bronchiolitis in young infancy.

In all cases, clinical data were collected using a structured questionnaire and further confirmed and supplemented with information collected during clinical interviews. The data were collected prospectively and carefully. Basic data were available for multivariate analyses when needed, and these were used to confirm the reliability and independence of associations revealed in the basic analyses. The ages at which the two post-bronchiolitis follow-up visits were arranged were selected to allow comparison of the results with those of other post-bronchiolitis studies.

Lung function testing was performed with established techniques to supplement the clinical findings. The data used to examine post-bronchiolitis lung function in relation to the *IL17A* and *IL17F* genotypes were collected prospectively for other purposes; hence, the studies were secondary analyses of the data. National population-based, age- and gender-specific and height-adjusted values for reference were available for the IOS and FVS measurements (244). No control groups with sufficient IOS or FVS data were available, and this is one of the main limitations of our study. The drop-out rate among the bronchiolitis patients until the follow-up at 5–7 years of age was 11.2%, and the drop-out rate until the follow-up at 11–13 years of age was 16.8%. Lung function testing was performed in 62.0% of the subjects at

the 5–7-year follow-up and in 64.5% of the subjects at the 11–13-year follow-up, which means that some of the cases of current asthma may have been missed. In long-term studies, drop-outs are fairly common. However, they may increase the risk of selection bias, since symptomatic patients attend follow-up visits more often than asymptomatic patients.

6.5.2 Genetics

From the genetic point of view, the results of the present cohort study are only exploratory. However, the design of this prospective cohort study is unique. Not only were the severity and viral aetiology of bronchiolitis evaluated in relation to *IL17A* and *IL17F* variants, but also post-bronchiolitis asthma and lung function at preschool and school age. The study population was of Finnish origin, and this ethnic homogeneity is a benefit for genetic studies. The homogeneity of the study population was further enhanced by setting the maximum age limit at the low value of 6 months for bronchiolitis that required hospitalisation, although the age limitation decreased the number of the bronchiolitis cases.

The main limitation of this thesis is the small sample size used for the genetic studies. Using a small sample size can lead to under-powered analyses and increase the risk of generating false-negative findings. Only three SNPs of the *IL17A* and three SNPs of the *IL17F* were examined but they were selected based on other studies. The outcome variables were analysed in relation to the genotypes, which were categorised as either wild or variant within the study group, and no external controls were recruited. The sample size for detecting associations between disease and SNP markers is known to be highly affected by, for example, disease prevalence and allele frequency (245). One possibility to increase the power of genetic studies is to combine different materials in case-control analyses which, as seen recently, increases the heterogeneity in terms of age, ethnicity and aetiology of bronchiolitis (246).

The functionality of the *IL17A* rs2275913, rs4711998 and rs8193036 SNPs and the *IL17F* rs763780, rs11465553 and rs7741835 SNPs was not measured in this study. However, the potential functionality (i.e. the effect of the mutation on the amino acid structure of the cytokine in question) was confirmed by consulting the genetic databases. Neither the production nor concentration of IL-17A or IL-17F as a consequence of the changed amino acid were studied. The association between the *IL17A* rs2275913 variant and lower post-bronchiolitis asthma risk and the

association between the *IL17F* rs763780 variant and higher post-bronchiolitis asthma risk were confirmed with adjusted analyses, which highlight the independence of the findings.

7 CONCLUSIONS

There were no significant differences in the allele frequencies of the *IL17A* rs2275913, rs4711998 or rs819303 SNPs or of the *IL17F* rs763780, rs11465553 or rs7741835 SNPs in the bronchiolitis cohort compared to the Finnish references available in two public registers: the 1000 Genomes Project (232) and the Genome Aggregation Database. In addition, neither the *IL17A* rs2275913 genotypes nor allele frequencies differed between the cases and three-month-old healthy infants from South-West Finland. This indicates that none of the variant alleles of the three *IL17A* SNPs or of the three *IL17F* SNPs is a genetic risk marker for severe bronchiolitis requiring hospitalisation.

Variations of *IL17A* or *IL17F* were not associated with the virus aetiology or severity of bronchiolitis in infants younger than 6 months of age. There were no significant associations between asthma occurrence at preschool age and the studied *IL17A* or *IL17F* polymorphisms.

At school age, the *IL17A* rs2275913 variant genotype protected against post-bronchiolitis asthma. In addition, we made a minor observation that the *IL17F* rs763780 variant genotype was associated with increased use of ICSs at 5–13 years of age, reflecting the persistence of asthma in this age group.

The wild genotype of *IL17A* rs2275913 was associated with exercise-induced airway resistance already at 5–7 years of age and later asthma at 11–13 years of age. No other associations were found between post-bronchiolitis lung function and the three *IL17A* SNPs and three *IL17F* SNPs investigated.

8 FUTURE ASPECTS

The findings on *IL17A* rs2275913 or *IL17F* rs763780 and post-bronchiolitis asthma are promising, but preliminary. The associations between bronchiolitis or post-bronchiolitis asthma and the predisposable genetic factors leading to the loss of function need to be further studied in multi-ethnic groups and larger populations. The variant genotype of *IL17A* rs2275913 was found to be protective against bronchial hyperresponsiveness at 5–7 years of age and asthma at 11–13 years of age. The variant genotype of *IL17F* rs763780 increased the risk of ICS use through the early school years.

In the future, research could focus on examining *IL17A* and *IL17F* variants other than those studied in this thesis. Future studies could also evaluate the effects of gene variations on the structure and production of IL-17A and IL-17F. The final step should involve a clinical study designed to evaluate the effects of any changes in the structure or production of the cytokines on clinical outcomes, such as severity of bronchiolitis and post-bronchiolitis hyperresponsiveness and asthma. In terms of utilising *IL17A* or *IL17F* as a marker for bronchiolitis patients, the clinical applications are not immediately clear.

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ORIGINAL PUBLICATIONS

PUBLICATION I

***IL-17A* gene polymorphism rs2275913 is associated with the development of asthma after bronchiolitis in infancy.**

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Original Article

IL-17A gene polymorphism rs2275913 is associated with the development of asthma after bronchiolitis in infancy

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IL, interleukin; ICS, inhaled corticosteroids; FEV1, forced expiratory volume in 1 s; HRMA, high resolution melting analysis; LOS, length of hospital stay; MAF, minor allele frequency; PCR, polymerase chain reaction; RSV, respiratory syncytial virus

ABSTRACT

Background: Interleukin-17 (IL-17A) is a mainly pro-inflammatory cytokine, and IL-17 signaling implicates in the development of allergic asthma. The polymorphism rs2275913 in the promoter region of the *IL-17A* gene has in previous studies been associated with asthma susceptibility. The objective was to evaluate the association between *IL-17A* rs2275913 (-197G>A) polymorphism and post-bronchiolitis asthma and/or allergic rhinitis in a prospective 11–13 years post-bronchiolitis follow-up.

Methods: 166 previously healthy full-term infants, hospitalized for bronchiolitis at age less than 6 months, were invited to follow-up visits at the ages of 5–7 years and 11–13 years. Asthma diagnoses and presumptive symptoms, allergic rhinitis and use of inhaled corticosteroids (ICS) were registered. Blood samples for *IL-17A* rs2275913 (-197G>A) polymorphism were obtained during hospitalization or at the 5–7 years control visit.

Results: There were no significant differences between children with the wild GG and variant GA or AA genotype in the severity of bronchiolitis during hospitalization or in the outcomes until the age 5–7 years. At 11–13 years of age, children with the variant GA or AA genotype had significantly less often current asthma, use of ICSs during last 12 months or allergic rhinitis than those with the wild GG genotype. The ICS use during last 12 months retained the statistical significance in adjusted analyses (adjusted OR 0.25), whereas current asthma and allergic rhinitis marginally lost it.

Conclusions: The *IL-17A* rs2275913 (-197G>A) polymorphism decreased the risk of post-bronchiolitis asthma at 11–13 years of age, but not earlier in life, in the present prospective, long-term follow-up study.

Introduction

Asthma constitutes a global pediatric problem, and genetic susceptibility seems to play an important role in its development.[1] Inhaled corticosteroids (ICS) for maintenance and bronchodilators for exacerbations are the established drugs for asthma treatment. Allergic rhinitis frequently co-exists with asthma and may even share a common genetic basis.[2]

Bronchiolitis is usually caused by respiratory syncytial virus (RSV) being characterized by virus-induced inflammation of the bronchioles.[3] Infants hospitalized with bronchiolitis are at increased risk of both recurrent wheezing and childhood asthma.[3] Allergic children may present with impaired antiviral responses leading to more pronounced inflammation during respiratory infection and further to higher risk of subsequent wheezing.[4]

The interleukin-17 (IL-17) family contains six members, and among them IL-17A and IL-17F share the highest protein sequence homology.[5] The IL-17 family members are pro-inflammatory cytokines predominantly secreted by T helper 17 (Th17) cells.[6] In addition, there is evidence that IL-17A induces local Th17 cell production in RSV bronchiolitis and some autoimmune diseases.[7,8]

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Th17 cells contribute to the accumulation of both eosinophils and neutrophils in the tissue, and IL-17A may have an impact on the balance between eosinophilic and neutrophilic inflammation in the airways.[9,10] Thus, the *IL-17A* gene is a potential candidate gene for post-bronchiolitis asthma susceptibility.

We have previously followed-up 166 children hospitalized for bronchiolitis at less than 6 months of age. The control visits were organized at 5–7 years[11] and 11–13 years of ages. Blood samples for genetic studies were obtained during hospitalization and at the 5–7 years visits.[12] In previous publications from this post-bronchiolitis cohort, *IL-10* polymorphism was associated with rhinovirus etiology of bronchiolitis,[13] atopy and asthma at pre-school age,[14] and asthma and lung function reduction at early school age.[15]

The aim of this study was to evaluate the associations between *IL-17A* rs2275913 (-197G>A) polymorphism and presence of asthma, use of asthma medication or presence of allergic rhinitis at 1.5 years, 5–7 years and 11–13 years of ages after hospitalization for bronchiolitis at age less than 6 months.

Methods

Design

During two study periods in 2001–2004, 187 eligible, previously healthy, full-term infants aged less than 6 months were hospitalized due to bronchiolitis in the department of Pediatrics, Tampere University Hospital, Finland.[13] Clinical data, collected during hospitalizations or at control visits were available from 166 patients,[11] and blood samples for genetic studies were available from 165 patients.[14]

Bronchiolitis was defined as lower respiratory infection associated with diffuse wheezes and/or crackles.[13] Viral etiology of bronchiolitis was studied with antigen detection and polymerase chain reaction (PCR) in nasopharyngeal aspirates.[13] Data on disease severity, like need of supplementary oxygen and feeding support, and length of hospital stay (LOS) were recorded during the inpatient care.[13] Information on atopic dermatitis were registered at the post-bronchiolitis control visit at 1.5 years of age.[16]

The children hospitalized for bronchiolitis were later invited to two follow-up visits. The first was arranged in 2008–2009 when the children were 5–7 years old.[11] Before the visit the parents completed a structured questionnaire comprising questions on doctor-diagnosed asthma and allergic rhinitis, and previous or current use of asthma medication and symptoms presumptive for asthma. The follow-up study included an interview of parents to check the questionnaire data, and bronchial hyper-reactivity was studied in children by an exercise challenge test with impulse oscillometry.[11] In addition, data were collected on allergic rhinitis and atopic dermatitis, and on use of corticosteroids and bronchodilators.

The second follow-up visit was arranged in 2014–2015 when the children were 11–13 years old. Before the visit the parents completed a structured questionnaire comprising questions on doctor-diagnosed asthma and allergic rhinitis, and on current use of asthma medication and symptoms presumptive for asthma, from the control visit at 5–7 years of age to the present. The follow-up also included interview of children and parents to check the questionnaire data, and a bronchodilation test. The best FEV1 (forced expiratory volume in 1 s) of three blows before and 15 min after the inhalation of 400 μg salbutamol (Ventolin Evohaler 0.1 mg/dos, GlaxoSmithKline, London, UK), measured with flow-volume spirometry (Vmax® V62J Autobox, Becton, Dickinson, NJ, USA) were analyzed. An increase of 12% or more in FEV1 was regarded as a positive test result meaning a reversible airway obstruction.

Definitions

At the control visit at age 5–7 years current asthma was defined as a continuous or intermittent ICS use for asthma during preceding 12 months, or alternatively, as reporting of doctor-diagnosed episodes of wheezing, prolonged cough or night cough during the preceding 12 months and a diagnostic finding in the exercise challenge test.[11] The parent-reported use of bronchodilators and doctor-diagnosed allergic rhinitis and atopic dermatitis were recorded if symptomatic during the past 12 months.[11]

At the control visit at age 11–13 years, current asthma was considered if the child had used ICSs continuously during the last 12 months, or alternatively, if the child had suffered from repeated wheezing or prolonged cough or night cough for four or more weeks during the last 12 months and in addition had a diagnostic increase of FEV1 in the bronchodilation test. The parent-reported use of bronchodilators and doctor-diagnosed allergic rhinitis and atopic dermatitis were recorded for the past 12 months.

Persistent asthma was defined as the presence of asthma at both the 5–7 years and 11–13 years follow-up studies.

Genetics

Genotyping of extracted DNA for the *IL-17A* rs2275913 (-197G>A) gene was performed by high resolution melting analysis (HRMA) (Roche Diagnostics Light Cycler 480, Basel, Switzerland).[17] HRMA PCR reactions were run at 95 °C for 10 min followed by 45 cycles amplification at 95 °C for 10 s, at 59 °C for 10 s and at 72 °C for 15 s. After PCR process final melting cycle conditions were as outlined by Roche: first heating to 95 °C and hold for 1 min, and cooling to pre-hold temperature (40 °C) to make sure that all PCR products have re-associated. In each run, known (sequenced) *IL-17* rs2275913 standards (wild GG type, and heterozygous variant GA and homozygous variant AA types) were used as controls.

Controls

The IL17A rs2275913 genotypes were determined in 405 controls recruited from a study called Steps to children's healthy development and wellbeing (Steps), which is a prospective birth cohort study of 1827 children. The control group comprised Finnish infants aged two to three months, who had not yet been vaccinated (except for oral rotavirus vaccine), who were healthy, ethnic Finns, and who visited the study clinic from 2008 to 2010.[18] In controls, genotyping from extracted DNA was performed by Sequenom massARRAY iPlex Gold system (Sequenom, CA, USA) in the University of Eastern Finland, Kuopio, Finland.[18]

Ethics

The study was carried out in accordance with the WMA Declaration of Helsinki. We obtained informed parental consent, including the use of samples for genetic studies on bronchiolitis and asthma risk both during hospitalization and at the control visits. The protocol of the study was approved by the Ethics committee of the Tampere University Hospital district, Tampere, Finland. The personal data of the study subjects were not given to the laboratory that performed the genetic studies, the Department of Medical Microbiology and Immunology, Turku, Finland.

Statistics

Statistical analyses were performed using the Statistic Package of Social Science (SPSS23.0, IBM, NY, USA). Chi square and Fisher's exact tests when appropriate, were used in the analyses of categorized variables. Student's t-test was used for normally distributed and Mann–Whitney test for non-normally distributed continuous variables. The results were expressed as frequencies, proportional frequencies, medians, means and standard deviations.

Logistic regression was used to evaluate the wild GG and variant GA or AA genotypes as risk factors for the outcomes at 5–7 years and 11–13 years of ages, first as non-adjusted, and then as adjusted for current age (continuous), sex, RSV positivity during bronchiolitis and atopic dermatitis at less than 12 months of age. The results were expressed as odds ratios (OR) and 95% confidence intervals (95% CI).

Results

Basic data

The mean age of the 165 bronchiolitis patients was 10.71 weeks (SD 6.82) during hospitalization, and 84 (50.6%) were boys. The wild genotype GG of *IL17A* rs2275913 was present in 48 (29.1%) children and the variant genotype in 117 (70.9%): GA in 87 (52.7%) and AA in 30 (18.2%) cases without any significant differences compared to controls (Table 1). The minor (A) allele frequency (MAF) was 0.45 (147/330) in the 165 bronchiolitis patients and 0.38 in controls ($p = 0.36$).

Hospitalization data

RSV was the causative agent in 113 (68.5%) cases. RSV was found in 33 (68.8%) cases if the child had the wild GG and in 81 (69.2%) if the child had the variant GA or AA genotype ($p = 0.95$). The mean LOS was 4.48 days (SD 3.19, range 0–22), being 4.61 days in children with wild GG and 3.97 days in children with variant GA or AA genotypes ($p = 0.32$).

There were no significant differences in the severity markers of bronchiolitis, including the need and duration of oxygen administration, or the need and duration of feeding support, between the children with wild GG and variant GA or AA genotypes (Data not shown).

Follow-up data at age 5–7 years

The wild genotype GG was present in 40 (28.6%) children and the variant genotype in 100 (71.4%): GA in 75 (53.6%) and AA in 25 (17.8%) cases. There were no significant differences in any wheezing, asthma or allergy parameters between children with wild GG and variant GA or AA genotypes (Table 2).

Follow-up data at age of 11–13 years

The wild genotype GG was present in 34 (27.4%) children and the variant genotype in 90 (72.6%): GA in 67 (54.0%) and AA in 23 (18.6%) cases. In non-adjusted analyses, children with the variant GA or AA genotype had significantly less often current asthma, or current allergic rhinitis, and reported less often use of ICSs in the last 12 months, compared with those with the wild GG genotype (Table 3).

In adjusted analyses, use of ICSs in the last 12 months (aOR 0.25) retained statistical significance, but current asthma, current allergic rhinitis and use of bronchodilators in last 12 months marginally lost it (Table 3).

Discussion

There are three main results in the present prospective, long-term follow-up study on the association of *IL-17A* rs275913 polymorphism with disease severity and development of asthma or allergic rhinitis in children hospitalized for bronchiolitis at age less than 6 months. First, we did not find any significant associations between the studied polymorphism and bronchiolitis severity during hospitalization or post-bronchiolitis outcome until 5–7 years of age. Second, we found a significant association between the studied polymorphism and asthma at the age of 11–13 years; asthma by different definitions was less common in those who had the variant GA or AA genotype of *IL-17A* rs2275913 polymorphism. The ICS use for asthma during the last 12 months remained as statistically significant in adjusted analyses. Third, we found some evidence for an association between the studied *IL17A* polymorphism and allergic rhinitis at 11–13 years of age.

The single nucleotide polymorphism (SNP) of *IL-17A* rs2275913 (-197G>A) in the promoter region of the *IL-17A* gene was selected

Table 1
Genotypes of IL17A rs2275913 polymorphisms in 165 children hospitalized for bronchiolitis at less than 6 months of age and in 405 population-based controls.

Genotypes	Bronchiolitis N = 165 (%)	Controls N = 405 (%)	*p* value vs. the wild GG genotype
Wild GG	48 (29.9)	146 (36.0)	–
Variant GA	87 (52.5)	181 (44.7)	0.072
Variant GA	30 (18.2)	78 (19.3)	0.563
Variant all, GA or AA	117 (70.9)	259 (64.0)	0.118
MAF	147/330 (44.5)	337/810 (38.3)	0.362

Distribution of three genotypes: $p = 0.187$ between bronchiolitis cases and controls.

Table 2
Clinical characteristic at the 5–7 years follow-up in relation to presence of the wild vs. variant genotype of IL-17A rs2275913 polymorphism.

Clinical characteristic	Wild GG genotype n = 40 (%)	Variant GA or AA genotype n = 100 (%)	*p* value	OR† (95% CI) for the variant genotype
Current asthma N = 19	8 (20.0)	11 (11.0) GA = 8, AA = 3	0.16	
Bronchodilators in last 12 months N = 26	11 (27.5)	15 (15.0) GA = 10, AA = 5	0.09	0.47 (0.19–1.13)
Inhaled steroids in last 12 months N = 18	8 (20.0)	10 (10.0) GA = 8, AA = 2	0.12	0.44 (0.16–1.22)
Allergic rhinitis in last 12 months N = 40	12 (30.0)	28 (28.0) GA = 20, AA = 8	0.81	0.91 (0.41–2.03)
Atopic dermatitis in last 12 months N = 42	13 (32.5)	29 (29.0) GA = 22, AA = 7	0.68	0.85 (0.39–1.87)

† aOR = OR adjusted for age, sex, RSV and atopy at age less than 12 months.

Table 3
Clinical characteristic at the 11–13 years follow-up in relation to presence of wild vs. variant genotype of IL-17A rs2275913 polymorphism.

Clinical characteristic	Wild GG genotype n = 34 (%)	Variant GA or AA genotype n = 90 (%)	*p* value	OR (95% CI) for the variant genotype	aOR[†] (95% CI) for the variant genotype
Current asthma N = 15	8 (23.5)	7 (7.8) GA = 7, AA = 0	**0.02**	**0.27 (0.09–0.83)**	0.32 (0.10–1.04)
Persistent asthma N = 9	5 (14.7)	4 (4.4) GA = 4, AA = 0	0.05	0.27 (0.07–1.07)	0.21 (0.04–1.02)
Inhaled steroids in last 12 months N = 11	6 (17.6)	5 (5.6) GA = 5, AA = 0	**0.04**	**0.28 (0.08–0.97)**	**0.25 (0.06–0.97)**
Bronchodilator in last 12 months N = 28	12 (35.3)	16 (17.8) GA = 11, AA = 5	**0.04**	**0.40 (0.16–0.96)**	0.44 (0.18–1.11)
Allergic rhinitis N = 19	9 (26.5)	10 (11.1) GA = 6, AA = 4	**0.03**	**0.35 (0.13–0.95)**	0.40 (0.13–1.22)
Atopic dermatitis N = 31	9 (26.5)	29 (29.0) GA = 22, AA = 7	0.82	0.90 (0.37–2.21)	0.95 (0.36–2.49)

The bold values represent that *p* values are significant at level <0.05.
[†] aOR = OR adjusted for age, sex, RSV and atopy at age less than 12 months.

based on previous studies, which suggested an association between that SNP and childhood asthma,[5,8,19] or between that SNP and asthma plus allergy.[20,21] The influence of different *IL-17A* variants on IL-17A production is still unknown, as is also the impact of the rs2275913 variant. In cell cultures, T cells from healthy individuals possessing the variant A allele of *IL-17A* rs2275913 produced more IL-17 than those without the A allele.[22] However, the result was opposite in the most recent study from Finland in 93 healthy infants aged 13 months.[18] Serum IL-17 was detectable in 6% of the variant AA homozygotes, in 33% of the variant GA heterozygotes, and in 75% of the wild GG homozygotes.

Dutch researchers combined clinical data from tracheal aspirates of bronchiolitis patients and experimental data from human cell cultures and an animal model of RSV infection and concluded that early local IL-17A production in the airways during RSV bronchiolitis facilitates neutrophil recruitment leading to neutrophilic inflammation in infant lungs.[23] Bronchiolitis, preschool asthma and severe asthma later may be merely associated with neutrophilic than eosinophilic inflammation.[24–26] IL-17A is, because of promoting neutrophil mobilization and further neutrophilic inflammation,[1,8] involved in host defense against bacteria, and possibly, also against viruses.[9]

The variant *IL-17A* genotypes were associated with a decreased risk of childhood asthma in a Tunisian case–control study,[5] which is in agreement with our results. In disagreement, the variant genotypes of *IL17A* genes were associated with an increased asthma and allergy risk in different populations,[19–21,27] including a study made in Chinese children.[8] The differences in the direction of the effect of the *IL-17A* rs2275913 SNP between our results and some other studies may be due to two reasons: first, simply ethnically different populations, or second, our selected study group with bronchiolitis in early infancy.

The prevalence of asthma is 7–9% in the child population at school age in Finland.[28] The 23.5% prevalence of current asthma was 3–4-fold in children with the wild GG *IL-17A* rs2275913 genotype and about the same (7.8%) in children with the variant GA or GG genotype in the present study. Since the polymorphism was not associated with wheezing or asthma until 5–7 years of age, the presence of the variant *IL-17A* rs2275913 genotype seemed to promote the natural tendency that many children with early-childhood asthma outgrow wheezing symptoms at school age. Evidently, asthma at preschool age and in adolescence have different risk factors, factors of innate immunity as an example.

There were certain limitations in the present study. The small number of patients means a risk of type-2 statistical error. On the other hand, we had a clear result that asthma risk was decreased at 11–13 years of age in those with variant *IL-17A* rs2275913 polymorphism, and we were able to confirm this result in analyses adjusted for relevant early-life confounding factors. Thus, the power of the study was sufficient for the conclusion that the variant-type *IL-17A* rs2275913 polymorphism is protective for post-bronchiolitis asthma in adolescence.

In conclusion, *IL-17A* rs2275913 polymorphism did not play any significant role in bronchiolitis that required hospitalization at less than 6 months, or in the post-bronchiolitis outcome until 5–7 years of age. Instead, the variant genotype of *IL-17A* rs2275913 polymorphism GA or AA was a significant protective factor for asthma in schoolchildren from the age 7 to 13 years.

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Conflict of interest
The authors have no conflict of interest to declare.

Authors' contributions
AH participated in data analyses and had responsibility for writing the manuscript. JT and QH had responsibility for the genetic analyses and they participated in writing the manuscript. EL, ST, PK and MH participated in the protocol development, patient screening and writing the manuscript. VP had responsibility for enrolling the control patients, arranging the laboratory analyses, and he participated in writing the manuscript. MK and KN had responsibility for protocol development, patient screening, data analysis and writing the manuscript. All authors read and approved the final manuscript.

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Note: there is an error in Table 1 where it should be 'variant AA' in the third row.

PUBLICATION
II

***IL17A* gene polymorphisms rs4711998 and rs8193036 are not associated with postbronchiolitis asthma in Finnish children.**

Nuolivirta K, Holster A, Teräsjärvi J, Lauhkonen E, Törmänen S, Helminen M, Koponen P, Korppi M, He Q.

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ACTA PÆDIATRICA
NURTURING THE CHILD

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BRIEF REPORT

IL17A gene polymorphisms rs4711998 and rs8193036 are not associated with postbronchiolitis asthma in Finnish children

Bronchiolitis is the most common cause of hospitalisation in infants with no severe underlying illness and bronchiolitis in infancy may lead to childhood asthma. There is no doubt that genetic susceptibility plays a role in this process. Allergic rhinitis is common in children with asthma, and asthma and allergies may even share a common genetic basis.

The IL-17 family members are pro-inflammatory cytokines predominantly secreted by T helper 17 (Th17) cells, and the IL-17A cytokine plays a role in asthma pathogenesis (1). Our prospective postbronchiolitis follow-up study revealed that *IL17A* rs2275913 single nucleotide polymorphism (SNP) was associated with postbronchiolitis asthma at 11–13 years of age, when the variant genotype and the minor allele were protective from asthma, but not at five to seven years of age or earlier (2).

The rs4711998 and rs8193036 SNPs of the *IL17A* gene were selected for this study, as the rs4711998 SNP was associated with asthma in Taiwanese children (3). We are not aware of any earlier studies on *IL17A* rs4711998 or rs8193036 polymorphisms in bronchiolitis or postbronchiolitis asthma or allergy.

IL17A rs8193036 polymorphism is functional by promoting DNA methylation and mRNA and protein expression in white blood cells (4), but no corresponding data are available for the functionality of *IL17A* rs4711998 polymorphism.

A meta-analysis on the impact of *IL17A* gene polymorphisms in asthma (5) comprised nine studies covering 3650 asthmatics and 3370 controls. This found that the *IL17A* rs4711998 SNP, which was included in our study, was a risk factor for asthma.

This study followed up a cohort of 187 previously healthy, full-term infants who were

Table 1 Findings on asthma and allergy at 11–13 years of age after hospitalisation for bronchiolitis at less than six months of age, presented in relation to *IL17A* rs4711998 and rs8193036 genotypes

***IL17A* rs4711998** **N = 123**	**GG (wild)** **N = 64**	**GA (variant)** **N = 51**	**AA (variant)** **N = 8**
Current asthma (N = 15)	5 (7.8%) p = 0.14*	9 (17.6%)	1 (12.5%) p = 0.66†
Persistent asthma (N = 9)	4 (6.3%) p = 0.24*	4 (10.7%)	1 (12.5%) p = 0.47†
Use of inhaled corticosteroids and bronchodilators in last 12 months (N = 11)	4 (6.3%) p = 0.24*	6 (10.7%)	1 (12.1%) p = 0.54†
Use of bronchodilators in last 12 months (N = 27)	14 (21.9%) p = 0.56*	11 (19.6%)	2 (25.0%) p = 0.56†
Allergic rhinitis symptoms in last 12 months (N = 19)	6 (9.5%) p = 0.05* p = 0.024¶	10 (17.9%)	3 (33.3%) p = 0.11†
***IL17A* rs8193036** **N = 122**	**TT (wild)** **N = 45**	**TC (variant)** **N = 59**	**CC (variant)** **N = 18**
Current asthma (N = 15)	8 (17.8%) p = 0.16‡	5 (8.5%)	2 (10.5%) p = 0.81§
Persistent asthma (N = 9)	5 (11.1%) p = 0.23‡	2 (3.4%)	2 (11.1%) p = 0.40§
Use of inhaled corticosteroids and bronchodilators in last 12 months (N = 11)	6 (13.3%) p = 0.20‡	3 (5.1%)	2 (11.1%) p = 0.51§
Use of bronchodilators in last 12 months (N = 28)	13 (28.9%) p = 0.23‡	12 (20.3%)	3 (16.7%) p = 0.40§
Allergic rhinitis symptoms in last 12 months (N = 19)	7 (15.6%) p = 1.00‡	10 (16.9%)	2 (10.5%) p = 0.44§

*p vs GA+AA.
†p vs GA+GG.
‡p vs TC+CC.
§p vs TC+TT.
¶p = 0.024 GG vs GA vs AA.

hospitalised for bronchiolitis at less than six months of age in 2001–2004 at Tampere University Hospital, Finland. We evaluated the associations of *IL17A* rs4711998 and rs8193036 polymorphisms with asthma, asthma medication and allergic rhinitis when they were 5–7 (n = 139) and 11–13 (n = 124) years of age (2).

The respiratory syncytial virus (RSV) was studied with antigen detection and polymerase chain reaction in nasopharyngeal aspirates obtained during hospitalisation in infancy.

Before the follow-up visits, the parents completed a structured postal questionnaire that covered doctor-diagnosed asthma, allergic rhinitis and previous or current use of asthma medication, like bronchodilators and inhaled corticosteroids (ICS), and symptoms presumptive for asthma (2). The parents were interviewed to check the questionnaire data. Bronchial hyper-responsiveness was studied in children with an exercise challenge test using impulse oscillometry at five to seven years, and the irreversibility of bronchial obstruction was studied with a bronchodilation test using flow-volume spirometry at 11–13 years.

At the control visits, current asthma was defined as continuous or intermittent ICS use for asthma during the preceding 12 months or as the presence of repeated doctor-diagnosed wheezing episodes, prolonged cough during the preceding 12 months and diagnostic findings in the exercise challenge or bronchodilation tests (2). Persistent asthma was defined as the presence of asthma at both of the five to seven and 11–13 years control visits. Allergic rhinitis had to be diagnosed by a doctor and symptomatic during the preceding 12 months.

High-resolution melting analysis (HRMA) was used to genotype 164 former bronchiolitis patients for the *IL17A* rs4711998 SNP and 162 patients for the *IL17A* rs8193036 SNP. The use of this method genotyping for the *IL17A* rs2275913 and rs8193036 SNPs has previously been published (4). The Ethics Committee of the Tampere University Hospital district approved the study protocol, including the genetic sampling and examinations.

We found that 84 of 166 (50.6%) bronchiolitis patients were boys, and RSV was the causative agent in 114 (68.7%) cases. There were no significant differences in RSV aetiology, the length of hospital stay or the need for feeding or oxygen support between the children with *IL17A* rs4711998 GG, GA and AA genotypes or with *IL17A* rs819 3036 TT, TC and CC genotypes (data not shown).

At five to seven years, 19 of 139 (13.7%) children had current asthma and 39 (28.1%) had allergic rhinitis, 18 (12.9%) children had used ICSs and bronchodilators and 26 (18.7%) had used bronchodilators in the last 12 months. There were no significant differences in asthma or allergy findings between the 81 children with *IL17A* rs4711998 GG, 74 with GA and nine with AA genotypes or between the 52 children with *IL17A* rs8193036 TT, 68 with TC and 19 with CC genotypes (data not shown).

At 11–13 years, 15 of 124 (12.2%) children had current asthma, nine (7.3%) had persistent asthma, 19 (15.4%) had allergic rhinitis, 11 (8.9%) had used ICSs and bronchodilators and 27 (22.0%) had used bronchodilators in last 12 months. There were no significant differences in any asthma or allergy findings between the 64 children with *IL17A* rs4711998 GG, 51 with GA and eight with AA genotypes or between the 45 children with *IL17A* rs8193036 TT, 59 with TC and 18 with CC genotypes (Table 1). The *IL17A* rs4711998 SNP was associated with the presence of allergic rhinitis in 9.5% of those with the homozygous GG genotype, 17.9% with the heterozygous GA genotype and 33.3% with the homozygous AA genotype ($p = 0.024$).

The result was clearly negative for the association of *IL17A* rs4711998 and rs8193 036 polymorphisms with the severity of bronchiolitis at less than six months of age and postbronchiolitis asthma at 11–13 years of age. The *IL17A* rs4711998 polymorphism was associated with allergic rhinitis.

FINANCE

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CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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PUBLICATION III

***IL17F* rs763780 single nucleotide polymorphism is associated with asthma after bronchiolitis in infancy.**

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REGULAR ARTICLE

ACTA PÆDIATRICA NURTURING THE CHILD WILEY

IL17F rs763780 single nucleotide polymorphism is associated with asthma after bronchiolitis in infancy

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Abstract

Aim: Interleukin-17F (IL-17F) is involved with asthma. The aim of this study was to evaluate the association of *IL17F* polymorphisms with childhood asthma after bronchiolitis in infancy.

Methods: We invited 166 children who were hospitalised for bronchiolitis at younger than 6 months of age to follow-up visits at 5-7 years and 11-13 years of ages. Asthma and allergy diagnoses, asthma-presumptive symptoms and use of inhaled corticosteroids (ICSs) were registered. Blood samples were available for *IL17F* rs763780 (T/C), rs11465553 (C/T) and rs7741835 (C/T) determinations in 165 cases.

Results: The presence of *IL17F* rs11465553 and rs7741835 variations showed no significant associations with any asthma or allergy outcome at either 5-7 years or 11-13 years of ages. Instead, children with the variant *IL17F* rs763780 genotype had used more often ICSs between the follow-up visits from 5-7 to 11-13 years (adjusted OR 3.58) than those with the wild genotype. Children with the variant *IL17F* rs763780 genotype reported more often doctor-diagnosed atopic dermatitis (adjusted OR 2.71) at 11-13 years of age than those with the wild genotype.

Conclusion: This prospective long-term follow-up study provided preliminary evidence on the association of the *IL17F* rs763780 polymorphism with asthma at school age after bronchiolitis in infancy.

KEYWORDS

atopic dermatitis, bronchiolitis, childhood asthma, Interleukin-17F, single nucleotide polymorphism

Abbreviations: HWE, Hardy-Weinberg equilibrium; ICS, inhaled corticosteroid; IL-17F, Interleukin-17F; MAF, minor allele frequency; OR, odds ratio; PCR, polymerase chain reaction; RSV, respiratory syncytial virus; SNP, single nucleotide polymorphisms; Th, T-helper.

1 | INTRODUCTION

Asthma is a multifactorial respiratory disease with a strong genetic component in its pathogenesis.[1] Allergic rhinitis frequently co-exists with asthma,[2] and atopic dermatitis is often linked with allergic rhinitis and allergic asthma.[3] T-helper (Th) 17 cells secrete the interleukin-17 (IL-17) cytokines, such as IL-17A to IL-17F, which are encoded by the *IL17A* and *IL17F* genes, respectively.[4,5] IL-17F is a pro-inflammatory cytokine that is involved with asthma.[16] The *IL17F* gene is located in a genomic region, which has been linked to asthma and asthma-related respiratory disorders in multiple-genome scans.[7] The *IL17F* gene was expressed in the airways of asthmatic adults,[8,9] and this expression was correlated with IL-17F production[9] and associated with asthma severity.[8]

Previous studies have documented an association between single nucleotide polymorphisms (SNPs) of the *IL17F* gene and the susceptibility to autoimmune and inflammatory diseases[10] including asthma in different populations.[9,11-13]

We have followed up 166 children hospitalised for bronchiolitis at <6 months of age. The control visits were arranged when the children were 5-7 years[14] and 11-13 years of ages.[15] Blood samples for genetic studies were obtained during hospitalisations in infancy and at the subsequent control visits.[16] The aim of this study was to evaluate the association of the *IL17F* rs763780, rs11465553 and rs7741835 polymorphisms with presence of asthma, use of asthma medication, and presence of allergic rhinitis and atopic dermatitis at 5-7 years and 11-13 years of ages after hospitalisation for bronchiolitis at younger than 6 months of age.

Key Notes

- Interleukin-17F, a pro-inflammatory cytokine encoded by the *IL17F* gene, is involved with asthma.
- In our post-bronchiolitis cohort, *IL17F* rs763780, rs11465553 or rs7741835 variations showed no significant associations with asthma at 5-7 or 11-13 years, but children with *IL17F* rs763780 variant genotypes, compared to wild genotypes, used more often ICSs for asthma between these ages (adjusted odds ratio 3.58).
- *IL17F* rs763780 variation may increase the risk of post-bronchiolitis asthma.

2 | MATERIAL AND METHODS

2.1 | Design

In 2001-2004, 187 previously healthy, full-term infants aged <6 months were hospitalised for bronchiolitis in the Department of Pediatrics, Tampere University Hospital, Finland.[17] Bronchiolitis was defined as lower respiratory infection associated with diffuse wheezes and/or crackles.[17,18] Viral aetiology of bronchiolitis, including respiratory syncytial virus (RSV), was studied with antigen detection and polymerase chain reaction (PCR) in nasopharyngeal aspirates.[17] The disease severity markers, such as the need and duration of oxygen administration, the need and duration of feeding support, or the length of hospital stay were registered during hospitalisation for bronchiolitis.[17]

Later, the children were invited to attend two follow-up visits. The first visit was arranged in 2008-2009 when the children were 5-7 years old[14] and the second visit in 2014-2015 when the children were 11-13 years old.[15] Before these visits, the parents completed a structured questionnaire comprising questions on doctor-diagnosed asthma, atopic dermatitis and allergic rhinitis, on symptoms presumptive for asthma, and on previous or current use of asthma medication. At the second follow-up visit, the questionnaire covered the period from the visit at 5-7 years of age to the visit at 11-13 years of age. Both follow-up studies included an interview of parents to check the questionnaire data. At the 5-7 years visit, bronchial hyper-reactivity was studied with an exercise challenge test using impulse oscillometry[19] and at the 11-13 years visit, bronchodilation test was done using flow-volume spirometry.[15] Current asthma was defined as continuous or periodical regular use of inhaled corticosteroids (ICS) for asthma during the preceding 12 months, or alternatively, as reporting of doctor-diagnosed episodes of wheezing, or prolonged cough or night cough for four or more weeks, during the preceding 12 months and in addition, as having a diagnostic finding in the exercise challenge test at the first or in the bronchodilation test at the second follow-up visit.[14] Persistent asthma was defined as the presence of asthma at both the 5-7 years and 11-13 years follow-up visits.[15]

Appropriate clinical data and the data on the *IL17F* rs763780, rs11465553 and rs7741835 polymorphisms were available in 165 patients hospitalised for bronchiolitis in infancy, and in 142 attending the 5-7 years and in 125 attending the 11-13 years follow-up study.

2.2 | Genetics

Capillary sequencing was used for the detection of the *IL17F* rs763780 and rs11465553 alleles. The primers designed to amplify the 156 bp area of interest were as follows: (forward) 5´-TTG CAG AGC ACT GGG TAA GG-3 and (reverse) 5´-ACC AAG GCT GCT CTG TTT CT −3 which were used for polymerase chain reaction (PCR) prior the sequencing. The primers were purchased from Sigma-Aldrich (Merck KGaA). Sequencing of the PCR product was done at the Institute for Molecular Medicine Finland laboratories, Helsinki, Finland.

High-resolution melting analysis (HRMA) was used to detect the *IL17F* rs7741835 alleles. HRMA was performed by LightCycler480 version 5.1 (Roche) with SensiFAST HRMA melting master kit (Bioline, London, UK). Primers for the SNP were designed with Primer-Blast design tool (http://www.ncbi.nlm.

nih.gov/tools/primer-blast/) and were purchased from Sigma-Aldrich Company. High-performance liquid chromatography (HPCL) quality primers used in the HRMA analysis were as follows: forward 5´- CAGCATCTAGCTTGTTCGCA −3´ and reverse 5´- ATGCAGCCTGATTGAGTAGGTT −3. In each run, known *IL17F* rs7741835 standards (wild, and heterozygous and homozygous variants) were used.

2.3 | Statistics

Statistical analyses were performed using the Statistic Package of Social Science (SPSS 25.0, IBM Corp). Chi-square and Fisher's exact tests, when appropriate, were used in the analyses of categorised variables. Logistic regression was used to evaluate the genotypes as risk factors for the outcomes at 5-7 years and 11-13 years of ages as adjusted for current age (continuous), sex and RSV positivity during bronchiolitis. The results were expressed as adjusted odds ratios (OR) and 95% confidence intervals (95% CI).

We compared the frequencies of all three studied *IL17F* SNPs between the original cohort of 166 bronchiolitis patients and 99 Finnish population-based controls from the 1000 Genome Project[20] The minor allele frequencies (MAFs) or genotype frequencies of the *IL17F* rs763780 and rs11465553 did not differ between cases and controls. Instead, the wild *IL17F* rs7741835 genotype was less common in cases than in controls (54.8% vs 67.7%, $P = 0.04$).

Deviations from the Hardy-Weinberg equilibrium (HWE) were studied with the HWE Calculator[21] and the alleles of the three SNPs were in the HWE.

2.4 | Ethics

The study was approved by the Ethics committee of the Tampere University Hospital district, Tampere, Finland. We obtained informed parental consent, including the use of samples for genetic studies on bronchiolitis and asthma risk both during hospitalisation and at the control visits. The personal data of the study subject were not given to the laboratories that performed the genetic studies, the Institute of Biomedicine, Turku, Finland and the Institute for Molecular Medicine Finland laboratories, Helsinki, Finland.

3 | RESULTS

3.1 | Data during hospitalisation

The mean age of the 165 bronchiolitis patients was 10.71 weeks (SD 6.82) during hospitalisation, and 84 (50.6%) were boys, as previously published.[22] The wild genotype TT of the *IL17F* rs763780 was present in 128 (77.6%), the wild genotype CC of the *IL17F* rs11465553 in 153 (92.7%) and the wild genotype CC of the *IL17F* rs7741835 in 90 (54.5%) children. The respective figures for the variant genotypes were 22.4% (rs763780, TC 36 and CC 1), 7.3% (rs11465553, CT 12 and TT 0), and 45.5% (rs7741835, CT 69 and TT 6).

RSV was the causative agent in 113 (68.5%) cases. RSV was found in 90 (70.3%) cases if the child had the wild TT and in 23 (62.2%) if the child had the variant genotype ($P = 0.35$) of the *IL17F* rs763780. The respective figures of RSV positivity were 104 (68.0%, wild CC) versus 9 (75.0%, variants, $P = 0.61$) for the *IL17F* rs11465553 and 60 (66.7%, wild CC) versus 53 (70.1%, variants, $P = 0.58$) for the *IL17F* rs7741835.

There were no significant differences in the severity markers of bronchiolitis, including the need and duration of oxygen administration, the need and duration of feeding support, or the length of hospital stay between children with wild and variant genotypes of the *IL17F* rs763780, rs11465553 or rs7741835 (Data not shown).

3.2 | Follow-up data at age of 5-7 years

The variant *IL17F* rs763780 genotype was present in 32 (22.5%), the variant *IL17F* rs11465553 genotype in 11 (7.7%) and the variant *IL17F* rs7741835 genotype in 60 (42.3%) children. The parents reported wheezing in 21.1% and bronchodilator use in 18.3% of the children. Nineteen (13.4%) children had doctor-diagnosed current asthma, and all but one of them used ICSs. The parents reported allergic rhinitis in 28.2% and atopic dermatitis in 29.6%. There were no significant differences in any of these wheezing, asthma or atopy parameters between children with wild versus variant genotypes of the three *IL17F* SNPs. (Table 1).

3.3 | Follow-up data at age of 11-13 years

The variant *IL17F* rs763780 genotype was present in 27 (21.6%), the variant *IL17F* rs11465553 genotype in 9 (7.2%) and the variant *IL17F* rs7741835 genotype in 51 (40.8%) children (Table 2). Doctor-diagnosed current asthma was present in 15 (12.0%) of children; 73.3% of them had used ICSs during the preceding 12 months, and 60.0% had doctor-diagnosed asthma already at the 5-7 years study (persistent asthma). In all, 12.8% of children had used ICSs at the time from 5-7 years to 11-13 years of age. Nearly, half, 44.0%, of children reported symptoms suggesting allergic rhinitis, but only 15.2% of the children had doctor-diagnosed allergic rhinitis. A fourth, 24.8%, reported doctor-diagnosed atopic dermatitis (Table 2).

Children with the variant *IL17F* rs763780 genotype presented with two significant findings (Table 2). They had used more often ICSs from the 5-7 years to the 11-13 years follow-up with adjusted OR of 3.58 (95% CI 1.11-11.5) than those with the wild genotype. The adjusted OR was 3.49 for ICS use during the last 12 months before the 11-13 years visit, but the finding was not statistically significant. In addition, children with the variant *IL17F* genotype reported more often doctor-diagnosed atopic dermatitis with adjusted OR 2.71 (95% CI 1.05-7.04) than those with the wild genotype (Table 2). There were no significant differences in asthma or allergy

TABLE 1 Clinical characteristics of the 142 former bronchiolitis patients at the 5-7 y follow-up in relation to presence of variant genotypes of the *IL17F* rs763780, rs11465553 and rs7741835 polymorphisms

Clinical characteristic	Variant rs763780 TC/CC genotype[1] n = 32	Variant rs11465553 CT/TT genotype[2] n = 11	Variant rs7741835 CT/TT genotype[3] n = 60
Wheezing in last 12 mo, N = 30	9 (28.1%)	1 (9.1%)	12 (20.0%)
aOR [95% CI]	1.63 [0.64-4.12]	0.39 [0.05-3.22]	0.90 [0.39-2.08]
Bronchodilator use in last 12 mo, N = 26	6 (18.8%)	1 (9.1%)	9 (15.0%)
aOR [95% CI]	1.02 [0.36-2.89]	0.45 [0.05- 3.75]	0.72 [0.29-1.77]
ICSs use in last 12 mo, N = 18	6 (18.8%)	0 (0)	6 (10.0%)
aOR [95% CI]	1.70 [0.55-5.25]	-	0.72 [0.24-2.11]
Current asthma, N = 19	7 (21.9%)	0 (0)	9 (15.0%)
aOR [95% CI]	2.23 [0.74-6.65]	-	1.43 [0.51-4.00]
Rhinitis in last 12 mo, N = 40	10 (31.3%)	3 (27.3%)	15 (25.0%)
aOR [95% CI]	1.28 [0.53-3.08]	1.01 [0.25-4.13]	0.71 [0.33-1.53]
Atopic dermatitis, N = 42	9 (28.1%)	3 (27.3%)	14 (23.3%)
aOR [95% CI]	0.82 [0.34-2.01]	0.98 [0.24-4.00]	0.59 [0.28-1.27]

Note: Variant homozygous genotype in one case[1], no case[2] and three cases[3].
aOR = OR adjusted for age, sex and RSV aetiology of bronchiolitis at age <6 mo.

parameters between children with the variant and wild genotypes of the *IL17F* rs11465553 or rs7741835 (Table 2).

4 | DISCUSSION

There are three main results in our prospective, long-term follow-up study on the association between the *IL17F* rs763780, rs11465553 or rs7741835 variations and the development of asthma or allergy at school age after bronchiolitis in infancy. First, children with the variant *IL17F* rs763780 genotype had used more often than those with the wild genotype ICSs between the ages of 5-7 and 11-13 years. Second, children with the variant *IL17F* rs763780 genotype reported more often than those with the wild genotype atopic dermatitis at 11-13 years of age. Third, there were no significant differences in asthma or allergy prevalence between children with wild and variant genotypes of the *IL17F* rs11465553 or rs7741835 at 5-7 or 11-13 years of ages.

The role of IL-17F in inflammation and inflammatory diseases are not fully known.[7] IL-17F can be detected in a wider range of tissues than IL-17A, which suggests that IL-17F presents with more diverse biological functions.[6] IL-17A and IL-17F share high degree of sequence homology and a common receptor, and they promote inflammatory activity of Th17 cells.[2] While Th2 cells are involved in allergy and asthma, Th1 and Th17 cells contribute to acute and chronic inflammation.[23] Th2 cytokines are able to enhance the biological activity of IL-17F, which suggests that the interaction of Th2 cytokines with IL-17F augments allergic inflammation.[6] In asthmatics, cells from bronchoalveolar fluid released IL-17F after allergen stimulation,[9] and overexpression of IL-17F had an additive effect on allergen-induced allergic inflammatory responses in an animal asthma model.[24] These findings suggest that IL-17F has a role in atopic asthma.

In adults, IL-17F seems to be associated with severe asthma, in which neutrophils are involved.[5] When the IL-17F expression was examined in nasal and bronchial biopsies, both nasal and bronchial IL-17F showed higher expression in 28 adults with chronic asthma than in controls.[25] In another study, the number of cells that expressed IL-17F in bronchial submucosa was higher in 165 asthmatic adults than in controls and the number of cells was associated with disease severity.[26] In addition, the number of cells that expressed IL-17F was positively correlated with the submucosal eosinophil count.[26] In mouse models, IL-17F induced corticosteroid-resistant inflammation.[27]

There are no published studies on IL-17F cytokine or *IL17F* gene in infants with bronchiolitis nor in children with wheezing or asthma. Our hypothesis was that IL-17F might play a role in bronchiolitis, since infant bronchiolitis, especially that caused by RSV, seems to be associated more often with neutrophilic inflammation than with atopic or eosinophilic inflammation.[28] In the present post-bronchiolitis cohort, the *IL17F* rs7741835 variant genotype was more common in cases than in population controls, which suggests that infants with this variation might be at risk for bronchiolitis hospitalisation. However, the *IL17F* rs7741835 showed no association with post-bronchiolitis hospitalisation. However, this polymorphism and two other *IL17F* gene polymorphisms showed no association with virus aetiology or disease severity of infant bronchiolitis.

TABLE 2 Clinical characteristics of the 125 former bronchiolitis patients at the 11-13 y follow-up in relation to presence of variant genotypes of the *IL17F* rs763780, rs11465553 and rs7741835 polymorphism

Clinical characteristic	Variant rs763780 TC/CC genotype n = 27	Variant rs11465553 CT/TT genotype n = 9	Variant rs7741835 CT/TT genotype n = 51
Current asthma, N = 15	6 (22.2%)	0 (0)	5 (9.8%)
aOR [95% CI]	2.70 [0.81-8.90]	-	0.69 [0.22-2.22]
Persistent asthma, N = 9	4 (14.8)	0 (0)	2 (3.9%)
aOR [95% CI]	3.77 [0.76-18.61]	-	0.38 [0.07-2.11]
Use of ICSs in last 12 mo, N = 11	5 (18.5%)	0 (0)	3 (5.9%)
aOR [95% CI]	3.49 [0.86-14.2]	-	0.51 [0.12-2.19]
Use of ICSs at the age of 5-13 y, N = 16	**7 (25.9%)**	0 (0)	5 (9.8%)
aOR [95% CI]	**3.58 [1.11-11.5]**	-	0.60 [0.19-1.90]
Doctor-diagnosed allergic rhinitis, N = 19	6 (22.2%)	2 (22.2%)	8 (15.7%)
aOR [95% CI]	1.88 [0.61-5.84]	2.09 [0.38-11.38]	1.09 [0.40-2.98]
Doctor-diagnosed atopic dermatitis, N = 31	**11 (40.7)**	1 (11.1)	14 (27.5)
aOR [95% CI]	**2.71 [1.05-7.04]**	0.34 [0.04-2.97]	1.38 [0.59-3.18]

Note: Variant homozygous genotype in one case[1], no case[2] and two cases[3].

The bold values to highlight the finding in the later follow-up.

aOR = OR adjusted for age, sex and RSV aetiology of bronchiolitis at age <6 mo.

Results on the association of the *IL17F* gene variations with asthma risk have been conflicting. When the *IL17F* rs763780 was determined in 867 subjects from Japan, the variant genotype, but only when homozygous, was associated with lower asthma risk.[9] When the *IL17F* rs763780 and rs13209590 genotypes were compared between 318 Chinese adults with asthma and 352 controls, the minor allele C of the *IL17F* rs763780 was associated with higher asthma risk but only in males.[8] When the *IL17F* rs763780 and rs2397084 variations were compared between 221 Chinese adults with asthma and 223 controls, the minor alleles and variant genotypes of the *IL17F* rs763780 were more common in cases than in controls.[29] In the present post-bronchiolitis cohort, the variant *IL17F* rs763780 genotype was associated with asthma at school age, but not earlier, which is in line with the recent Chinese observation in adults with asthma.[29]

Published data on IL-17F or *IL17F* gene are scarce in atopic dermatitis or allergic rhinitis. In a Korean case-control study, serum IL-17F levels were higher in 228 children with atopic dermatitis than in 62 controls, and the level correlated with disease severity.[4] A Chinese study offered preliminary evidence that different haplotypes containing the *IL17F* rs13192563 SNP may either weaken or strengthen the association between allergic rhinitis and asthma.[2] However, we did not study the rs13192563 polymorphism. Among 160 Japanese adults with atopic dermatitis and 103 controls, the *IL17F* rs763780 showed no association with dermatitis.[30] In the present study, the variant *IL17F* rs763780 genotype increased the risk for atopic dermatitis at 11-13 years of age but not earlier.

Fifty polymorphisms and two insertions/deletions have been detected in the *IL17F* gene.[7] In this study, we focused on three functional *IL17F* rs763780, rs11465553 and rs7741835 SNPs, which are located in the coding region of the gene leading to amino acid changes in the IL-17F protein.[7] The altered IL-17F antagonises the wild-type IL-17F activity as was documented in cultures of human bronchial epithelial cells.[9] There is recent experimental evidence on the association of the *IL17F* rs7741835 with IL-17F production in cultured peripheral mononuclear cells.[13] There are no studies on the functionality of the *IL17F* rs11465553 SNP.

The main limitation to our present study is the small sample size for a genetic study, consisting of 125 to 142 children with bronchiolitis in early infancy. Less than 15% of them, depending on the used definition, presented with school-age asthma. We compared children with wild genotypes to those with variant genotypes within the cohort, which is an acceptable design in long-term follow-up studies. In addition, we compared the three studied *IL17F* SNPs between our 166 bronchiolitis patients and 99 Finnish population-based controls from the 1000 Genome Project.[20] The *IL17F* rs763780 that was associated with asthma and allergy in the present study did not differ between cases and population controls.

The main strengths of the present study are the prospective design, long-term follow-up and careful data collection during both bronchiolitis hospitalisation and at the follow-up visits. The homogeneity of the study population of the present study, that is ethnically Finnish children, is a benefit for a genetic study. Three *IL17F* polymorphisms were studied, including the rs763780 SNP with presence of earlier but conflicting results on the association with asthma in adults. The *IL17F* rs11465553 SNP has been less studied, and there are no previous studies on the association of the *IL17F* rs7741835 with asthma susceptibility. Our study is the only one having this far evaluated the *IL17F* SNPs as risk factors of post-bronchiolitis asthma.

5 | CONCLUSION

The variant genotype of the *IL17F* rs763780 was associated with more post-bronchiolitis asthma needing ICS therapy between 5-7 and 11-13 years of ages. Our results suggest that *IL17F* rs763780 variation may be a risk factor of severe post-bronchiolitis asthma in school-aged children.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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PUBLICATION IV

Interleukin 17A **gene variations and lung function at school age after bronchiolitis in infancy.**

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ACTA PÆDIATRICA NURTURING THE CHILD WILEY

BRIEF REPORT

Interleukin 17A gene variations and lung function at school age after bronchiolitis in infancy

Interleukin 17A (IL-17A) is a pro-inflammatory cytokine of the IL-17 family predominantly secreted by T helper 17 cells. IL-17A is involved with the pathogenesis of inflammatory diseases such as asthma but seems to decrease the disease severity of bronchiolitis.[1] In our previous studies, the *IL17A* rs2275913 single nucleotide polymorphism (SNP) was associated with asthma risk at 11-13 years of age, but not earlier at 5-7 years of age, in the cohort of originally 166 children hospitalised for bronchiolitis at younger than 6 months of age.[2] The *IL17A* rs4711998 and rs8193036 SNPs were also studied, and they did not show any such associations at either age.[3]

At 5-7 years of age, 103 children of this post-bronchiolitis cohort performed impulse oscillometry (IOS) including measurement of respiratory system resistance at 5Hz (Rrs5) before and after exercise and after bronchodilation (BD).[4] Baseline IOS was pathological (Rrs5> 5th percentile of references) in eight cases (7.8%) and post-BD IOS in no case.[4] An increased responsiveness to exercise (>35% increase in Rrs5) or to BD (>35% decrease in Rrs5) was found in 5% and 11%, respectively.[4]

At 10-13 years of age, 89 children of this post-bronchiolitis cohort performed flow-volume spirometry (FVS) including FEV1 (forced expiratory volume in one sec) and FVC (forced vital capacity) before and after BD. FEV1 was pathological (<5th percentile of references) in 25% before and in 18% after BD.[5] The respective figures for FEV1/FVC were 25% and 10%. An increased responsiveness to BD (>12% increase in FEV1) was found in 8%.[5]

The present study is a secondary analysis of IOS data from 5-7 years and of FVS data from 11-13 years visits in relation to the *IL17A* rs4711998, rs8193036 and rs2275913 genotypes. Data on the *IL17A* rs4711998, rs8193036 and rs2275913 SNPs were available for 98, 99 and 98 of those 103, who performed IOS at 5-7 years of age. The respective figures were 83, 82 and 84 for those 89 who performed FVS at 11-13 years of age. Other *IL17A* SNPs were not studied.

SPSS Statistics for Windows (IBM Corp.) was used in statistical analyses. Both IOS and FVS parameters were studied as continuous variables with analysis of variance. Multivariate analyses of covariance were adjusted for maternal smoking in infancy and respiratory syncytial virus aetiology of bronchiolitis during hospitalisation (IOS and FVS data), and for current asthma and sex-specific, height-related body mass index for age *z*-scores (FVS data). The results are presented as means, standard deviations (SD) and adjusted 95% confidence intervals (95% CI).

There were no significant differences in baseline or post-BD IOS results between children with wild versus variant genotypes of the *IL17A* rs4711998, rs8193036 and rs2275913 (Data not shown). In the 24 children with the wild genotype GG of the *IL17A* rs2275913, exercise induced a greater increase in airways resistance (ΔRrs5+ 1.53 vs +0.22, crude and adjusted $P = .002$) compared to 74 cases with the variant AG or AA genotype. No such associations were found for the *IL17A* rs4711998 or rs8193036. There were no significant differences in responses to BD between children with wild and variant genotypes in any of the three *IL17A* SNPs (Data not shown).

In non-adjusted analyses, both baseline FEV1/FVC and post-BD FEV1/FVC were lower in the 39 children with the variant *IL17A* rs4711998 GA or AA genotype than in those 44 with the wild GG genotype. The mean of the baseline FEV1/FVC was 91.4 in those with the variant and 95.1 in those with the wild genotype ($P = .03$), and the means of post-BD FEV1/FVC were 94.4 and 97.7, respectively ($P = .02$). The adjusted 95%CIs marginally overlapped, which means that statistical significances were lost (Table 1). There were no significant differences in baseline or post-BD FEV1 or FEV1/FVC between children with wild and those with variant genotypes of the *IL17A* SNPs rs2275913 and rs8193036 (Table 1).

Thus, we found some evidence that the variant genotype of the *IL17A* rs4711998, compared to the wild genotype, was associated with lower FEV1/FVC at both baseline and post-BD measurements at 11-13 years in children who were hospitalised for bronchiolitis in early infancy. However, the observations were not independent from asthma and overweight. In addition, we made an interesting finding suggesting a link between increased bronchial responsiveness at early school age to asthma at teenage. The wild genotype of the *IL17A* rs2275913, compared to the variant genotype, was associated with a greater exercise-induced increase in airways resistance in IOS at 5-7 years. The same wild genotype was associated with higher prevalence of current asthma and more use of inhaled corticosteroids at 11-13 years.[2] Thus, different variations even in the same gene may expose to asthma and bronchial hyper-responsiveness, and to reduced lung function.

The main limitations of this post-bronchiolitis study on the association of *IL17A* variations and lung function were the small

TABLE 1 Baseline and post-bronchodilator parameters in flow-volume spirometry, expressed as continuous per cent-of-predicted values, in relation to the *IL17A* wild and variant genotypes

IL17A genotype	Baseline FEV1 Mean (SD) 95% CI	Post-bronchodilator FEV1 Mean (SD) 95% CI	Baseline FEV1/FVC Mean (SD) 95% CI	Post-bronchodilator FEV1/FVC Mean (SD) 95% CI
rs2275913 Wild GG n = 23	88.7 (10.0) 84.4-93.0	91.8 (10.9) 87.1-96.5	91.8 (9.4) 87.8-95.9	94.2 (6.8) 91.3-97.2
rs2275913 Variant GA or AA n = 61	89.5 (11.8) 86.5-92.5	93.0 (12.3) 89.9-96.2	94.1 (7.1) 92.3-95.9	97.0 (6.6) 95.3-98.7
rs8193036 Wild TT n = 28	88.4 (12.3) 83.6-93.2	92.3 (13.6) 87.0-97.6	92.4 (8.8) 89.0-95.8	95.3 (6.8) 92.7-97.9
rs8193036 Variant TC or CC n = 54	89.1 (10.4) 86.3-92.0	92.6 (11.1) 89.6-95.6	93.9 (7.4) 91.9-95.9	96.8 (6.8) 94.9-98.6
rs4711998 Wild GG n = 44	90.4 (11.2) 87.0-93.8	93.7 (12.2) 90.0-97.4	95.1 (7.8) 92.7-97.4	97.7 (6.5) 95.7-99.7
rs4711998 Variant GA or AA n = 39	88.6 (11.0) 85.0-92.2	91.9 (11.4) 88.3-95.6	91.4 (7.4) 89.0-93.8	94.4 (6.4) 92.3-96.5

Note: The analyses were adjusted for respiratory syncytial virus aetiology of bronchiolitis and sex-specific, height-related body mass index for age z-scores at the study visit.

Abbreviations: CI, confidence interval; FEV1, forced expiratory volume in one sec; FVC, forced vital capacity.

sample size for genetic analyses and that only three *1L17A* polymorphisms were studied. Therefore, the study is exploratory and the results are preliminary. The main strength of the study is the prospective, over ten-year follow-up after bronchiolitis in early infancy including objective lung function measurements at 5-7 and 11-13 years of ages.

CONFLICT OF INTEREST

The authors declared no conflicts of interest.

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PUBLICATION V

Interleukin 17F polymorphisms showed no association with lung function at school age after infant bronchiolitis.

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BRIEF REPORT

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Interleukin-17F (IL-17F) is a pro-inflammatory cytokine of the IL-17 family. As is well-known, IL-17 cytokines are involved with the pathogenesis of inflammatory diseases such as asthma, but they seem to reduce the disease severity of bronchiolitis.[1] In our previous studies, *IL17F* rs763780 single nucleotide polymorphism (SNP) was associated with the risk of chronic asthma at school age in the cohort of originally 166 children hospitalised for bronchiolitis in infancy.[2] Instead, the presence of *IL17F* rs11465553 or rs7741835 variations was not associated with asthma at school age.[2]

At 5-7 years of age, 103 children of this post-bronchiolitis cohort performed impulse oscillometry before and after exercise and after bronchodilation, as described previously.[3] In impulse oscillometry, sound waves are superimposed at various frequencies on normal tidal breathing, and the disturbances in flow and pressure are measured and used to calculate parameters for estimating resistance to airflow and reactance of lung tissue. In baseline, impulse oscillometry was pathological (resistance at 5 Hz > 5th or reactance at 5 Hz < 5th percentile of references in age- and height-adjusted *z*-scores) in 20% but after bronchodilation in only one child, and bronchial reactivity to exercise was increased (>35% rise in resistance at 5 Hz) in 16%.[3]

At 10-13 years of age, 89 children of this post-bronchiolitis cohort performed flow-volume spirometry, including forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) before and after bronchodilation, as described previously.[4] FEV1 expressed as per cent-of-predicted values was pathological (<5th percentile of tolerance limit) in 25% before and in 18% after bronchodilation. The respective figures for FEV1/FVC were 25% and 10%. An increased responsiveness to bronchodilation (>12% increase in FEV1) was found in 8%.[4]

The present study is a secondary analysis of impulse oscillometry data from 5 to 7 years and flow-volume spirometry data from 10 to 13 years in relation to the *IL17F* rs763780, rs7741835 and rs11465553 genotypes. Data on these three *IL17F* SNPs were available for 100/103 cases at 5- to 7-year visits and for 84/89 cases at 10- to 13-year visits.

SPSS Statistics for Windows (IBM Corp.) was used in statistical analyses. Both impulse oscillometry and flow-volume spirometry parameters were studied as continuous variables with analysis of variance adjusted for maternal smoking when the child was <12 months old, for respiratory syncytial virus aetiology of bronchiolitis during hospitalisation (oscillometry and spirometry data), and for current asthma and sex-specific, height-related body mass index-for-age *z*-scores (spirometry data). The results are presented as means, standard deviations (SDs) and 95% confidence intervals (CIs).

There were no significant differences in baseline impulse oscillometry between children with wild vs variant genotypes of the *IL17F* rs763780, rs11465553 or rs7741835 at the 5- to 7-year visit (data not shown). One significant association was found in post-bronchodilation oscillometry. Concerning the *IL17F* rs11465553, the measurements in seven carriers of the variant genotype CT suggested mild peripheral obstruction, that is higher resistance at 5Hz (mean −0.82 vs −1.78 *z*-scores, $P = .01$) and lower reactance at 5Hz (−0.16 vs 0.38 $P = .04$), compared to 93 wild CC genotype carriers. No child had the variant homozygous TT genotype.

There was only one significant difference in parameters of flow-volume spirometry between children with wild vs variant genotypes of the three studied *IL17F* polymorphisms at the 10- to 13-year visit (Table 1). The mean baseline FEV1/FVC was 93.2 (95% CI 91.4-94.9) in 79 children with the wild *IL17F* rs11465553 CC genotype compared to 98.4 (95.1-101.8, $P = .02$) in those five with the variant CT genotype. There was no difference in FEV1/FVC ratio in post-bronchodilation measurements (Table 1). Again, no child had the variant homozygous TT genotype. Other analyses comparing baseline or post-bronchodilation FEV1 or FEV1/FVC in relation to the *IL17F* rs11465553, rs763780 or rs7741835 did not show any significant associations (Table 1).

We consider the results of this secondary analysis on the association between the three studied *IL17F* polymorphisms and post-bronchiolitis lung function negative. At 5-7 years of age, there were parameters in impulse oscillometry that suggested mild peripheral obstruction after bronchodilation in children with the variant genotype of the *IL17F* rs11465553. On the contrary, at 10-13 years of age, the FEV1/FVC ratio after bronchodilation did not differ between carriers of the variant and wild genotypes. Furthermore, the baseline FEV1/FVC was lower in children with the wild genotype, showing an opposite direction for the carriers of variant genotype between control visits. Therefore, no evidence of persistent post-bronchodilation lung function reduction was found in relation to the *IL17F* rs11465553 genotypes. All other analyses at both ages, including those for the *IL17F* rs763780 and rs7741835, gave negative results. However, this interpretation is highly speculative, since the number of the children with any

TABLE 1 Baseline and post-bronchodilator parameters in flow-volume spirometry in former bronchiolitis patients at 10-13 y of age expressed as continuous per cent-of-predicted values in relation to the *IL17F* wild and variant genotypes

IL17F genotype	Baseline FEV1 Mean (SD) 95% CI	Post-bronchodilation FEV1 Mean (SD) 95% CI	Baseline FEV1/FVC Mean (SD) 95% CI	Post-bronchodilation FEV1/FVC Mean (SD) 95% CI
IL17F rs7741835				
Wild CC	89.0 (11.0)	91.7 (11.8)	94.7 (7.3)	97.0 (6.3)
n = 52	85.9-92.0	88.4-94.9	92.7-96.8	95.2-98.7
Variant CT or TT	89.8 (11.8)	94.4 (12.1)	91.4 (8.3)	95.1 (7.2)
n = 32	85.5-94.0	90.0-98.7	88.4-94.4	92.5-97.7
IL17F rs11465553				
Wild CC	89.4 (11.3)	93.0 (11.9)	93.2 (7.9)*	96.0 (6.8)
n = 79	86.9-91.9	90.4-95.7	91.4-94.9	94.5-97.6
Variant TC	87.8 (12.0)	87.4 (10.6)	98.4 (2.7)*	99.6 (4.7)
n = 5	72.9-102.7	74.2-100.6	95.1-101.8	93.7-105.4
IL17F rs763780				
Wild TT	89.2 (11.9)	92.4 (12.2)	93.1 (7.9)	95.8 (6.8)
n = 65	86.3-92.1	89.4-95.5	91.1-95.0	94.1-97.5
Variant TC or CC	89.2 (9.1)	93.6 (11.0)	94.8 (7.5)	97.9 (6.3)
n = 19	85.2-94.0	88.3-98.9)	91.2-98.4	94.8-100.9

Note: Analysis of variance adjusted for maternal smoking when the child was <12 mo old, respiratory syncytial virus aetiology of bronchiolitis during hospitalisation, current asthma and current sex-specific, height-related body mass index-for-age *z*-scores.

The numbers of homozygous variant genotypes were one for *IL17F* rs7741845 (TT), zero for *IL17F* rs11465553 (CC) and one for *IL17F* rs763780 (CC).

Abbreviations: FEV1, forced expiratory volume in one second; FVC, forced vital capacity.

*P-value = .02 between wild and variant genotypes.

variation was very small, and there were no cases with homozygous variations.

The main limitation of this post-bronchiolitis study is the small sample size for genetic analyses, carrying a risk of type 2 errors. In addition, only three *IL17F* polymorphisms were determined. The main strength of the study is prospective, over 10-year follow-up with careful collection of the data during hospitalisation for bronchiolitis and at subsequent scheduled visits. We consider the current negative results as preliminary with minor clinical significance. Since IL-17F plays a role in the pathogenesis of asthma and chronic obstructive lung disease, the connection between IL-17F genetics and post-bronchiolitis outcome, such as reduced lung function, needs to be further studied in larger cohorts including more polymorphisms.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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