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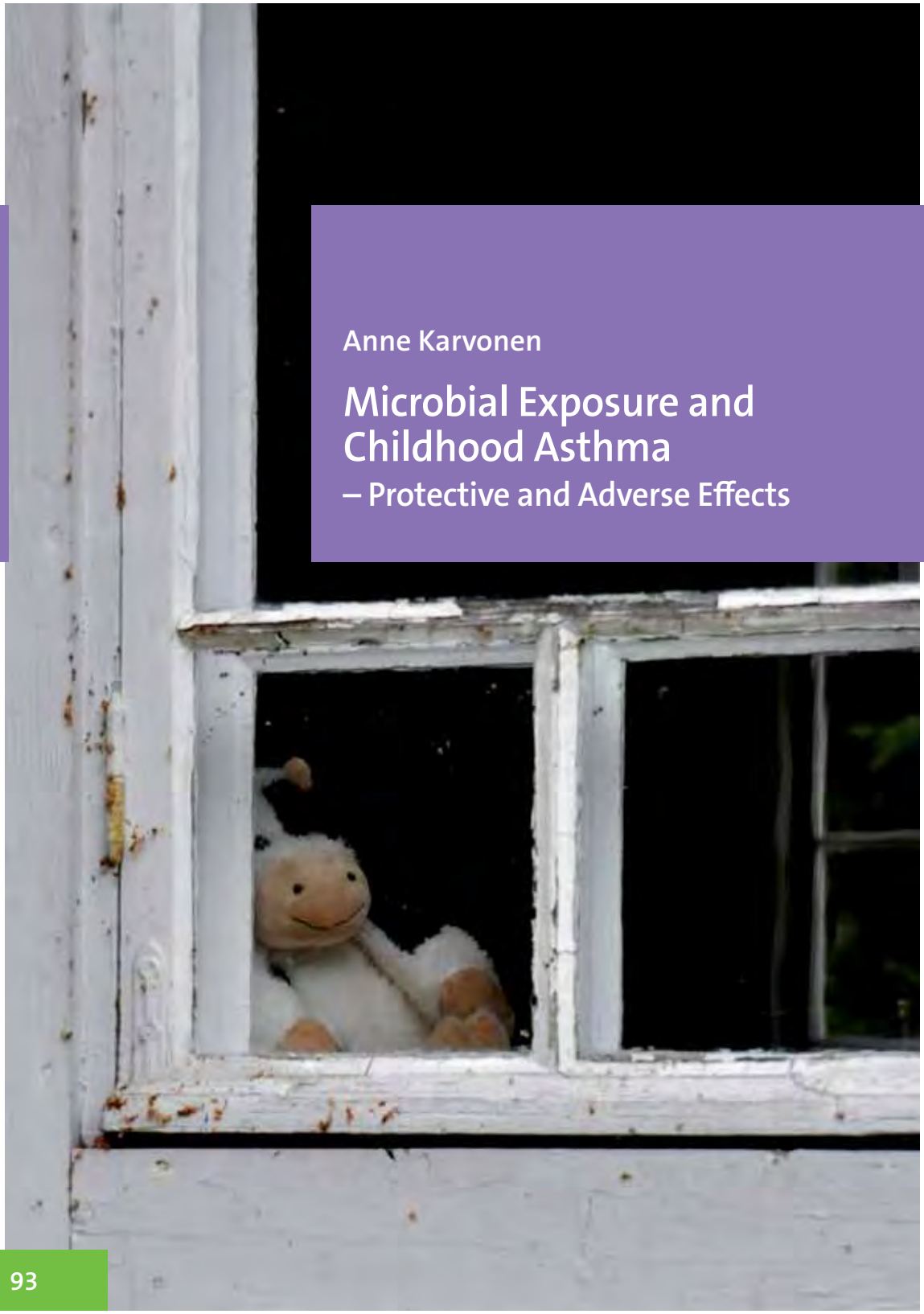

Anne Karvonen

Microbial Exposure and Childhood Asthma

– Protective and Adverse Effects

RESEARCH 93/2012

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Microbial Exposure and Childhood Asthma

Protective and Adverse Effects

ACADEMIC DISSERTATION

To be presented with the permission of Faculty of Health Sciences, University of Eastern Finland for public examination in Medistudia ML3, Yliopistonranta 1A, on Friday 16th of November, at 12:00

National Institute for Health and Welfare,
Department of Environmental Health, Kuopio, Finland
and
University of Eastern Finland,
Faculty of Health Sciences, Kuopio, Finland

NATIONAL INSTITUTE
FOR HEALTH AND WELFARE

Cover photo: Maria Valkonen

ISBN 978-952-245-751-6 (printed)
ISSN 1798-0054 (printed)
ISBN 978-952-245-752-3 (pdf)
ISSN 1798-0062 (pdf)
URN:ISBN: 978-952-245-752-3
http://urn.fi/URN:ISBN: 978-952-245-752-3

Juvenes Print – Finnish University Print
Tampere, Finland 2012

441 729
Painotuote

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Dedicated to Aki, Eetu and Elias

Abstract

Anne Karvonen. Microbial Exposure and Childhood Asthma – Protective and Adverse Effects. National Institute for Health and Welfare (THL). Research 93/2012. 162 pages. Kuopio, Finland 2012.
ISBN 978-952-245-751-6 (printed); ISBN 978-952-245-752-3 (pdf)

Asthma and other allergic diseases such as atopic dermatitis and allergic rhinitis are the most prevalent chronic diseases in children in developed countries. Changes in the living environment, such as changes in microbial exposure, may partly be behind this phenomenon. The aim of this thesis was to evaluate the association of exposure during infancy to moisture and mould damage and environmental microbes in the home with asthmatic symptoms, infections and allergic diseases during the first six years of life.

The study populations belonged to the PASTURE and LUKAS birth cohorts. Recruitments of 1133 PASTURE study children and 442 LUKAS study children were done between 2002 and 2005. House dust samples were collected for microbial analyses at the age of two months. In the LUKAS study, home inspection for moisture damage was carried out at the average age of five months. The data on children's health and potential confounders were collected by questionnaires up to the age of six years. Atopic sensitisation to 19 allergens was determined from serum samples at the age of one and six years.

Severe moisture damage (adjusted odds ratio (aOR) 3.84, 95% confidence interval (95%CI) 1.03–14.29) and visible mould (aOR 5.33, 95%CI 1.39–20.46) in the child's bedroom in infancy increased the risk of asthma both during the first six years of life and at the age of six years. The associations with asthma were more pronounced among atopic children than non-atopic children, and were similar in the early and latter part of the follow-up. Moisture and mould damage in the home in infancy were not associated with respiratory infections or atopic sensitisation to inhalant allergens up to the age of six years. No consistent associations were found with moisture damage or mould in the bathrooms or other interior spaces.

The quantity score for microbial exposure in infancy, i.e. the sum of markers for fungi (ergosterol), gram-positive (muramic acid) and gram-negative (endotoxin) bacteria, was significantly (inverted U-shape) associated with asthma up to the age of six years: the highest risk was found at the medium levels (aOR 3.45, 95%CI 1.07–11.15 for 3rd quintile) and the lowest risk at the highest level (aOR 0.19, 95%CI 0.02–1.75 for 5th quintile). Adjusting for amount of dust or diversity score i.e. the sum of detected qPCRs for two bacterial and six fungal genera, species or groups, did not change the estimates. No significant associations were found between the microbial quantity score and respiratory symptoms, atopic dermatitis or atopic sensitisation up to the age of six years. The microbial diversity score in in-

fancy was inversely associated with the risk of wheezing during the first six years of life.

The single microbial markers including also individual genera, species or groups in infancy were mostly non-significantly associated with health up to the age of six years and the shape of the association varied. Protective effects of the amounts of dust, markers for fungi (extracellular polysaccharides of *Aspergillus* and *Pencillium* spp. (EPS)) and gram-negative bacteria (endotoxin) on asthma and respiratory symptoms were seen especially among non-farmers up to the age of two years. Due to high correlations between different microbial markers, their effects could not be clearly separated from each other.

In conclusion, severe moisture damage and visible mould in the areas of the house, where the children spend most of their time, increase the risk of persistent asthma in children. The score for quantity of environmental microbial exposure not only predicted asthma better than single microbial markers, but also the associations were independent of microbial diversity and amount of dust. These results underline the importance of severe moisture damage and visible mould as significant threats to respiratory health. In contrast, the quantity and diversity of environmental microbial exposure may have protective effects on asthma.

Keywords: Allergic rhinitis, asthma, atopic dermatitis, atopy, bacteria, children, cohort, endotoxin, EPS, ergosterol, exposure, fungi, indoor, microbial exposure, mould, muramic acid, specific IgE

Tiivistelmä

Anne Karvonen. Microbial Exposure and Childhood Asthma – Protective and Adverse Effects [Mikrobialtistus ja lapsuuden astma – Suojaavat ja haitalliset vaikutukset]. Terveyden ja hyvinvoinnin laitos (THL). Tutkimus 93/2012. 162 sivua. Kuopio, Finland 2012.
ISBN 978-952-245-751-6 (painettu); ISBN 978-952-245-752-3 (pdf)

Astma ja muut allergiset sairaudet kuten atooppinen ekseema ja allerginen nuha ovat lasten yleisimpiä pitkäaikaissairauksia kehittyneissä maissa. Elinympäristön kuten mikrobialtistuksen muutos voi osittain olla ilmiön takana. Tämän väitös-kirjan tarkoituksena oli selvittää kodin kosteus- ja homevaurioiden sekä elinympäristöstä peräisin olevan mikrobialtistuksen yhteyttä lasten astmaattisiin oireisiin, infektioihin ja allergisiin sairauksiin kuuden ensimmäisen elinvuoden aikana.

Tutkimusaineisto koostui PASTURE- ja LUKAS-syntymäkohorteista. PASTURE-tutkimukseen otettiin 1133 lasta ja LUKAS-tutkimukseen 442 lasta vuosina 2002–2005. Huonepölynäytteet kerättiin mikrobianalyysejä varten lasten ollessa kahden kuukauden ikäisiä. Kodin kosteusvauriokuntoarvio tehtiin LUKAS-tutkimuksessa lasten ollessa keskimäärin viiden kuukauden ikäisiä. Tietoja lasten terveydestä ja mahdollisista sekoittavista tekijöistä kerättiin kyselylomakkeilla kuuteen ikävuoteen asti. Atooppinen herkistyminen 19 allergeenille määritettiin seeruminäytteistä yhden vuoden ja kuuden vuoden iässä.

Vakavat kosteusvauriot (vakioitu ristitulosuhde (vOR) 3.84, 95% luottamusväli (95%LV) 1.03–14.29) ja näkyvä home (vOR 5.33, 95%LV 1.39–20.46) lapsen makuuhuoneessa varhaislapsuudessa lisäsivät astmaan sairastumisen riskiä sekä kuuden ensimmäisen elinvuoden aikana että kuuden vuoden iässä. Tämä yhteys näkyi erityisesti atooppisesti herkistyneillä lapsilla kuin ei-atooppisilla lapsilla ja yhteys oli samanlainen sekä seurannan alussa että lopussa. Kosteus- ja homevauriot eivät olleet yhteydessä hengitystieinfektioihin tai atooppiseen herkistymiseen kuuden vuoden aikana. Kylpyhuoneiden ja muiden asuintilojen kosteus- ja homevauriot eivät olleet yhteydessä terveyteen.

Varhaislapsuuden mikrobialtistuksen kokonaismäärän ts. homeiden (ergosteroli), gram-positiivisten bakteerien (muramiinihappo) ja gram-negatiivisten bakteerien (endotoksiini) määrää kuvaavien markkereiden summa oli tilastollisesti merkitsevästi yhteydessä astmaan kuuden ensimmäisen elinvuoden aikana (yhteys muodoltaan nurinkäännetty U): suurin riski astmaan sairastumiselle oli keskimääräisellä mikrobialtistuksen kokonaismäärällä (vOR 3.45, 95%LV 1.07–11.15, kolmas viidennes) ja matalin riski suurimmalla mikrobialtistuksen kokonaismäärällä (vOR 0.19, 95%LV 0.02–1.75, ylin viidennes). Pölyn määrällä tai mikrobialtistuksen moninaisuudella, ts. qPCR-menetelmällä havaittujen kahden bakteeri- tai kuuden homemikrobilajien, -sukujen tai -ryhmien summa, vakiointi ei vaikuttunut tuloksiin. Varhaislapsuuden mikrobialtistuksen kokonaismäärä ei ollut yhteydessä hengitystieoireisiin,

atooppiseen ekseemaan tai atooppiseen herkis-tymiseen kuuden ensimmäisen elinvuoden aikana. Mikrobialtistuksen moninai-suus suojasi hengityksen vinkunalta.

Varhaislapsuuden yksittäiset mikrobimarkkerit sisältäen mikrobiryhmät, -suvut tai -lajit olivat pääosin ei-tilastollisesti merkitsevästi yhteydessä terveyteen kuuden ensimmäisen elinvuoden aikana ja niiden yhteyden muoto vaihteli. Kokonaispölyn määrä, homeiden (*Aspergillus* ja *Penicillium* homeiden ekstrapolysakkaridit (EPS)) ja gram-negatiivisten bakteerien (endotoksiini) määrää kuvaavat markkerit suojasivat erityisesti ei-maanviljelijöiden lapsia astmaan sairastumiselta ja hengitystieoireilta kahden ensimmäisen elinvuoden aikana. Yksittäisten mikrobi-markkereiden itsenäistä yhteyttä terveyteen ei voitu selvästi erotella, koska niiden pitoisuudet olivat riippuvuussuhteessa pölynmäärään ja muiden mikrobimarkke-reiden pitoisuuksiin.

Vakavat kosteusvauriot ja näkyvä home huoneissa, joissa lapsi oleskelee eniten, lisää astmaan sairastumisen riskiä. Elinympäristön mikrobialtistuksen kokonaismäärä ennusti paremmin astmaan sairastumisen riskiä kuin yksittäiset mikrobimarkkerit ja havaittu yhteys oli riippumaton mikrobiston moninaisuudesta tai kokonaispölyn määrästä. Tämän tutkimuksen tulokset osoittavat, että kodin kosteusvauriot ja näkyvä home ovat merkittävä terveysuhka. Elinympäristön mikrobialtistuksen kokonaismäärä ja moninaisuus sitä vastoin voivat vähentää astmaan sairastuvuutta.

Avainsanat: Allerginen nuha, altistuminen, astma, atooppinen ekseema, atopia, bakteeri, endotoksiini, EPS, ergosteroli, home, kohorttitutkimus, lapset, mikrobialtistus, muramiinihappo, sisäilma, spesifinen IgE

Contents

Original publications

List of original papers

This thesis is based on the following original articles referred to in the text by their Roman numerals:

I Anne M. Karvonen, Anne Hyvärinen, Marjut Roponen, Matthias Hoffmann, Matti Korppi, Sami Remes, Erika von Mutius, Aino Nevalainen, Juha Pekkanen. Confirmed Moisture Damage at Home, Respiratory Symptoms and Atopy in Early Life: A Birth-Cohort Study. *Pediatrics* [http://www.pediatrics.org/cgi/content/full/124/2/e329] Electronic Pages. 2009; 124 (2): e329-338.

II Anne M. Karvonen, Anne Hyvärinen, Matti Korppi, Harald Renz, Petra I. Pfefferle, Jon Genuneit, Juha Pekkanen. Moisture damage in the home and asthma, respiratory symptoms and infections, atopic dermatitis, and atopy up to age of 6 years: a birth cohort study with home inspections. *Submitted.*

III Anne M. Karvonen, Anne Hyvärinen, Urike Gehring, Matti Korppi, Gert Doekes, Josef Riedler, Charlotte Braun-Fahrländer, Sondhja Bitter, Susanne Schmid, Leea Keski-Nisula, Marjut Roponen, Vincent Kaulek, Jean-Charles Dalphin, Petra I. Pfefferle, Harald Renz, Gisela Bühele, Erika von Mutius, Juha Pekkanen and PASTURE study group. Exposure to microbial agents in house dust and wheezing, atopic dermatitis and atopic sensitization in early childhood: a birth cohort study in rural areas. *Clinical & Experimental Allergy*. 2012; 42 (8): 1246-1256.

IV Anne M. Karvonen, Anne Hyvärinen, Helena Rintala, Matti Korppi, Martin Tàubel, Gert Doekes, Urike Gehring, Harald Renz, Petra I. Pfefferle, Jon Genuneit, Leea Keski-Nisula, Sami Remes, Jussi Lampi, Erika von Mutius, Juha Pekkanen. Quantity and diversity of environmental microbial exposure and development of asthma: a birth cohort study. *Submitted.*

Abbreviations

aOR	adjusted odds ratio
EIA	enzyme immunoassay
EPS	fungal extracellular polysaccharides antigens of *Penicillium* and *Aspergillus* species
EPSU	EPS units
EU	endotoxin units
ERMI	environmental relative moldiness index
GC-MS-MS	gas chromatography tandem mass spectrometry
GEE	general estimating equations
GM	geometric mean
GSD	geometric standard deviation
IOM	Institute of Medicine
kU/L	kilo units per liter
LAL	Limulus amebocyte lysate
LPS	lipopolysaccharide
$LPS_{10:0–16:0}$	amount (mol) of LPS in each sample; the number of mol of 3-hydroxy fatty acids of $C_{10:0}$ to $C_{16:0}$ carbon chain length divided by four
OR	odds ratio
PASTURE	Protection against Allergy Study in Rural Environments
PRR	pattern recognition receptors
RSV	respiratory synctial virus
rFC	recombinant Factor C

qPCR	quantitative polymerase chain reaction
sIgE	specific immunoglobulin E
Th	T-helper lymphocytes

1 INTRODUCTION

Asthma and allergic diseases such as atopic dermatitis and allergic rhinitis are the most prevalent chronic diseases in western children and have been increasing during recent decades (Asher et al. 2006). Due to a rapid increase in the incidence of asthma it has been proposed that genetic factors (i.e. inheritable factors) can not be the only factor behind the process (Garn and Renz 2007). Thus, an explanation for the rapid growth in the incidence has been sought in the changes in living environments, where microbial exposure may have a critical role (von Mutius and Vercelli 2010). The effect of microbial exposure seems to be beneficial or harmful depending on timing, duration, source, amount and diversity of microbial exposure.

Over twenty years ago, a so-called hygiene hypothesis was for the first time introduced (Strachan 1989) suggesting that very hygienic living conditions can lead to the development of allergic diseases. Originally the focus of the hypothesis was on respiratory infections, but since then the focus has changed to exposure to non-pathogenic microbes in the environment and in the gut (Garn and Renz 2007). Hygienic environment decreases the exposure to microbes and parasites that, it has been speculated, are involved in the maturation of the immune system especially in early life (von Mutius and Vercelli 2010). The absence of these exposures leads to Th2-oriented reactivity, instead of Th1-oriented reactivity, and finally to Th2-type over-reactions to environmental allergens, and further to allergic manifestations (Braun-Fahrlander 2003).

It has been noticed that people living on farms, especially those with livestock, have less atopy than the people in urban areas or in other rural environments (von Mutius and Vercelli 2010). The exact reasons for this lower atopy prevalence are still unclear, but there seems to be a close relationship with livestock contacts. Many studies, especially those done among farmers, have suggested that higher environmental microbial exposure in early childhood is associated with the lower risk of atopy and allergic diseases in later life (von Mutius and Vercelli 2010). In most previous epidemiological studies, only a few markers, mainly endotoxin, have been applied to characterize microbial exposure (Heederik and von Mutius 2012), which naturally gives a very incomplete picture of the total microbial exposure. Recently the importance of microbial diversity has been highlighted (Ege et al. 2011).

In contrast to the potential protective effect of environmental microbial exposure, many studies have shown (World Health Organization 2009), that moisture damage, mould and dampness in the building are associated with respiratory symptoms such as wheezing and cough, asthma exacerbation and even the development of asthma. When people spend 90% of their time indoors, the impact of good indoor air quality is important. It has been suggested that microbes, microbial toxins and volatile compounds trigger these respiratory manifestations. However, the evidence is conflicting

(Tischer et al. 2011c, World Health Organization 2009). Most previous studies have been cross-sectional, which makes it difficult to explore causality; in addition, the exposure assessment has mostly been based on self-reporting, which may cause a reporting bias. The association between home dampness and adverse health effects may be not as high as has been reported in cross-sectional studies with self-reported exposure assessments.

The main problems are very similar for both research arms, that is, in the research of the potential protective farming microbes, and in the research of the potential harmful microbes in moisture-damaged homes. Both study fields are need of new research modes including more objective microbial exposure assessment, better characterization of exposing microbes and reliable monitoring of health outcomes. This is the aim of the current thesis.

2 REVIEW OF THE LITERATURE

2.1 Asthma and allergies in children

2.1.1 Phenotypes of asthma in children

Asthma is not a single disease entity, but rather a matter of several phenotypes (Lötvall et al. 2011). However, characterizing and defining different asthma and wheezing phenotypes is difficult (Global Initiative for Asthma (GINA) 2007, Wenzel 2006). For preschool asthma, the European Respiratory Society Task Force recently recommended differentiating between multi-trigger wheeze and episodic wheeze (Brand et al. 2008). The Tucson Birth Cohort Study revealed that childhood wheezing can be classified into four main categories: early-onset transient, persistent non-atopic wheezing, persistent atopic wheezing, and late-onset wheezing (Henderson et al. 2008, Martinez et al. 1995). Persistent atopic and late-onset wheezing usually lead to school-age asthma.

The most common phenotype in young children is early-onset transient wheezing, which is usually defined as the onset of wheezing before the age of three years that stops at some point before the age of six (Martinez et al. 1995). Wheezing episodes are usually associated with viral infections. The reason for the discontinuation of wheezing by age is not known; the suggested reasons include matured immunity and decreased reactivity, and simply the growth of the airways. Early-onset persistent wheezing is defined as wheezing symptoms which start before the age of three years and continue up to the age of six years or later. Persistent wheezing can be classified into two subgroups, non-atopic and atopic persistent wheezing. Wheezing which starts after the age of three years is called late-onset wheezing. Early-onset atopic persistent and late-onset wheezing phenotypes are associated with the development of asthma at school age and in later life.

2.1.2 Risk factors for asthma and allergy, and hygiene hypothesis

The general risk factors in childhood for persistent asthma even up to adulthood have been explored in birth cohorts with a high number of children and in prospective cohorts with children who have been hospitalized due to bronchiolitis or wheezy bronchitis (Piippo-Savolainen et al. 2009). These risk factors are doctor-diagnosed parental asthma, doctor-diagnosed atopic dermatitis and/or food allergy in the child, recurrent wheezing in infancy, early-childhood atopic sensitisation to inhalant allergens or blood eosinophilia, maternal smoking during pregnancy and early-childhood passive smoking, wheezing apart from cold and doctor-diagnosed allergic rhinitis (reviewed in Guilbert et al. 2004, Piippo-Savolainen and Korppi 2008). There are also other factors such as breastfeeding and exposure to pets, which may have dif-

ferent effects on asthma depending on the age and immunological maturity of the child (reviewed in Piippo-Savolainen et al. 2009). Also, parental education level, male sex in early age and female sex in later age predicts asthma (reviewed in Castro-Rodriguez 2011). In addition, postbronchiolitis studies have revealed that bronchiolitis caused by rhinoviruses rather than respiratory synctial virus (RSV) is associated with later asthma (reviewed in Piippo-Savolainen et al. 2009).

The hygiene hypothesis was for the first time introduced by Strachan (1989) over twenty years ago, postulating that very hygienic living conditions increase the risk of allergic diseases. The idea of the hypothesis was risen from the observation that people who lived in overcrowding and unhygienic conditions had less allergic rhinitis and atopic dermatitis (Strachan 1989). Originally the focus of the hypothesis was on respiratory infections, but since then the focus has changed to exposure to non-pathogenic microbes in the environment and in the gut (Garn and Renz 2007).

An hygienic environment decreases the exposure to microbes and parasites, which, it has been speculated, are involved in the maturation of the immune system especially in early life (von Mutius and Vercelli 2010). T lymphocytes, like T-helper-1 (Th1) and T-helper-2 (Th2) cells are essential components of adaptive immunity regulating different cellular responses and the production of different mediators, including cytokines and other mediators of innate immunity (Martinez and Holt 1999). Exposure to the high concentrations of microbial agents with pro-inflammatory properties stimulates the maturation of Th1-oriented immunity, instead of the atopic Th2-oriented immunity (Braun-Fahrlander 2003). The pattern recognition receptors (PRR) are essential components of innate immunity, and both PRRs and cytokines are involved in the maturation of adaptive immunity either in the Th1 direction towards non-allergic or in the Th2 direction towards allergic reaction patterns (Garn and Renz 2007). A plausible cellular mechanism behind hygiene hypothesis is that the absence of exposures to microbes and parasites leads to an imbalance of the immune system which increases the risk of allergic manifestations via Th2-oriented reactivity, instead of Th1-oriented reactivity (Vercelli 2006).

2.2 Observed moisture damage, mould or dampness in homes — adverse health effects in children

Several reviews (Bornehag et al. 2001, Bornehag et al. 2004, Institute of Medicine 2004, Mendell et al. 2011, Sahakian et al. 2008, Tischer et al. 2011a, World Health Organization 2009) have summarized the findings from numerous studies and concluded that moisture damage, mould and dampness in the dwellings increase the risk of respiratory symptoms. Although numerous studies have been published, the causal exposure is still unknown.

It has been suggested that the crucial exposures that are related to adverse health effects in dwellings with moisture damage, mould or dampness, may be exposures to micro-organisms or their fragments, toxins, allergens and microbial, other volatile

organic compounds (World Health Organization 2009) or non-biologic emissions such as formaldehyde and 2-ethyl-1-hexanol (Mendell et al. 2011). It has, however, not been possible to identify a single causal factor or agent that would be able to explain the health effects associated with occupying dwellings with moisture damage or mould.

It is quite likely that multiple exposures are involved. The competition between microbes for space and available nutrients leads to changes in the characteristics of the microbes: for example co-cultivation of two microbes in relatively low concentrations has been shown to lead to the formation of highly toxic compounds (Markkanen Penttinen et al. 2009). The exposure may change not only because of the metabolic activity of microbes, but also due to several factors such as the moisture conditions in the structure, ambient temperature and relative humidity, air flows and pressure and availability of nutrients affecting on microbial growth and release of contaminants. In addition, studies that have included identification of microbial species, groups or genera with cultivation method have not found consistent effects, while the results using quantitative polymerase chain reaction (qPCR) determining both viable and non-viable microbes remain preliminary (Mendell et al. 2011).

Studies with data on moisture damage and mould or dampness in homes and respiratory symptoms and infections, allergic diseases, or atopy in children are included in the current review of the literature.

2.2.1 Moisture damage and respiratory symptoms and asthma

Evidence on the adverse health effects of moisture damage, mould or dampness in the dwelling is the most convincing for respiratory symptoms and exacerbation of asthma, as were shown in extensive reviews by Bornehag and his colleagues (Bornehag et al. 2001, Bornehag et al. 2004). A recent systematic review of 61 publications on children showed that visible mould increases significantly the risk of wheezing (adjusted Odds Ratio (aOR) 1.68, 95% Confidence Interval (95%CI) 1.48–1.90) and asthma (aOR 1.49, 95%CI 1.28–1.72) (Tischer et al. 2011a). Furthermore, a review (Mendell et al. 2011) of 103 peer-reviewed stdies with children and adults confirmed the conclusions of earlier reviews (Institute of Medicine 2004, World Health Organization 2009) that evidence of associations between moisture damage or mould and respiratory symptoms or asthma exacerbation were sufficient.

Since the most recent reviews (Mendell et al. 2011, Tischer et al. 2011c), 13 articles on wheezing and current asthma in children have been published. Most of the studies supported the previous findings, but unfortunately the designs were mostly cross-sectional with no follow-up data available (Boneberger et al. 2011, Chen et al. 2011, Civelek et al. 2011, Kasznia-Kocot et al. 2010, Kim et al. 2011, Lam et al. 2011, Shpakou et al. 2011, Sun and Sundell 2011, Tischer et al. 2011b, Tsai et al. 2011, Visser et al. 2010). No association was found in two birth cohort studies between objectively observed moisture damage or mould and dampness and wheezing

(Rosenbaum et al. 2010) or between measured mould surface area and the number of respiratory illnesses (stuffy nose, cough, wheeze or shortness of breath) during first two years of life (Dales et al. 2010). Despite these two recent studies, sufficient evidence exists that moisture damage, mould or dampness in dwellings increase children's risk for respiratory symptoms.

The evidence is less clear for the association between moisture damage, mould or dampness in dwellings and the development of asthma (Institute of Medicine 2004). Two strongly designed studies involving children revealed that moisture damage, mould or dampness in the home increase the risk of development of asthma (Jaakkola et al. 2005, Pekkanen et al. 2007). Three years ago, the WHO review concluded that there is sufficient evidence for this association between moisture damage, mould and dampness in the dwelling and the development of asthma in children (World Health Organization 2009). A recent extensive review including 13 articles on asthma development (Mendell et al. 2011) agreed with this conclusion.

Most recently, an incident case-control study (Hwang et al. 2011), a cohort study with a five-year follow-up (Larsson et al. 2011) and a meta-analysis of European birth cohorts (Tischer et al. 2011c) confirmed the earlier observations on the development of new asthma. However, there were some limitations in these studies. In the Larsson and her colleagues study (Larsson et al. 2011), mould odour was the only indicator of moisture damage, mould or dampness which was associated with asthma development in a cohort study with five years follow-up Also, moisture damage, mould or dampness in the home was significantly associated with asthma only in children aged less than two years (Tischer et al. 2011c). Wheezing children, even those with asthma diagnoses, include typically also those wheezing children with early transient phenotype, who do not develop persistent or chronic asthma later. In a recent cohort study, inspector-observed moisture damage, mould or dampness was not associated with asthma development in children in a seven-year follow-up (Reponen et al. 2011). Thus, moisture damage, mould or dampness are associated with asthma exacerbations and may be associated with asthma development in children, but cohort studies with long follow-ups are needed to confirm or rule out the long-term effects.

Mechanisms behind the adverse health effects have been evaluated in toxicological studies. Mechanisms that may be related to respiratory symptoms and even to the development of asthma may be non-specific inflammation and oxidative stress (reviewed in World Health Organization 2009). When the human body is repeatedly exposed to moisture damage, mould or dampness, the natural immune defences are activated. This may lead to overreaction in immune responses with the increased and prolonged production of inflammatory mediators and tissue damage and finally, to chronic airway inflammation manifesting as asthma (reviewed in World Health Organization 2009). The adverse effects may not be related to one causal agent, but may be rather caused by many agents and their productive compounds. For example, based on findings from toxicological studies, unknown highly toxic compounds

produced by two or more microbes during co-cultivation seemed to induce oxidative stress with cytotoxic, genotoxic and inflammogenic effects (Markkanen Penttinen et al. 2009). These mechanisms may be involved in the development of asthma and therefore, more research is needed to define the findings. However, the difficulties in identifying the cause and effect relationships of health outcomes associated with moisture damage, mould or dampness motivate the search for causal agents in other than viable microbes.

In conclusion, based on numerous previous studies from different countries and climatic areas presented in previous reviews and meta-analysis, moisture damage, mould and dampness have harmful effects on respiratory health, but the causative factors and mechanisms are still unresolved. The objective exposure assessment such as home inspections for moisture damage, mould or dampness may enhance to discover the causal agents which are related to health outcomes. Thus, the objective exposure assessment may amplify the knowledge of exposure which is related to adverse health effects.

2.2.2 Moisture damage in the home — location

Few previous studies have explored moisture damage, mould or dampness separately in the bedroom of the child (Mohamed et al. 1995, Pekkanen et al. 2007, Pirastu et al. 2009, Ponsonby et al. 2000, Strachan and Carey 1995, Strachan et al. 1990, Tham et al. 2007, Verhoeff et al. 1995, Wickens et al. 1999) or in bedrooms in general (Garrido et al. 2010, Naydenov et al. 2008), in the main living area (Belanger et al. 2003, Emenius et al. 2004a, Emenius et al. 2004b, Lawson et al. 2005, Pekkanen et al. 2007, Ponsonby et al. 2000, Venn et al. 2003, Verhoeff et al. 1995, Wickens et al. 1999) or in the main living area including bathrooms (Bornehag et al. 2005, Kim et al. 2011, Larsson et al. 2011, Rydjord et al. 2007) or only in the kitchen (Miyake et al. 2007). Also, some studies have used the definition of the occupied rooms of the dwelling (Jaakkola et al. 2010, Jaakkola et al. 1993) without information about which rooms were included. Moreover, most of these studies have reported different rooms separately using different exposure assessments in these rooms and thus, the comparisons between the rooms are impracticable.

Three studies (Pekkanen et al. 2007, Verhoeff et al. 1995, Wickens et al. 1999) have used the same exposure assessment in different rooms (Table 1), but one of these studies did not present the estimates (Wickens et al. 1999). A nested incident case-control study with an objective home inspection (Pekkanen et al. 2007) concluded that the rooms have to be analyzed separately since no associations were found between moisture damage or mould in the bathrooms and other interior spaces and health, and the whole house estimates were weaker than in the main living area, in the kitchen or in the child's bedroom. In that study, most of the houses were detached one-family homes. Consistent with these results, a case-control study

(Verhoeff et al. 1995) found that the effect estimates for asthma were weaker for the whole house than for the living room or the child's bedroom. However, this difference was observed only when parent-reported moisture damage, mould or dampness were used in the analyses and not when damage was objectively observed (Verhoeff et al. 1995). In addition, no estimates were reported from the bathrooms or elsewhere in the house and no information on the type of the homes was available.

Two cross-sectional studies (Ponsonby et al. 2000, Strachan et al. 1990) found fairly similar estimates for asthma or wheezing and presence of mould in the child's bedroom, based on home inspection and parent-report. In contrast, two recent publications from cohort studies (Reponen et al. 2011, Tischer et al. 2011c) with follow-ups until the age of 7–10 years, did not find any associations between moisture damage, mould or dampness in the home at early age and the development of asthma in older age. This might be partly due to the fact that moisture damage, mould and dampness were assessed for the whole home and not separated to specific rooms.

Although there is only limited evidence from previous studies (Pekkanen et al. 2007, Verhoeff et al. 1995) that the location of the moisture damage in the home is important, current evidence underlines the need to explore this further. Specifically, future studies should use more specific estimates of exposure to moisture damage and not only use the estimate for the whole house, especially in the case of one family houses.

2.2.3 Objective *versus* self-reported moisture damage in the home

Most of the previous studies were based on parent-reported symptoms or diseases as well as parent-reported moisture damage, mould or dampness in the home, and in addition, were either cross-sectional with no follow-up or prospective cohorts with short follow-ups (Mendell et al. 2011). When the occupants' reports on dampness or mould growth are subjective, there is a risk of an artificial association between home dampness and adverse health effects (Strachan and Elton 1986).

At least 14 different studies have included home inspection for moisture damage, mould or dampness (Tables 1–4) (Cho et al. 2006, Dales et al. 2010, Douwes et al. 1999, Emenius et al. 2004b, Hägerhed-Engman et al. 2009, Koskinen et al. 1999, Mohamed et al. 1995, Nafstad et al. 1998, Naydenov et al. 2008, Pekkanen et al. 2007, Ponsonby et al. 2000, Rosenbaum et al. 2010, Rydjord et al. 2007, Strachan et al. 1990, Tavernier et al. 2005, Verhoeff et al. 1995). However, two of these studies did not show the results (Rydjord et al. 2007, Tavernier et al. 2005). In addition, there are even fewer studies that have compared results from home inspections for moisture damage with results of parent-reported damage (Emenius et al. 2004b, Koskinen et al. 1999, Nafstad et al. 1998, Naydenov et al. 2008, Ponsonby et al. 2000, Rydjord et al. 2007, Strachan et al. 1990, Verhoeff et al. 1995).

Two case-control studies based on birth cohorts found that both self-reported and objectively observed moisture damage, mould or dampness were associated with

health (Emenius et al. 2004a, Nafstad et al. 1998). Nafstad and his colleagues found also that the association was stronger with health if both the parent- and inspector-reported moisture damage, mould or dampness (Nafstad et al. 1998).

In contrast, two case-control studies based on cross-sectional studies found that inspectors observed more mould odour (Hägerhed-Engman et al. 2009, Naydenov et al. 2008) or damp stains (Naydenov et al. 2008) than parents. In contrast, parents reported more visible signs of dampness (Hägerhed-Engman et al. 2009) and visible mould (Naydenov et al. 2008) than did the investigator. Objectively observed moisture damage, mould and dampness were not associated with health (Hägerhed-Engman et al. 2009, Naydenov et al. 2008). This might be partly due to the fact that moisture damage, mould and dampness were assessed for the whole house and not separated to specific rooms (Hägerhed-Engman et al. 2009). However, due to non-professional investigators, no association was found when the objectively observed visible mould and damp stains were restricted to the child's or parents' bedrooms (Naydenov et al. 2008), and only parent-reported moisture damage, mould and dampness were associated with health (Naydenov et al. 2008).

A case-control study found that inspectors observed less dampness and mould than parents reported (Verhoeff et al. 1995). In that study, however, a good agreement between these two measurements (parents *vs.* inspectors) was found and no indications on over-reporting by the parents of cases was noticed (Verhoeff et al. 1995). Another case-control study revealed that self-reported and inspector observed moisture damage, mould or dampness were well correlated (Rydjord et al. 2007). In contrast, another matched case-control study found that parents of asthmatic children reported more dampness than did the parents of non-asthmatic children (Tavernier et al. 2005). However, in that study, home inspectors did not find any differences in dampness between homes of asthmatic and non-asthmatic children, and no results between inspector-observed moisture damage and health were shown. The authors suggested that awareness of the importance of indoor air quality in the respiratory health had impact on the reporting.

2.2.4 Reported moisture damage and other adverse health effects

Moisture damage, mould or dampness in dwellings may also cause other adverse health effects than respiratory tract symptoms or asthma. Other effects include symptoms of upper airway irritation (blocked or itching of the nose, sore throat or hoarseness), ocular irritation and general symptoms (headache, joint pain, nausea and fatigue) (Park and Cox-Ganser 2011). There is also some evidence that moisture damage, mould or dampness may be associated with respiratory infections and allergic manifestations such as allergic rhinitis, atopic dermatitis and sensitisation (World Health Organization 2009).

The WHO review concluded that there is sufficient evidence to show an association between moisture damage, mould and dampness and respiratory tract infections

with or without otitis (World Health Organization 2009). However, common cold was classified as having limited or suggestive evidence for an association in another recent review (Mendell et al. 2011). The conclusion of the latter review (Mendell et al. 2011) was partly based on the meta-analysis (Fisk et al. 2007) of 19 publications, of which 12 studies were made among children (Biagini et al. 2006, du Prel et al. 2006, Karevold et al. 2006, Koskinen et al. 1999, Li and Hsu 1996, Pettigrew et al. 2004, Rylander and Megevand 2000, Spengler et al. 2004, Stark et al. 2003, Strachan 1988, Yang et al. 1999, Yang et al. 1997). In children, moisture damage, mould or dampness in homes increased the risk of respiratory infections 1.5-fold (aOR 1.48, 95%CI 1.33–1.65) (Fisk et al. 2010). Based on the previous studies, moisture damage, mould and dampness seem to increase the risk of respiratory tract infections such as sinusitis, otitis, croup, bronchitis, pneumonia and tonsillitis in both adults and children.

The evidence for allergic rhinitis was classified as limited or suggestive in the review of the WHO (World Health Organization 2009), but sufficient in a recent review with updated data (Mendell et al. 2011). A recent systematic review (Tischer et al. 2011a) including ten original studies (Biagini et al. 2006, Brunekreef et al. 1989, Chen et al. 2003, Dong et al. 2008a, Dong et al. 2008b, Ibargoyen-Roteta et al. 2007, Koskinen et al. 1999, Li and Hsu 1996, Li and Hsu 1997, Stark et al. 2005) and one meta-analysis (Antova et al. 2008), estimated that visible mould increases the risk of allergic rhinitis (aOR 1.39, 95%CI 1.28–1.51) (Tischer et al. 2011a). After that review, eight studies (Civelek et al. 2010, Jaakkola et al. 2010, Lam et al. 2011, Shpakou et al. 2011, Sun and Sundell 2011, Tischer et al. 2011b, Tsai et al. 2011) and a meta-analysis (Tischer et al. 2011c) have reached rather similar estimates for allergic rhinitis or rhinoconjunctivitis.

Table 1. Summary of studies with objectively observed moisture damage and dampness in the home and asthma or wheezing in children.

AUTHOR	YEAR	STUDY DESIGN	AGE[¥]	N	AGE[£]	TYPE OF OBSERVATION	PLACE	EXPOSURE	PREV.[$]	ASTHMA	WHEEZE
Emenius et al.	2004a	case-control in a birth cohort (BAMSE)	2	540	first winter	home inspection	other than bathrooms or showers	sign of dampness[a]	25 %		1.6 (1.0-2.5)[h]
Hägerhed-Engman et al.	2009	nested case-control (DBH)	3-8	400	3-8	home inspection	excluding crawlspaces and cold attics	mild (grade 1-2)[b]	7 %	0.82 (0.28-2.42)	
								severe (garde 3)[b]		0.82 (0.05-12.33)	
Rosenbaum et al.	2010	birth cohort (AUDIT)	1	103	3.3 mo	walk-through home inspection	not known	dampness[c]	71 %		1.32 (0.54-3.22)[i]
Koskinen et al.	1999	cross-sectional	< 7	57	< 7	inspection by building engineers	not known	signs of moisture[d]	57 %		0.33 (0.07-1.52)[j]
		cross-sectional	7-15	147	7-15		not known	signs of moisture[d]	53 %		2.05 (0.45-9.27)[j]
Mohamed et al.	1995	case-control	9-11	154	9-11	author-observed	child's bedroom	damp damage	-	4.9 (2.0-11.7)	
Øie et al.	1999	incident case-control based on the OSLO birth cohort study	<2	344	<2	objectively observed	not known	dampness	-	2.4 (1.25-4.44)[k]	
Pekkanen et al.	2007	matched case-control	1-7	362	1-7	inspection by building engineers	whole house	any or suspected moisture damage	86 %	0.63 (0.28-1.45)	
							main living area	minor moist. damage[e]	20 %	2.11 (1.06-4.21)	
							main living area	major moist. damage[f]	11 %	2.46 (1.09-5.55)	
							main living area	area of the damage, m^2	4 %	1.36 (0.91-14.32)[l]	
							child's bedroom	moisture damage[g]	29 %	1.97 (1.00-3.90)	
							kitchen	moisture damage[g]	72 %	1.41 (0.80-2.47)	
							bathroom	moisture damage[g]	72 %	0.70 (0.39-1.25)	
							other interior spaces	moisture damage[g]	24 %	0.77 (0.40-1.46)	
Verhoeff et al.	1995	case-control	6-12	516	6-12	inspection by trained investigators	whole house	dampness	47 %	0.98 (0.68-1.41)	
							living room	dampness	16 %	0.94 (0.59-1.51)	
							child's bedroom	dampness	16 %	1.00 (0.61-1.64)	

[¥]age of the children (years) when health assessment was done and for prospective studies at the end of the follow-up, N=number of children, [£]age of the children (years if no other indicated) when exposure assessment was done, [$] prevalence of moisture problem [a]any other sign of dampness than mould spots on surface material/tile joints, [b] Black areas on parquet flooring or bubbly, loosening floor covering, justification of grades not shown in the article, [c] Visible dampness/ water (standing water, water damage, plumbing leaks), [d] Signs of moisture (signs of leakage, moist spots, detachment of paint or other surface, material, discolouring materials, [e] a need for repair of surface materials with area of damage < 1 m^2 or a need for repair of a structural component with area of damage < 0.1 m^2, [f] a need for repair of surface materials with area of damage ≥1 m^2 or a need for repair of a structural component with area of damage ≥ 0.1 m^2 or a need to renewal of functional element, [g] minor and major damage are pooled together (see [e] and [f]), [h] for recurrent wheeze, [i] unadjusted estimates, adjusting for confounders did not change the estimates, [j] for bronchitis, [k] for bronchial obstruction at least twice or one episode lasting more than a month, [l]for a 1 m^2 change in area of damage

Table 2. Summary of studies with objectively observed visible mould in the home and asthma or wheezing in children.

AUTHOR	YEAR	STUDY DESIGN	AGE[§]	N	AGE[£]	TYPE OF OBSERVATION	PLACE	EXPOSURE	PREV.[$]	ASTHMA	WHEEZE
Emenius et al.	2004a	case-control in a birth cohort (BAMSE)	2	540	first winter	home inspection	bathroom/shower	mould spots[a]	14 %		1.0 (0.5-1.7)[b]
Ponsonby et al.	2000	birth cohort (SIDS)	7	863	1 mo	objectively observed by interviewer	child's bedroom	mould	-	1.26 (0.87-1.81)	
Rosenbaum et al.	2010	birth cohort (AUDIT)	1	103	3.3 mo	walk-through home inspection	not known	visible mould	25 %		0.90 (0.35-2.29)[c]
Pekkanen et al.	2007	matched case-control	1-7	362	1-7	objectively observed	whole house	visible mould	49 %	1.24 (0.73-2.11)	
							main living area	mould spots	6 %	4.01 (1.12-14.32)	
							main living area	visible mould	2 %	1.95 (0.69-5.47)	
							kitchen	visible mould/ mould spots	25 %	1.13 (0.63-2.04)	
							bathroom	visible mould	26 %	0.81 (0.44-1.49)	
							other interior spaces	visible mould	12 %	0.86 (0.37-2.00)	
Strachan et al.	1990	cross-sectional	7	330	7	observed	child's bedroom	mould	12 %		3.25 (1.60-6.60)
Verhoeff et al.	1995	case-control	6-12	516	6-12	objectively observed by trained investigators	whole house	mould	32 %	1.18 (0.78-1.77)	
							living room	mould	8 %	1.10 (0.58-2.10)	
							child's bedroom	mould	5 %	0.68 (0.29-1.63)	

[§]age of the children (years) when health assessment was done and for prospective studies at the end of the follow-up, N=number of children, [£]age of the children (years if no other indicated) when exposure assessment was done, [$]prevalence of mould problem, [a]mould spots on surface material/tile joints, [b]for recurrent wheeze, [c]unadjusted estimates, adjusting for confounders did not change the estimates

Table 3. Summary of studies with objectively observed combined moisture or mould damage and dampness in the home and asthma or wheezing in children.

AUTHOR	YEAR	STUDY DESIGN	AGE[§]	N	AGE[£]	TYPE OF OBSERVATION	PLACE	EXPOSURE	PREV.[$]	ASTHMA	WHEEZE
Reponen et al.	2011	cohort study (CCAAPS)	7	176	<1	home inspection	not known	class 1[a]	35 %	1.0 (0.46-2.34)	
								class 2[b]	10 %	0.3 (0.03-2.04)	
		cross-sectional design in cohort study (CCAAPS)	7	176	7	home inspection	not known	class 1[a]	68 %	0.8 (0.29-4.52)	
								class 2[b]	11 %	1.1 (0.29-4.52)	
Cho et al.	2006	cross-sectional design in cohort study (CCAAPS)	1	640	<1	home inspection	not known	class 1[a]	51 %		1.2 (0.9-1.7)[f]
								class 2[b]	5 %		2.1 (1.2-3.6)[f]
Hägerhed-Engman et al.	2009	nested case-control (based on cross sectional study, DBH)	3-8	400	3-8	home inspection	whole house excluding crawlspaces and cold attics	mild (grade 1-2)[c]	25% mild & severe	0.86 (0.47-1.60)	
								severe (grade 3)[c]		0.28 (0.5-1.52)	
Nafstad et al.	1998	incident case-control based on OSLO birth cohort	<2	502	<2	objectively observed & parent reported	not known	moisture or mould problems[d]	27 % of the cases and 14% of the controls	3.8 (2.0-7.2)[g]	

[§]age of the children (years) when health assessment was done and for prospective studies at the end of the follow-up, N=number of children, [£]age of the children (years) when exposure assessment was done, [$] prevalence of moisture problem

[a] either minor indications of mould and/or water damage or history of mould and/or water damage, [b] at least 0.2m^2 of visible mould or mould and water damage combined, [c] visible spots of mould, stain of dampness or discoloured stains on walls / ceiling (the justification of grades not shown in the article), [d] presence of water damage, damp stain or visible mould/mildew growth during last 2 yrs, [e]sign of mould or other moisture-related damages, [f] for recurrent wheeze, [g] for bronchial obstruction at least twice or one episode lasting more than a month

Table 4. Summary of studies with objectively observed mould odour in the home and asthma or wheezing in children.

AUTHOR	YEAR	STUDY DESIGN	AGE[$]	N	AGE[£]	TYPE OF OBSERVATION	PLACE	EXPOSURE	PREV.[$]	ASTHMA	WHEEZE
Rosenbaum et al.	2010	birth cohort (infants at high risk) (AUDIT)	1	103	3.3 mo	walk-through home inspection	not known	mould/musty/ damp odour	37 %		1.32 (0.58-3.02)[a]
Hägerhed-Engman et al.	2009	nested case-control (based on cross-sectional study, DBH)	3-8	400	3-8	home inspection, excluding crawlspaces and cold attics (the justification of grades not shown in the article)	mouldy odour whole home or at least 1 room	mild (grade 1-2)	39 % mild & severe	0.99 (0.54-1.84)	
							mouldy odour whole home or at least 1 room	severe (grade 3)		0.57 (0.27-1.26)	
							mouldy odour along the skirting board	mild (grade 1-2)	47 % mild & severe	1.30 (0.73-2.29)	
							mouldy odour along the skirting board	severe (grade 3)		1.28 (0.60-2.73)	
Pekkanen et al.	2007	matched case-control	1-7	362	1-7	inspection by building engineers	whole house	mould odour, some	5 %	1.35 (0.42-4.36)	
							whole house	mould odour, clear	3 %	4.12 (0.65-26.01)	
							main living area	mould odour	16 %	2.96 (0.62-14.19)	

[$]age of the children (years) when health assessment was done and for prospective studies at the end of the follow-up, N=number of children, [£]age of the children (years if no other indicated) when exposure assessment was done, [$]prevalence of mould odour, [a]unadjusted estimates, adjusting for confounders did not change the estimates

Based on these previous studies, moisture damage, mould and dampness seem to be associated with allergic rhinitis in children. Sufficient evidence of the association between moisture damage, mould or dampness and eczema was found in a recent review (Mendell et al. 2011) including four studies on moisture damage, mould or dampness in children's homes (Hägerhed-Engman et al. 2009, Miyake et al. 2007, Pirastu et al. 2009, Tham et al. 2007) and one in adults. Most studies have a problem in differentiating between eczema and atopic eczema. Non-specified dermatitis was the health outcome used in most of the studies, and atopic dermatitis was used only in the birth cohort study, which was also the only prospective study (Miyake et al. 2007). In addition to these studies, previous and recent cross-sectional (or case-control) studies taht were not included in the review, have found mostly a non-significant tendency for an association with atopic dermatitis (du Prel et al. 2006, Garrido et al. 2010, Ibargoyen-Roteta et al. 2007, Koskinen et al. 1999, McNally et al. 2001, Pirastu et al. 2009, Shpakou et al. 2011, Simoni et al. 2005, Yang et al. 2000), eczema or itchy or flexural rash (Apfelbacher et al. 2011, Austin and Russell 1997, Bornehag et al. 2005, Chen et al. 2003, Schäfer et al. 2008, Sun and Sundell 2011, Tham et al. 2007). Thus, the evidence in the case of atopic dermatitis is mostly based on cross-sectional studies and thus, more prospective studies are needed to clarify the suggested association.

The evidence on the association for atopy or allergy was classified as inadequate or insufficient in the review of the WHO (World Health Organization 2009), but limited or suggestive in the recent review (Mendell et al. 2011). In the studies concerning children, atopy was mostly defined as positive results in specific IgE determinations or skin prick tests (Cho et al. 2006, du Prel et al. 2006, Schäfer et al. 1999). Although a pooled study of 12 cross-sectional studies showed that moisture damage, mould or dampness in the home increased the risk of atopic sensitisation to inhalant allergens (Antova et al. 2008), the evidence from prospective studies has not supported the finding (Cho et al. 2006, Iossifova et al. 2009, Tischer et al. 2011c). Thus, moisture damage, mould and dampness may not be associated with atopy, however, more prospective studies are needed.

In conclusion, moisture damage, mould or dampness seem to be associated with respiratory infections, otitis media and allergic rhinitis, but not with atopic sensitisation or atopic dermatitis.

2.3 Indoor exposure to environmental non-pathogenic microbes and health effects in children

The environmental non-pathogenic microbes belong to the groups of gram-negative bacteria, gram-positive bacteria and fungi. Indoor exposure to these microbes can be estimated by measuring viable or non-viable microbes, their components or products

in air samples or house dust samples. In large epidemiological studies, the most used collection method has been house dust sampling using either vacuuming or settled dust. Endotoxin is the most studied microbial agent from house dust in epidemiological studies on asthma and atopy (Doreswamy and Peden 2011). Endotoxin measured with the Limulus amebocyte lysate bioassay (LAL) is a marker of biological activity of lipopolysaccharide (LPS) of gram-negative bacteria. In addition, there are some other microbial markers, such as EPS, ergosterol and β-D-glucan for which there is a limited number of studies available. The studies with microbial exposure measurements from house dust samples from the homes and respiratory symptoms and allergic diseases estimates presented separately in children are included in the current review of the literature.

When exploring the health effects of microbial exposures, it is important to clearly identify the time points in the child's life span, e.g. when the exposure has occurred and when the health effect has been measured. Environmental exposures can have different effects depending on the timing of the exposure in relation to the child's age, growth, development and immune maturity (Liu 2002). This concerns especially the effects on the immune system, which continues to evolve during the whole childhood, and is dependent on the interaction between genes and environment.

2.3.1 Endotoxin in house dust and its association to health

The outer layer of gram-negative bacteria contains LPS, which is commonly called endotoxin. Endotoxin has usually been measured by using LAL (Douwes et al. 1995), but to date, there are some studies that have used the Recombinant Factor C (rFC) assay to measure endotoxin (McKenzie et al. 2011). Endotoxin measured with LAL or rFC describes the biological activity of LPS of gram negative bacteria.

The associations between environmental endotoxin concentrations and the development of asthma and atopy in children have been evaluated extensively (von Mutius and Radon 2008). Endotoxin is a well-known respiratory irritant (Smit et al. 2010), especially among adults in the workplaces with substantially high endotoxin exposure. In line with this, most studies in children have found an increased risk for wheezing in early life as was shown in a meta-analysis of observational studies (Mendy et al. 2011).

Nine cohort studies (Table 5) have examined the association between endotoxin exposure during the first year of life and wheezing in the first three years of life. Five have found a significant positive association (Bolte et al. 2003, Gehring et al. 2001, Gillespie et al. 2006, Park et al. 2001a, Perzanowski et al. 2006), although one only during the second year of life (Perzanowski et al. 2006). In other studies, one has found a suggestion for an inverse association i.e. children living in homes with higher amounts of endotoxin at early age had less wheezing(Wood et al. 2011), and four publications from two cohort studies have found no association (Campo et al. 2006, Rullo et al. 2009, Rullo et al. 2008, Ryan et al. 2009). In addition, one of these cohort studies found that high endotoxin levels in dogowners' homes was associated with reduced risk of wheezing in infants (Campo et al. 2006) as well as coexposure to high endotoxin levels and high traffic exposure synergistically increased the risk of wheezing at the age of three years (Ryan et al. 2009).

Five cohort studies with exposure assessment during the first two years of life and follow-ups from four to ten years have mostly found no associations with asthma (Table 6). One of these studies found a significant inverse association with asthma (Douwes et al. 2006a) and one found a suggestion for an inverse association (Sordillo et al. 2010). In contrast, two studies found a suggestion of positive association with asthma (Celedon et al. 2007, Reponen et al. 2012) while one did not find any association (Bertelsen et al. 2009). Concerning wheezing, there are three studies, one of which has found a significant association between higher endotoxin exposure in infancy and more wheezing at the age of seven years (Celedon et al. 2007) and two have found no association (Douwes et al. 2006b, Sordillo et al. 2010).

Associations between endotoxin exposure and asthma and/or wheezing have been evaluated in 17 studies with exposure assessment after the age of two years and these studies have mostly been studies with a cross-sectional design (Table 7). Four studies found a significant inverse association (among German children only, Tischer et al.) (Carlsten et al. 2011, Gehring et al. 2008, Sordillo et al. 2010, Tischer et al. 2011a) and two found a suggestion for an inverse association (El-Sharif et al. 2006, Gehring et al. 2002). Two studies found a positive association with asthma or wheezing (Litonjua et al. 2002, Tavernier et al. 2005) or one study found a suggestion of a positive association (among Dutch children only) (Tischer et al. 2011a). Another eight studies did not find any association (Ege et al. 2007, Hsu et al. 2011, Moniruzzaman et al. 2012, Nicolaou et al. 2006, Rennie et al. 2008, Simpson et al. 2006, Wickens et al. 2002, Yilmaz et al. 2009). Due to high correlations between levels of endotoxin and other microbial markers and the amounts of dust, it has been difficult to separate the specific effect of endotoxin.

There is only one study (Bolte et al. 2003), that has tried to separate the effects on endotoxin in children with atopic history. The study found that high endotoxin exposure increased the risk of repeated wheezing especially in children with atopic parents. In addition, three studies (Braun-Fahrlander et al. 2002, Iossifova et al. 2009, Schram-Bijkerk et al. 2005) have compared atopic asthma/wheeze with all

Table 5. Summary of studies evaluating the association between endotoxin in house dust in infancy and asthma, wheezing and/or atopy in young children in cohort studies (follow-up until the age of three years or less).

PUBLICATION	YEAR	STUDY DESIGN	AGE[1]	N	AGE[2]	SAMPLE	UNIT*	ASTHMA	WHEEZING	ATOPY
Bolte et al.	2003	birth cohort (LISA)	2	1942	3 mo	mother's mattress	/g	low incidence of asthma	++	+
Böttcher et al.	2003	birth cohort	2	219	3-12 mo	child's mattress / carpet	/g	not studied separately	not studied	-- in Sweden
							/g		not studied	0 in Estonia
Campo et al.	2006	cohort (CCAAPS)	mean age 12.5 mo ±0.8 mo	532	6 mo	baby's primary activity room floor	/g	not studied	0	+
Codispoti et al.	2010	cohort (CCAAPS)	3	549	8 mo	baby's primary activity room floor	/g	not studied	not studied	medium levels +, high levels -
Ege et al.	2008	birth cohort (PASTURE)	birth	922	2 mo	mother's mattress	?	not studied	not studied	0
Gehring et al.	2001	birth cohort (LISA)	6 mo	1884	3 mo	mother's mattress	/g	not studied	++	not studied
			1		3 mo	mother's mattress	/g	not studied	++	not studied
			6 mo		3 mo	infant's mattress	/g	not studied	0	not studied
			1		3 mo	infant's mattress	/g	not studied	0	not studied
Gillespie et al.	2006	birth cohort[£]	15 mo	881	3 mo	child's bedroom floor	/g	not studied	++	0
Park et al.	2001	birth cohort[§]	1	404	3 mo	family room floor	/g	not studied	++	not studied
Perzanowski et al.	2006	birth cohort	1	301	1	bedroom floor	/m^2	not studied	0	not studied
			1				/g	not studied	0	not studied
			2				/m^2	not studied	0	not studied
			2				/g	not studied	++	not studied
			3				/m^2	not studied	0	not studied
			3				/g	not studied	0	not studied
Rullo et al.	2009	birth cohort	2.5	99	<6 mo	infant's bed and bedroom floor	/g	not studied	0	0
Rullo et al.	2008	birth cohort	8 mo	104	<6 mo	infant's bed and bedroom floor	/g	not studied	0	not studied
Ryan et al.	2009	cohort (CCAAPS)	3	483	8 mo	baby's primary activity room floor	/g	0	0	not studied
Wood et al.	2011	birth cohort (URECA)	1	443	3 mo	bed dust	?	not studied	-	-

[1]age of the children (years if no other indicated) when health assessment was done and for prospective studies at the end of the follow-up,[2]age of the children (years if no other indicated) when exposure assessment was done, N= number of children, Unit=concentration (/g) or load (m^2), *concentration (/g) is in original papers either grams or milligrams, no association (0), suggestive positive association (+), significant positive association (++), suggestive inverse association (-), significant inverse association (--), [£]The New Zealand Asthma and Allergy Birth Cohort study, [§]Epidemiology of Home Allergens and Asthma

Table 6. Summary of studies evaluating the association between endotoxin in house dust in infancy and atopy, wheezing and/or asthma in children in cohort studies with longer follow-up (follow-up between ages four and ten years).

PUBLICATION	YEAR	STUDY DESIGN	AGE[1]	N	AGE[2]	SAMPLE	UNIT*	ASTHMA	WHEEZING	ATOPY
Bertelsen et al.	2009	birth cohort (ECA)	10	260	2	living room floor	/g	0	not studied	0
Celedón et al.	2007	birth cohort[§]	7	440	2-3 mo	family room/ living room floor	/g	+	++	-
Chen et al.	2008	two birth cohorts (LISA & GINI)	6	2108	3 mo	mother's/ child's/ parent's mattress	/g	not studied	not studied	0
Douwes et al.	2006	birth cohort (PIAMA)	4	690	3 mo	living room floor	/m^2	--	0	0
			4		3 mo	living room floor	/g	0	0	0
			4		3 mo	infant's mattress dust	/m^2	0	0	0
			4		3 mo	infant's mattress dust	/g	0	0	0
Sordillo et al.	2010	birth cohort[§]	7	406	1-3 mo	family room floor and upholstered chair	/g	-	0	0
Reponen et al.	2012	cohort (CCAAPS)	7	289	8 mo	baby's primary activity room floor	/g	+	not studied	not studied

[1]age of the children (years) when health assessment was done and for prospective studies at the end of the follow-up, N= number of children, [2]age of the children (years if no other indicated) when exposure assessment was done, Unit=concentration (/g) or load (m^2), *concentration (/g) is in original papers either grams or milligrams, no association (0), suggestive positive association (+), significant positive association (++), suggestive inverse association (-), significant inverse association (--), [§]Epidemiology of Home Allergen and Asthma study

Table 7. Summary of studies evaluating the association between endotoxin in house dust and asthma, wheezing and/or atopy in children in cross-sectional studies and other studies.

PUBLICATION	YEAR	STUDY DESIGN	AGE[1]	N	AGE[2]	SAMPLE	UNIT*	ASTHMA	WHEEZING	ATOPY
Braun-Fahrländer et al.	2002	cross-sectional (ALEX)	mean age 9.5 years SD±1.2 years	812	at the same time	child's mattress	/m^2	only for atopic and nonatopic asthma/wheeze		--
							/g	only for atopic and nonatopic asthma/wheeze		-
Carlsten et al.	2011	cross-sectional design in a birth cohort	7	334	7	mix of dust samples from four sites	/g	--	-	-
Ege et al.	2007	cross-sectional (PARSIFAL)	5-13	440	5-13	child's mattress	/g	0	0	-
El-Sharif et al.	2006	case-control	6-12	110	6-12	child's mattress	/g	not studied	-	0
						living room floor	/g	not studied	-	-
Gehring et al.	2002	cross-sectional	5-10	454	5-10	living room floor	/m^2	-	0	-
Gehring et al.	2007	nested case-control study (AIRALLERG)	2-4	1069	2-4	child's mattress	/m^2	not studied	not studied	--
			2-4		2-4	child's mattress	/g	not studied	not studied	-
			2-4		2-4	living room floor	/m^2	not studied	not studied	-
			2-4		2-4	living room floor	/g	not studied	not studied	0
Gehring et al.	2008	cross-sectional (ISAAC Phase II)	9-12	840	9-12	living room floor	/m^2	-	-	0
						living room floor	/g	-	--	-
Hsu et al.	2011	case-control study	3-9	98	3-9	settled dust from bed and surfaces that were above floor in the child's major and minor activity room	/g	0	0	0
Litonjua et al.	2002	siblings in a cohort study[§]	median age 6.87	226	median age 2.87	living room floor and kitchen floor	/g	not studied	++	not studied

Table 7. Continues.

PUBLICATION	YEAR	STUDY DESIGN	AGE[1]	N	AGE[2]	SAMPLE	UNIT*	ASTHMA	WHEEZING	ATOPY
Moniruzzaman et al.	2012	case-control	3-8	400	3-8	child's bedroom floor	/g	0	not studied	0
						living room floor	/g	0	not studied	0
Nicolaou et al.	2006	cross-sectional	15-16	128	15-16	child's mattress	/m^2	0	not studied	++
						child's mattress	/g	0	not studied	+
Perkin & Strachan	2006	cross-sectional	primary school children	879	at the same time	living room floor	/g	not reported	not reported	0
Rennie et al.	2008	case-control	6-13	197	6-13	child's mattress	/g	0	not studied	not studied
			6-13	197	6-13	play area	/g	0	not studied	not studied
Simpson et al.	2006	birth cohort	5	442	5	living-room floor	/m^2	not studied	0	--
Sordillo et al.	2010	cross-sectional design in a birth cohort[§]	7	360	7	family room floor	/g	--	--	--
				299		child's mattress	/g	-	-	0
Tavernier et al.	2005	matched case-control	4-17	200	4-17	living room floor	/g	++	not studied	not studied
Tischer et al.	2011	nested case-control study in birth cohorts	6	346[#]	5	child's mattress	/m^2	--	0	not studied
				332[$]		child's mattress	/m^2	+	0	not studied
Wickens et al.	2002	cross-sectional	7-10	282	7-10	living room floor	/m^2	0	0	0
							/g	0	0	0
Yilmaz et al.	2009	case-control	7-14	200	7-14	living room carpet	/m^2	0	0	0
							/g	0	0	0

[1]age of the children (years) when health assessment was done and for prospective studies at the end of the follow-up, N= number of children, [2]age of the children (years if no other indicated) when exposure assessment was done, Unit=concentration (/g) or load (m^2),*concentration (/g) is in original papers either grams or milligrams, no association (0), suggestive positive association (+), significant positive association (++), suggestive inverse association (-), significant inverse association (--), [#]German children (LISA & GINI), [$] Dutch children (PIAMA),[§]Epidemiology of Home Allergens and Asthma

non-asthmatics/non-wheezers, which leads to biased estimates (Pekkanen et al. 2012), and thus, the results cannot be compared. There is a lack of knowledge about children who might be more susceptible to endotoxin exposure than others.

High environmental endotoxin levels have been consistently associated with less atopic sensitisation. Twelve cohort studies and 15 studies with cross-sectional study designs have examined this association (Tables 5–7). Of these studies, six found a significant inverse association (Braun-Fahrlander et al. 2002, Böttcher et al. 2003, Gehring et al. 2007, Simpson et al. 2006, Sordillo et al. 2010) and the other six a suggestion of a similar inverse association (Carlsten et al. 2011, Celedon et al. 2007, Ege et al. 2007, El-Sharif et al. 2006, Gehring et al. 2002, Gehring et al. 2008, Wood et al. 2011). Birth cohort studies with endotoxin exposure in early infancy and short follow-up time (Table 5) found an inverse association (only in Swedish children in Böttcher et al. 2003) or a suggestion of the inverse association (Wood et al. 2011). In contrast, a birth cohort study found a suggestion for a positive association (Bolte et al. 2003) which was pronounced in children with atopic parents. Four cohort studies did not find any associations (only in Estonian children in Böttcher et al. 2003) (Ege et al. 2008, Gillespie et al. 2006, Rullo et al. 2009). Four cohort studies assessing exposure with dust samples in infancy and follow-ups from four to ten years (Table 6) did not find any associations (Bertelsen et al. 2009, Chen et al. 2008, Douwes et al. 2006b, Sordillo et al. 2010) and only one study found a tendency for an inverse association (Celedon et al. 2007). On the other hand, studies with cross-sectional design (Table 7) have mostly found an association or at least a tendency between the high endotoxin exposure and lower prevalence of atopy in school-aged children (Braun-Fahrlander et al. 2002, Carlsten et al. 2011, Ege et al. 2007, El-Sharif et al. 2006, Gehring et al. 2002, Gehring et al. 2007, Gehring et al. 2008, Simpson et al. 2006, Sordillo et al. 2010). Thus, environmental endotoxin exposure after infancy seems to be inversely associated with atopic sensitisation in school-aged children. This conclusion is based on mostly cross-sectional studies due to the lack of knowledge of cohort studies with longer follow-ups.

Only a few studies have examined the association of endotoxin exposure with other allergic outcomes. Six studies have evaluated the association of endotoxin exposure with atopic dermatitis. One birth cohort study found an association in six-month follow-up (Gehring et al. 2001), but not in longer follow-ups (Bolte et al. 2003, Chen et al. 2009). No association was found in another birth cohort (Wood et al. 2011) and one cross-sectional study found an inverse association (Karadag et al. 2007). In addition, a birth cohort study showed that increasing endotoxin load decreased the risk of eczema, but significantly only in children with the genotype CC of the CD14 gene (Simpson et al. 2006). Moreover, nine studies have examined the association of endotoxin with allergic rhinitis or allergic rhinoconjunctivitis. One birth cohort study and one cross-sectional study found an inverse association (Braun-Fahrlander et al. 2002, Celedon et al. 2007) and two a suggestion of an inverse association (Gehring et al. 2008, Marinho et al. 2007). One cohort study with a

three-year follow-up found inverse associations between low or high endotoxin exposure and allergic rhinitis, but exposure to medium levels of endotoxin increased the risk of allergic rhinitis (Codispoti et al. 2010). One cross-sectional study found a suggestion of a positive association (Gehring et al. 2002) and one did not find any association (Moniruzzaman et al. 2012). Thus, the role of endotoxin exposure in the development of atopic dermatitis is weaker than in the case of asthma or atopic sensitisation and the development of atopic dermatitis may be dependent on genetic factors. More prospective studies are needed to examine the association of endotoxin and allergic rhinitis.

2.3.2 Other markers for bacteria in house dust

Another marker of gram-negative bacteria is 3-hydroxy-fatty acids (3-OH-FAs), which are found in Lipid A, the immunostimulatory part of LPS (Saraf et al. 1997). The 3-OH-FAs with shorter chain ($C_{10:0}$-$C_{14:0}$) lengths correlate well with the endotoxin test, LAL (Saraf et al. 1997). 3-OH-FAs are measured with gas chromatography tandem mass spectrometry (GC-MS-MS).

The preliminary evidence from two children's studies indicates that a higher level of 3-OH-FAs might be associated with less asthma and/or wheezing (Hyvärinen et al. 2006b, Sordillo et al. 2010). In atopy, a higher level of 3-OH-FAs tended to be a protective factor when measured in dust samples from living room floors and a risk factor when measured in dust samples from mattresses (Sordillo et al. 2010). These few observations from the studies need to be confirmed in further research.

N-acetyl-muramic acid is a component of peptidoglycan, which is found in the cell wall of bacteria. The amount of peptidoglycan is much higher in gram-positive bacteria (30–70% of the cell wall) than in gram-negative bacteria (<10% of cell wall (Starr et al. 1981). Muramic acid is also determined with gas chromatography tandem mass spectrometry (GC-MS-MS). Although previous studies have not found any association between muramic acid and atopy (Eder et al. 2006, Sordillo et al. 2010, van Strien et al. 2004), there is some tentative evidence that a high level of muramic acid in dust from mattresses might be inversely associated with wheezing and asthma (Sordillo et al. 2010, van Strien et al. 2004).

2.3.3 Markers for fungi in house dust

Extracellular polysaccharide (EPS) of *Penicillium* and *Aspergillus* measured with enzyme immunoassay (EIA) (Douwes et al. 1999) is a marker of exposure to these moulds. EPS concentrations have been significantly associatiated with amounts of culturable fungi in house dust (Chew et al. 2001, Douwes et al. 1999). Although *Penicillium* and *Aspergillus spp.* belong among the most common viable fungal genera occurring in indoor air, there is preliminary evidence (Table 4) that EPS may protect against atopic sensitisation (Douwes et al. 2006b, Gehring et al. 2007),

wheezing and/or asthma in children (Douwes et al. 1999, Douwes et al. 2006b, Ege et al. 2007, Tischer et al. 2011b). Only one cross-sectional study has evaluated the association between indoor EPS and doctor-diagnosed atopic dermatitis, and there was no significant association (Karadag et al. 2007). All these observations need further confirmation using prospective studies.

Other fungal markers are ergosterol (fungal biomass) measured with gas chromatography tandem mass spectrometry (GC-MS-MS) (Sebastian and Larsson 2003) and β-D-glucan measured with either LAL, EIA or ELISA (Douwes et al. 1996).

The preliminary evidence from three children's studies shows that ergosterol might increase the risk of asthma or recurrent wheezing (Hyvärinen et al. 2006b, Wood et al. 2011), but decrease the risk of atopy (Wood et al. 2011).

Six studies (Table 5) have examined the association of β-D-glucan exposure with wheezing and/or asthma (Bertelsen et al. 2009, Douwes et al. 2006b, Ege et al. 2007, Hsu et al. 2011, Iossifova et al. 2007, Tischer et al. 2011b). Three of these studies found no association (Bertelsen et al. 2009, Ege et al. 2007, Hsu et al. 2011) and two studies found a tendency towards an inverse association (only in German children in Tischer et al.)(Douwes et al. 2006b, Tischer et al. 2011a) and one a tendency to a positive association (only Dutch children) (Tischer et al. 2011a). One cohort study found that low levels of β-D-glucan increased the risk of recurrent wheezing, but the high levels of β-D-glucan decreased the risk of wheezing (Iossifova et al. 2007). In addition, six studies have evaluated the association of β-D-glucan exposure with atopy and three of them did not find any association (Bertelsen et al. 2009, Douwes et al. 2006b, Hsu et al. 2011). One study found significant (Gehring et al. 2007) and two others a suggestion (Ege et al. 2007, Iossifova et al. 2007) of an inverse association. Although β-D-glucan is a fungal marker mainly originating from fungi, there may also be other sources, such as in some bacteria and higher plants. In addition, concentrations of β-D-glucan differ widely between different fungal species (Iossifova et al. 2008). Preliminary evidence suggests there might be some association between β-D-glucan and ergosterol and respiratory symptoms (Douwes 2005).

Table 8. Summary of studies evaluating the association between EPS in house dust and asthma, wheezing and/or atopy in children.

AUTHOR	YEAR	STUDY DESIGN	AGE[1]	N	AGE[2]	SAMPLE	UNIT*	ASTHMA	WHEEZING	ATOPY
Douwes et al.	1999	case-control	6-12	60	6-12	living room floor	/g	+	0[a]	not studied
						bedroom	/g	--	-[a]	not studied
						child's mattress	/g	0	--[a]	not studied
Douwes et al.	2006	birth cohort (PIAMA)	4	690	3 mo	living room floor	/m^2	--	0	--
			4		3 mo	living room floor	/g	0	0	0
			4		3 mo	infant's mattress	/m^2	0	0	0
			4		3 mo	infant's mattress	/g	0	0	0
Ege et al.	2007	cross-sectional (PARSIFAL)	5-13	440	5-13	child's mattress	/g	--	-	0
Ege et al.	2008	birth cohort (PASTURE)	birth	922	2 mo	mother's mattress	?	not studied	not studied	0
Gehring et al.	2007	nested case-control (AIRALLERG)	2-4	1069	2-4	child's mattress	/m^2	not studied	not studied	-
			2-4		2-4	child's mattress	/g	not studied	not studied	+
			2-4		2-4	living room floor	/m^2	not studied	not studied	-
			2-4		2-4	living room floor	/g	not studied	not studied	0
Tischer et al.	2011	nested case-control	6	346[#]	5	child's mattress	/m^2	--	0	not studied
			6	332[$]	5	child's mattress	/m^2	+	0	not studied
			6	346[#]	5	living room floor	/m^2	-	0	not studied
			6	332[$]	5	living room floor	/m^2	+	0	not studied

[1]age of the children (years if no other indicated) when health assessment was done and for prospective studies at the end of the follow-up, [2]age of the children (years if no other indicated) when exposure assessment was done, N= number of children, Unit=concentration (/g) or load (m^2), *concentration (/g) is in original papers either grams or milligrams, no association (0), suggestive positive association (+), significant positive association (++), suggestive inverse association (-), significant inverse association (--), [#]German children (LISA & GINI), [$]Dutch children (PIAMA), [a]bronchitits

Table 9. Summary of the studies evaluating the association between β-D-glucan in house dust and asthma, wheezing and/or atopy.

AUTHOR	YEAR	STUDY DESIGN	AGE[1]	N	AGE[2]	SAMPLE	UNIT*	ASTHMA	WHEEZING	ATOPY
Bertelsen et al.	2009	birth cohort (ECA)	10	260	2	living room floor	/g	0	not studied	0
Douwes et al.	2006	birth cohort (PIAMA)	4	690	3 mo	living room floor	/m^2	-	0	0
			4		3 mo	living room floor	/g	0	0	0
			4		3 mo	infant's mattress	/m^2	0	0	0
			4		3 mo	infant's mattress	/g	0	0	0
Ege et al.	2007	cross-sectional (PARSIFAL)	5-13	440	5-13	child's mattress	/g	0	0	-
Gehring et al.	2007	nested case-control (AIRALLERG)	2-4	1069	2-4	child's mattress	/m^2	not studied	not studied	--
			2-4		2-4	child's mattress	/g	not studied	not studied	0
			2-4		2-4	living room floor	/m^2	not studied	not studied	-
			2-4		2-4	living room floor	/g	not studied	not studied	0
Hsu et al.	2011	case-control study	3-9	101	3-9	settled dust from bed and surfaces	/g	0	0	0
Iossifova et al.	2007	cohort study (CCAAPS)	13 mo	574	8 mo	baby's primary activity room floor	/g	not studied	inverted U-shape	-
							/m^2	not studied	inverted U-shape	-
Tischer et al.	2011	nested case-control	6	346[#]	5	child's mattress	/m^2	-	-	not studied
			6	332[$]	5	child's mattress	/m^2	+	-	not studied
			6	346[#]	5	living room floor	/m^2	-	-	not studied
			6	332[$]	5	living room floor	/m^2	+	-	not studied

[1]age of the children (years if no other indicated) when health assessment was done and for prospective studies at the end of the follow-up,[2]age of the children (years if no other indicated) when exposure assessment was done, N= number of children, Unit=concentration (/g) or load (m^2), *concentration (/g) is in original papers either grams or milligrams, no association (0), suggestive positive association (+), significant positive association (++), suggestive inverse association (-), significant inverse association (--), [#]German children (LISA & GINI), [$]Dutch children (PIAMA)

2.3.4 Other markers of microbial exposure

Microbial exposure can be determined by using qPCR. Only one prospective study (Reponen et al. 2011) and two cross-sectional studies (Vesper et al. 2007, Vesper et al. 2008) have so far used qPCR to evaluate the association between fungal exposure at home and asthma in children, but none have used this method to study the association between bacterial exposure and asthma or other allergic diseases. The studies mentioned above have used the Environmental Relative Mouldiness Index (ERMI), which is a relative scale between mould groups of water damage related moulds and non-damage related moulds, and describes the mould burden in homes using 36 different fungal qPCRs. The prospective study found that a high ERMI value in infancy was associated with increased risk of asthma at the age of seven years, but no association was found between high ERMI value at the age of seven years and asthma at the same age (Reponen et al. 2011). QPCR was also applied in a school that suggested an association between fungal exposure and respiratory symptoms (Cai et al. 2011, Simoni et al. 2011). These preliminary results need to be confirmed by other prospective studies.

Recent studies have applied a new approach, microbial indices (Ege et al. 2011, von Hertzen et al. 2010) to better describe the total scope of microbial exposure. In the study of microbial diversity, children from farms had a greater variety of microbial diversity than children from rural areas (Ege et al. 2011). In addition, the microbial diversity was inversely associated with asthma, but not with atopy. One can assume that not only is the quality of microbial exposure i.e. the wider microbial diversity relevant to the development of the immune system, but also the amount of the microbial exposure. An index constructed on the basis of the amount of three different microbial components was tested in a small Finnish study, and a higher amount of microbial exposure tended to decrease the risk of atopy (von Hertzen et al. 2010). More research of new methods to describe and analyse microbial exposure is warranted in the research of allergic diseases.

2.4 Farming effect — protection from allergic diseases in children

Children who grow up on farms have lower prevalence of atopy, allergic rhinitis and asthma (von Mutius and Vercelli 2010) than children who live on other rural or urban environments. A recent meta-analysis estimated that the prevalence of asthma in people from a farming environment was approximately 25% lower than in other people (Genuneit 2012).

Numerous cross-sectional studies have mostly consistently found that a farming environment is associated with less allergic rhinitis or rhinoconjunctivitis and atopic sensitisation, but not as strongly as with asthma (Naleway 2004). The protective

effect of a farming environment in childhood seems to be persistent if maternal exposure to cowsheds during pregnancy is followed by children's exposure in the early years (Douwes et al. 2008, Remes et al. 2003, Riedler et al. 2001). Moreover, children from farming families with livestock have less atopic sensitisation in adulthood (Lampi et al. 2011).

Farm exposure, such as maternal contact with different farm animal species and working in hay barns, seems to activate the innate immune system in *utero* (Pfefferle et al. 2010) and the influence may continue during the first years of life (Riedler et al. 2001). A potential explanation for the observed effects may be exposure to microbes including a greater variety and higher levels of fungi and bacteria (Ege et al. 2011). According to hygiene hypothesis (Strachan 1989), exposure to high concentrations of microbial agents with pro-inflammatory properties stimulates the maturation of Th1-oriented immunity, instead of the atopic Th2-oriented immunity, which leads towards allergic reaction patterns (Braun-Fahrlander et al. 2002). It has been hypothesized that maternal exposure to farm animals might act as a natural model of immunotherapy to the child (Schaub et al. 2009). It is clear that the mechanisms behind the protective effects are complex and are likely to include gene–environment interactions and epigenetic changes (Lluis and Schaub 2012).

Consumption of unboiled farm milk has been associated with a lower risk of allergic diseases (Braun-Fahrländer and von Mutius 2011, Illi et al. 2006, Loss et al. 2011, Perkin and Strachan 2006, Riedler et al. 2001, Waser et al. 2007), but this was not found in a Finnish cross-sectional study on atopic sensitisation (Remes et al. 2003). Farm milk has been found to be associated with allergic diseases independently of other farm related factors (Braun-Fahrländer and von Mutius 2011). Unboiled farm milk contains several milk components, such as a concentration of pathogenic bacteria and whey proteins, which occur more in unboiled than in boiled farm milk or shop milk (Lluis and Schaub 2012). However, the mechanism and causes behind the farm milk effect are unknown (Braun-Fahrländer and von Mutius 2011). It has been suggested that the association may be mediated by another mechanism than the stable exposure due to independent effects on asthma from other farm related factors and the effect has also been seen independently of living on a farm (Lluis and Schaub 2012). However, the consumption of unpastured farm milk is not encouraged for protection against allergic diseases since raw milk may increase the risk of severe infections (Braun-Fahrländer and von Mutius 2011).

To date, there are only a few cohort studies focusing on rural or farming environments. In the PASTURE study, which is one of two birth cohorts in the present thesis, maternal consumption of boiled farm milk during pregnancy was associated with more atopic sensitisation to cow's milk, but maternal contact with livestock and hay was associated with less atopic sensitisation to seasonal allergens in the cord blood (Ege et al. 2008). Another cohort among school-aged children found that farmers had less atopy, less incident atopic sensitisation and they were more likely to lose atopic sensitisation during the three-year follow-up (Horak et al. 2002). More

prospective studies are needed to clarify the mechanisms behind the effects, timing of exposure and the factors behind the observed farming effect.

3 AIMS OF THE STUDY

The aim of the thesis was to evaluate the association of early-life exposure to moisture damage or mould and microbial exposure in the home with asthmatic symptoms, infections and allergic diseases during the first six years of life.

The specific aims were:

1. To examine the association between objectively determined moisture damage and mould in homes during infancy and wheezing symptoms, infections and atopic dermatitis until the age of 18 months and atopic sensitisation at the age of one year (Study I)
2. To investigate the association between objectively determined moisture damage and mould in homes during infancy and wheezing symptoms, infections, atopic dermatitis and allergic rhinitis up to the age of six years and atopic sensitisation at the age of six years (Study II)
3. To assess the microbial exposure at the age of two months and onset of asthma, wheezing symptoms, atopic dermatitis and sensitisation during the first two years of life (Study III)
4. To examine the association between microbial exposure at the age of two months and development asthma, wheezing symptoms, infections, atopic sensitisation, atopic dermatitis and allergic rhinitis up to the age of six years (Study IV)

4 MATERIALS AND METHODS

4.1 Study design

The study populations consisted of two prospective birth cohorts (PASTURE and LUKAS studies), which have been followed using practically similar protocols. PASTURE is a multicenter birth cohort study in Europe and LUKAS is a Finnish birth cohort. Study children have been followed-up from the third trimester of pregnancy. The follow-up data that has been used in this thesis is presented in Table 10.

Table 10. The time points for health outcomes measured by using questionnaires and the time points for exposure assessment (age in years if no other indicated).

Health outcomes	2 mo	1	1.5	2	3	4	5	6	Study[#]
Doctor-diagnosed wheezing[$]		X	X						I
Doctor-diagnosed asthma, ever[£]		X	X	X	X	X	X	X	II-IV
Current asthma^		X	X	X	X	X	X	X	II, IV
Wheezing apart from cold		X	X	X	X	X	X	X	I-IV
Any wheezing		X	X	X	X	X	X	X	I-IV
Cough apart from cold		X	X	X	X	X	X	X	I, II, IV
Nocturnal cough apart from cold		X	X	X	X	X	X	X	I, II, IV
Otitis		X	X	X	X	X	X	X	I, II
Laryngitis		X	X	X	X	X	X	X	I, II
Pneumonia		X	X	X	X	X	X	X	II
Common cold with fever			X	X	X	X	X	X	I, II
Common cold without fever			X						I
Fever (>38.5°C) with no resp. symptoms			X						I
Doctor-diagnosed atopic dermatitis, ever		X	X	X	X	X	X	X	I-IV
Doctor-diagnosed allergic rhinitis, ever								X	II
Rhinoconjunctivitis						X	X	X	II
Serum, sIgEs		X						X	I-IV
Exposure assessment									
Home inspection for moisture damage	X------X								I, II
Dust sampling with vacuuming	X								III, IV

[$] Doctor-diagnosed asthma and/or asthmatic bronchitis, [£] Doctor-diagnosed asthma once or asthmatic bronchitis at least twice during the follow-up, ^Doctor-diagnosed asthma ever with current medication/symptom at the age of 6 years

[#]The follow-up data until the age of 1.5 years (I) and until the age of 2 years (III), otherwise six years

4.1.1 Study population — PASTURE study (III)

The Protection against Allergy – STUdy in Rural Environments (PASTURE)(von Mutius and Schmid 2006) is an ongoing multi-center birth cohort study in five European countries: Austria, Finland, France, Germany and Switzerland. In the period of 2002–2005, pregnant women who lived on farms and equal size of pregnant women who lived in rural areas were invited to the study.

In total 1133 children were included in the study between August 2002 and March 2005 (Figure 1). The inclusion criteria of the mothers were living on a farm with livestock or in rural areas, a maternal age of 18 years or older, singleton pregnancy, delivery in a hospital, no plans to move from the study area and common mother tongue of the country. Exclusion criteria after delivery were premature delivery (<37 weeks of gestation), home delivery, congenital abnormalities in the infants, and failure to obtain cord blood samples. A family could participate in the study only with one child. The families were not recruited if they did not have a phone.

The study protocol for PASTURE was approved by the Research Ethics Committees in each of the study centers. A written informed consent was obtained from the parents of participating children.

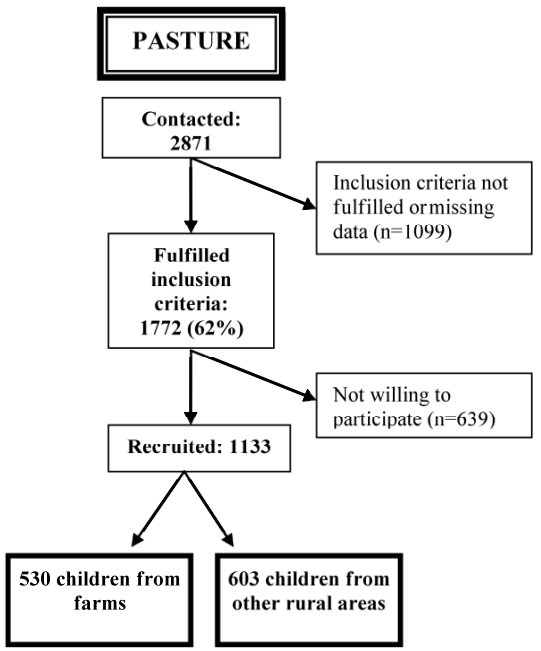

Figure 1. Recruitment of the study population in the PASTURE study.
(Modified from Ege et al. 2008)

4.1.2 Study population — LUKAS study (I, II and IV)

The LUKAS study consists of two Finnish ongoing birth cohort studies (Karvonen et al. 2009): the LUKAS1 study is the Finnish part of the PASTURE cohort and the LUKAS2 is its extended cohort (Figure 2). In the LUKAS1 study, 214 children were recruited between September 2002 and May 2004. All pregnant women who lived on farms with livestock and an equal number of non-farming women who lived in rural areas were invited to the study at 20–34 weeks of gestation in the areas of four central hospitals in Eastern and Middle Finland (Kuopio, Jyväskylä, Joensuu and Iisalmi).

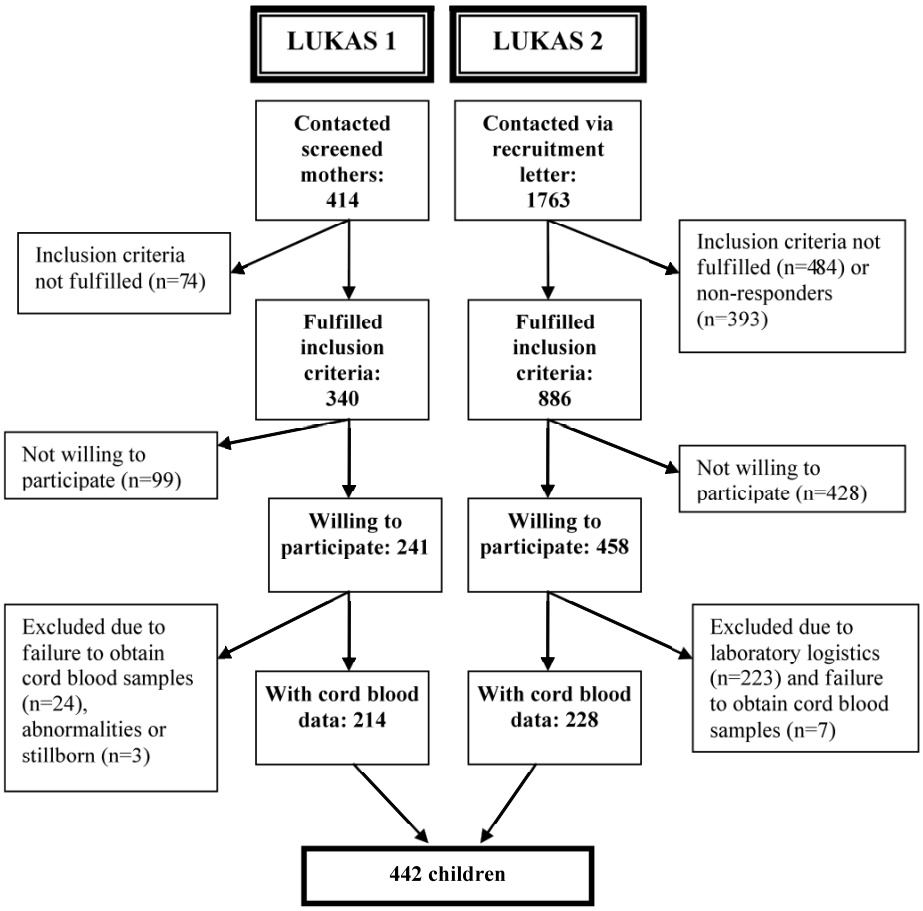

Figure 2. Recruitment of the study population in the LUKAS study: inclusions, exclusions and refusals. (Modified from Study I, Figure 1)

The LUKAS2 study includes 224 children (Figure 2). The inclusion and exclusion criteria of the LUKAS2 were similar to those of the PASTURE study, though without selection by the parental occupation or area of living. All the women who were estimated to give birth in the Kuopio University Hospital between May 2004 and May 2005 and who did not live in apartments were invited to participate in the study. For logistic reasons, the women delivering at the end of a week (from Thursday afternoon to Sunday morning) were excluded from the extended cohort.

The study protocol of LUKAS1 and LUKAS2 was approved by the Research Ethics Committee of the Hospital District of Northern Savo, Kuopio, Finland. A written informed consent was obtained from the parents of participating children in each cohort.

4.2 Questionnaires

The first questionnaire was administered during the third trimester of pregnancy, and follow-up data were collected when the children were 2, 12, 18 and 24 months old, and thereafter annually. All the questionnaires were self-administrated by parents, except the two-month questionnaire in the PASTURE study (and in the LUKAS 1 study), which study nurses administered during a home site visit while interviewing the parents. The two-month questionnaire enquired about information on the end of the pregnancy, the mode of delivery, birth weight and height, use of medication by the mother or the child, breastfeeding, dog and cat ownership and staying indoors, mother working in or child staying in a cow shed or hay barn, and second-hand smoking. The follow-up questionnaires after the two-month questionnaire enquired about the symptoms and diseases of the children as well breastfeeding, the consumption of food and farm milk, the use of medication, second-hand smoke, dog and cat ownership and staying indoors, mother working in or child staying in a cow shed and hay barn, and day care attendance for the time period after the preceding questionnaire. The response rates for each follow-up were from 80 to 95%.

4.2.1 Health outcomes

Asthma was defined in two ways. In Study I, asthma was called doctor-diagnosed wheezing and it was defined as doctor-diagnosed asthma and/or asthmatic (obstructive) bronchitis at least once up to the age of 18 months. In the other Studies (II, III and IV), asthma was defined as the first parent-reported doctor-diagnosed asthma and/or parent-reported second doctor-diagnosed asthmatic (or obstructive) bronchitis during the two-year (III) or the six-year follow-up (II and IV) (incident asthma).

‘Current asthma’ was defined as asthma ever up to the age of six years together with reported asthma medication or wheezing during the past 12 months at the age of six years (II and IV).

'Atopic dermatitis' was defined as the first parent-reported doctor-diagnosed atopic dermatitis at the two-year (III) or six-year follow-up (II and IV) (incident atopic dermatitis).

Allergic rhinitis was defined as parent-reported doctor-diagnosed allergic rhinitis ever in life (from the six-year follow-up questionnaire) (II). Allergic rhinoconjunctivitis, which was enquired about at the four-year follow-up questionnaire onward, was defined as parent-reported sneezing, a runny or blocked nose apart from cold accompanied by itchy, watery eyes (II).

Wheezing apart from cold, any wheezing, cough apart from cold and nocturnal cough was defined as a parent-reported symptom, which occurred less than once a month or more frequently for the time period after the preceding questionnaire.

Otitis media, laryngitis (I, II) and pneumonia (II) were defined as a positive answer for the question about having a given infection for the time period after the preceding questionnaire. The number of episodes of fever (≥38.5°C) without other symptoms was defined as at least one episode (I). The number of episodes of common cold with fever was defined as at least two episodes of the given infection (I, II) and the number of episodes of common cold without fever was defined as at least three episodes (I). Laryngitis, pneumonia and otitis media were parent-reported diagnoses; only laryngitis and pneumonia at the 12-month questionnaire were doctor-diagnosed diagnosis (I and II).

4.2.2 Confounders and determinants

Data on housing characteristics were collected with a questionnaire during the home investigation for moisture problems and with two questionnaires filled out when the study children were two months old: a paternal questionnaire and a questionnaire related to dust sampling.

All models were adjusted for study center (only in III) or study cohort (I, II and IV), living on a farm, gender, the maternal history of allergic diseases (asthma, atopic dermatitis or allergic rhinitis), number of older siblings (≥2, 1 vs. 0) and smoking during pregnancy (I–IV).

The models were also tested for additional confounding factors. If a confounder changed the estimates of the moisture damage in the kitchen or main living area more than 10%, respective outcome were adjusted for the factor. These factors were the paternal history of allergic diseases, parental educations, the age of mother, birth weight, the mode of delivery, second-hand smoke, dog and cat ownership and staying indoors, mother working in a cow shed and hay barn during pregnancy, the presence of a dung hill near the residence, breastfeeding, the consumption of farm milk and day care attendance, and in addition (only in III, IV), season and floor type of dust sampling and moisture damage or mould in the living area (kitchen, living room or bedrooms). In Study III, the determinants of house dust samples were evaluated,

and the models of health outcomes were additionally tested for these factors (see III, Supplement Table S1 and S2).

4.3 Specific IgEs

Venous blood samples were collected at the age of one and six years. Specific immunoglobulin E (sIgE) to 19 common allergens were analysed using the Allergy Screen Test Panel for Atopy (Mediwiss Analytic, Moers, Germany) (Herzum et al. 2005). All the measurements were done in Marburg, Germany. The 13 inhaled allergens tested were two house dust mites (*Dermatophagoides pteronyssinus and D. farinae*), seven pollen (alder, birch, European hazel, grass pollen mixture, rye, mugwort and plantain), cat, horse and dog dander, and the mould *Alternaria alternata*. Six food allergens were hen's egg, cow's milk, peanut, hazelnut, carrot and wheat.

Atopy was defined as any sIgE concentration of 0.35 kU/L or greater at age one year (I, III, IV). The cut-off level was 0.70 kU/L at the age of six years (II, IV). Atopic sensitisation to inhalant allergens was defined as one or more sIgE to inhalant allergens as 0.35 kU/L or greater, and sensitisation to food allergens as one or more sIgE to food allergens as 0.35 IU/L or greater at the age of one year and 0.70 kU/L or greater at the age of six years.

In addition, the same 19 specific IgEs were determined from the mothers during the delivery and from the fathers during the two-month home site visit in the LUKAS1 study and around the first birthday of his child in the LUKAS2 study. Parental atopy (II) was defined as maternal and/or paternal atopic sensitisation to any of the tested allergens (cut-off level $\geq$3.50 kU/L).

4.4 House dust samples

Two months after delivery dust samples were collected from living room floors by fieldworkers in the PASTURE study including the LUKAS1 study and by parents in the LUKAS2 cohort. The living room was defined as the room where the family spends most of their time in the evenings after dinner. At the same follow-up, dust samples were taken from the mother's mattress. A similar protocol, the same type of vacuum cleaners (Miele 516) and nylon dust sample socks were used in each study centre in the PASTURE cohort and in the LUKAS2 cohort.

In the PASTURE study (III), the vacuumed area and time were either 1 m^2 of wall-to-wall carpet (for two minutes) or 4 m^2 of smooth floor (for four minutes). In the case of a smooth floor with a rug, an additional sample from a 1 m^2 area of the rug was vacuumed (for two minutes). The whole surface of the mother's mattress was vacuumed for two minutes after removing duvets or blankets. The collected dust samples were used for EPS and endotoxin analyses.

In the LUKAS1 study (IV), an additional living room floor dust sample for the analyses of microbial markers other than those mentioned above was collected from another part of the rug (2 m^2 vacuumed for 4 minutes) or smooth floor (4 m^2 vacuumed for 4 minutes).

In the LUKAS2 (IV), a living room floor dust sample was collected from a rug by vacuuming an area of 1 m^2 for two minutes or in the absence of a rug, an area of 4 m^2 for two minutes from the smooth floor. All the microbial analyses were done using one sample per house.

4.4.1 Determination of EPS, endotoxin and β-D-glucan

The samples were stored at -20°C without sieving until extracted in Utrecht, The Netherlands (Schram et al. 2005). The levels of fungal EPS of *Penicillium* spp. and *Aspergillus* spp. were measured with an antigen-specific sandwich enzyme immunoassay (Douwes et al. 1999), the levels of endotoxin by the kinetic chromogenic Limulus Amebocyte Lysate test (Bio Wittaker, Walkersville, MD) (Douwes et al. 1995) and the levels of β-D-glucan were measured with a β(1,3)-D-glucan-specific inhibition enzyme immunoassay (Douwes et al. 1996).

In Study III, the detection limit of the amount of house dust was 20 mg. In the case of the nondetectable amounts of dust (eight from mothers' mattresses, and 11 from living room floors), a value of 13 mg (two-thirds of the detection limit) was assigned, and the levels of EPS and endotoxin in these samples were excluded from further data analyses. The detection limit of EPS was 141 EPSU/g. In the case of the nine samples with nondetectable amounts of EPS, a value of 94 EPSU/g was assigned. All samples contained detectable levels of endotoxin. In the case of missing data on the loads or concentrations of EPS or endotoxin in the rug sample, the given load or concentration was replaced from smooth floor samples. Levels were expressed as endotoxin units (EU) and EPS units (EPSU) both per square meter of sampled area (load) and per gram of sampled dust (concentration).

In Study III, the levels were expressed as units per square meter of sampled area of a rug (load) or area of the smooth floor in the case of absence of a rug.

4.4.2 Determination of chemical markers and quantification of microbial species, groups or genera

The dust samples for chemical markers and for qPCR were first sieved and then stored at -20°C until being analysed at the National Institute for Health and Welfare (THL), Kuopio. Ergosterol (as a marker of fungal biomass), $LPS_{10:0–16:0}$ (based on 3-hydroxy fatty acids with chain lengths $C_{10:0}$–$C_{16:0}$) (as a marker of Gram-negative bacteria) and muramic acid (as a marker of Gram-positive bacteria) were determined in house dust using gas chromatography tandem mass spectrometry (GC-MS-MS). Sample preparation for ergosterol (Lappalainen et al. 2008) as well as for muramic

acid and 3-hydroxy fatty acids have been described previously (Lappalainen et al. 2012).

The qPCR-method (Brinkman et al. 2003, Haugland et al. 1999, Haugland et al. 2004, Rintala et al. 2004, Torvinen et al. 2010) was applied to determine the load (cells/m^2) of two gram-positive bacteria genera, *Mycobacterium* spp. and *Streptomyces* spp., and six fungal species, genera or groups: *Aspergillus fumigatus* / *Neosartorya fischeri*; *Cladosporium* spp.; the combined assay group for *Penicillium* spp., *Aspergillus* spp. and *Paecilomyces variotii* (=PenAsp group); *Stachybotrys chartarum*; *Trichoderma viride* / *atroviride* / *koningii* (=Trichoderma viride group); and *Wallemia sebi*. The analyses were done at THL, Kuopio.

4.5 Home inspections for moisture damage

In the LUKAS1 study, either of the two trained civil engineers of the study group inspected the home for moisture damage and visible mould when the child was approximately 2.0 months old (SD 0.3 months, range 0.8–3.1 months). The engineers had several years of experience in the use of a standard protocol (Nevalainen et al. 1998). In the LUKAS2 study, one civil engineer (who was one of the two engineers in LUKAS1) inspected all the homes when the child was 8.4 months old on average (SD 6.9 months, range 1.1–25.0 months). Overall, home inspections were performed before the age of six months in 312 (79.5%) children.

The homes were inspected for signs of moisture in the surfaces and the structures using a pre-designed checklist (Nevalainen et al. 1998, Pekkanen et al. 2007). The information recorded during the home visit was focused on the whole house and details of every individual damaged area were recorded and allocated in the analyses into four areas (Pekkanen et al. 2007): kitchen; main living area; bathroom; and other areas. Main living area consisted of bedrooms, including the child's bedroom, living rooms and the main hallways connecting these rooms. Moisture damage was classified into three classes, i.e. no damage, minor damage and major damage, based on a six-point "need for repair" estimation scale (Nevalainen et al. 1998) and the area of the damage. Classes 0 and 1 meant damage with no need for repair or only cosmetic repair; class 2 meant a repair of surface materials needed; class 3 meant a repair of structural components needed; and classes 4 and 5 meant more extensive repair needed. "No damage" was defined as need-for-repair classes 0 or 1 (Pekkanen et al. 2007). "Major damage" was defined in three different cases: 1) a need-for-repair class 2 with the area of damage $\geq$1 m^2, 2) a need-for-repair class 3 with the area of damage $\geq$0.1 m^2, or 3) a need-for-repair class 4 or 5. Other damage in the given area was classified as "minor damage". During the home inspection, observed mould was also categorized separately in the four areas of the home into three classes: no mould, spots of mould and visible mould. Due to the small numbers of

homes with visible mould, the mould observations were categorized into two classes (any visible mould vs. no mould) in the given area in Study I.

4.6 Statistical analyses

Bivariate analyses were conducted by Pearson χ^2 – test or Fisher's exact test in categorized variables (I–IV). In Study III, the loads and concentrations of EPS and endotoxin and the amounts of dust were not normally distributed and were therefore transformed using a natural logarithm (ln-transformed). In addition, mean values were expressed as geometric means (GM) with their geometric standard deviations (GSD) as well as in the case of determinants, geometric means with their 95% confidence intervals (CIs) (III). ANOVA with ln-transformed data was used to analyze associations between levels of microbial exposure in the different classes of determinants (III). Correlations between microbial markers were calculated as Spearman's correlation coefficients (III, IV).

Logistic regression was used to analyse the associations between moisture damage and mould and doctor-diagnosed wheezing, respiratory symptoms and infections in Study I. It was similarly used to analyse the association between moisture damage and mould or microbial exposure and atopic sensitisation to allergens at the age of one year and six years (I-IV) or doctor-diagnosed allergic rhinitis ever (II).

Generalized estimating equations (GEE) with an exchangeable correlation structure to account for correlation between repeated measures within subjects were used to determine associations between moisture damage and mould or microbial exposure and repeated measures of parent-reported wheezing and cough at ages 1, 1.5, 2, 3, 4, 5 and 6 years (II–IV), except when the follow-up time was two years in Study III. In addition, common cold with fever from the 18-month questionnaire onward, otitis and laryngitis and allergic rhinoconjunctivitis from the four-year questionnaire onward were analyzed using GEE models in Study II.

Discrete-time hazard models were used to evaluate the association between moisture damage and mould or microbial exposure and the onset of asthma, current asthma or atopic dermatitis (II–IV).

The results were presented as adjusted odds ratios (aORs) with their 95% confidence intervals (95%CI).

Analyses of single microbial markers and diversity score (IV)

In the analysis of single microbial markers in Study IV, the amounts of dust and microbial markers were generally divided into three categories using tertiles as cut-offs. For *Wallemia sebi*, the lowest category consisted of levels below the limit of detection (percentage of samples below detection limit was 37.4%) and the rest of the values were evenly divided into two groups (medium and high). Due to high numbers of non-detected values for *Stachybotrys chartarum* and the assay group for

Aspergillus fumigatus / *Neosartorya fischeri* these were dichotomized at the detection level. For the microbial diversity score all markers measured by qPCR were dichotomized at the detection level. Microbial diversity was roughly estimated by calculating a diversity score as the sum of all the dichotomized variables of the measured qPCR markers (range 0–8). Due to a low number of observations with low and high diversity, the microbial diversity score was categorized into four classes: 0–4, 5, 6 and 7–8.

Analyses of quantity score (IV)

The microbial quantity score was calculated as the sum of three markers for the three different microbial groups, i.e. for total fungal (ergosterol and β-D-glucan), gram-negative (endotoxin and $LPS_{10:0–16:0}$) and gram-positive (muramic acid) bacteria (one marker per group). Single markers were first divided into five categories using quintiles as cut-offs with scores 0, 1, 2, 3 and 4 from lowest to highest and then summed. The following markers were used to create four different microbial quantity scores: 1) ergosterol, endotoxin and muramic acid; 2) β-D-glucan, endotoxin and muramic acid; 3) ergosterol, $LPS_{10:0–16:0}$ and muramic acid; and 4) β-D-glucan, $LPS_{10:0–16:0}$ and muramic acid. The created variables, with ranges from 0 to 12, were divided into five categories using quintiles as cut-offs for statistical analyses. Linear and quadratic trends in the associations between microbial diversity or quantity scores and health outcomes were tested using polynomial contrasts (Davis 2010).

5 RESULTS

5.1 Moisture damage and mould in the home and adverse health effects (I, II)

In the LUKAS study, home inspection data for moisture damage and mould at early age and follow-up data on health outcomes until the age of 18 months were available for 396 (90%) of 442 children (I). Data on six-year follow-up were available for 377 (85%) of the children (II).

Out of 396 homes of the study children, 55 (14%) had observed major moisture damage in the main living area and ten of these (18%) were confirmed by parents in the one-year questionnaire. The respective figures for minor damage in the main living area were 73 (18%) and seven (10%). Out of the 268 non-damaged homes observed by inspector, 258 (96%) were confirmed by parents.

5.1.1 Asthma and respiratory symptoms

The presence of moisture damage in the kitchen and visible mould in the main living area or in the child's bedroom increased the risk of doctor-diagnosed asthma in early childhood (I, Table 2). The moisture damage in the kitchen increased the risk with a dose-response. After the six-year follow-up, the association remained significant concerning major or highest severity of the moisture damage or visible mould in children`s bedroom (aOR 3.84, 95%CI 1.03–14.29 for major moisture damage, aOR 2.75, 95%CI 1.15–6.62 for highest severity of moisture damage and aOR 5.33, 95%CI 1.39–20.46 for visible mould), although dose-response relationships were no longer observed (II, Table 1). Similar associations were seen for current asthma at the age of six years (II, Table 1).

The presence of moisture damage in the whole house or in the kitchen increased the risk of wheezing apart from cold in infancy (I, Table 2). After the six-year follow-up, significant associations were mainly seen for the main living area and for the child's bedroom (II, Table 2). In general, the associations between respiratory symptoms and moisture damage and mould in the main living area or in the child's bedroom or in the kitchen did not change over time, but there was a tendency for weaker associations among children aged three years or older than among those under three years old (II, Table 3).

Mostly non-significant associations were found between moisture damage and mould in the bathrooms, in the other interior spaces or in the classification of the whole house and asthma or respiratory symptoms in adjusted analyses both in in-

fancy (I, Table 1) and in the six-year follow-up (II, Supplement Table 2). No significant associations were found with mould odour (I, Table 2, II, Table 1 and 2).

No effect modifications were found for gender, the parental history of allergic diseases, cat ownership, dog ownership and cat and/or dog ownership (I). In addition, results were not notably changed in the analyses by excluding the children who had moved up to the age of 18 months.

There was a tendency that atopic children, i.e. children who were sensitized at least to one allergen at the age of one year or six years, had an increased risk of asthma when living in homes with moisture damage and mould (II, Table 4). No such associations were found among children with atopic parents (II, Table 4).

5.1.2 Atopic dermatitis, allergic rhinitis and atopic sensitisation

Positive, but not significant, associations were found between moisture damage or mould in the main lving area or in the child's bedroom and doctor-diagnosed atopic dermatitis or allergic rhinitis or allergic rhinoconjunctivitis (II, Supplement Table 4).

Several indicators of moisture problems were associated with atopic sensitisation to cat dander at the age of one year, visible mould as being the most significant (I, Table 4). However, no such associations were found at the age of six years with atopic sensitisation to inhalant allergens (II, Supplement Table 4) or with atopic sensitisation to cat allergen (data not shown).

5.1.3 Infections

Moisture damage or mould were not significantly associated with respiratory infections in infancy (I, Table 3) or during the six-year follow-up, except with common cold with or without fever, (I, Table 3) otitis media (I, Table 3 and II, Supplement Table 3) and laryngitis (II, Supplement Table 3). The presence of visible mould in the child's bedroom increased the risk of otitis media (II, Supplement Table 3). A similar tendency was seen with the major moisture damage and presence of visible mould in the main living area (II, Supplement Table 3).

In contrast, in the 18-month follow-up, otitis media tended to be inversely associated with the presence of visible mould in the whole house (I, Table 3) as were common colds with and without fever with the presence of visible mould in the kitchen (I, Table 3). Laryngitis was inversely associated with minor moisture damage in the main living area and child's bedroom in the six-year follow-up (II, Supplement Table 3).

5.2 Microbial exposure and health (III, IV)

In the PASTURE study, the number of children who had data on house dust samples, health outcomes and confounders was approximately 980 (86%) (III). In the LUKAS study, the corresponding figure was approximately 360 (81%) (IV). How-

ever, in the statistical models, including the indices of microbial exposure, the number was approximately 310 (70%).

In the PASTURE study, living room floor samples, which were included in the analyses, were almost solely vacuumed from a rug in Finland and from smooth floor in France (III, Table 1). The amount of dust and the levels of EPS and endotoxin from the living room floor were quite similar across centres when stratified by floor type (III, Table 1), except in France due to a low number of rugs. In every centre, the amount of dust and the loads of EPS and endotoxin were higher in the farmers' homes than in non-farmers' homes on the living room floor (III, Figure 1) and in the mother's mattress (data not shown). Determinants of the amount of dust and the levels of EPS and endotoxin from the living room floor are shown in the Supplement Tables S1 and S2 in Study III.

Table 11. The Spearman correlations of microbial markers per sampled area (/m^2).

		Mother's mattress			Living room floor	
		Amount of dust	EPS	Endotoxin	Amount of dust	EPS
Mother's mattress	EPS	.71				
	Endotoxin	.58	.60			
Living room floor	Amount of dust	.18	.09	.15		
	EPS	.18	.26	.20	.86	
	Endotoxin	.20	.22	.30	.82	.77

In the PASTURE study, the correlations between the amount of house dust and the loads of EPS and endotoxin were high in dust samples from the living room floor and from the mother's mattress (Table 11). Also, the correlation between the loads of EPS and endotoxin in dust from the living room floor was high and the corresponding correlation was slightly lower for dust samples from the mother's mattress. Both EPS and endotoxin loads between the two sampled locations were correlated only poorly. The correlations between the amount of dust and concentrations of EPS or endotoxin were all much lower (Table 12) than those between the amount of dust and the loads for microbial markers (Table 11).

In the LUKAS study, the description of the amount of dust, the single microbial markers and the indices of microbial exposure in dust from the living room floor are shown in Study IV, Table 1 while the correlations are presented in Study IV, Supplement Table E1.

Table 12. The Spearman correlations of microbial markers per gram of dust (/g).

		Mother's mattress			Living room floor	
		Amount of dust	EPS	Endotoxin	Amount of dust	EPS
Mother's mattress	EPS	.09				
	Endotoxin	-.14	.34			
Living room floor	Amount of dust	.18	.04	.08		
	EPS	.05	.51	.15	.09	
	Endotoxin	.06	.36	.33	-.08	.22

5.2.1 Asthma and respiratory symptoms

Single microbial markers and the amount of dust

In the LUKAS study with the six-year follow-up (IV), the associations of single microbial markers with asthma and respiratory symptoms were mostly non-significant, while the shape of the association curve varied (Figure 3 and 4). These models were adjusted for study cohort, farming status, gender, maternal history of allergic diseases (hay fever, atopic dermatitis and/or asthma), smoking during pregnancy, the number of older siblings, paternal history of asthma, major moisture damage in the home and the living area of the home, and additionally with paternal education (respiratory symptoms and asthma) and in analyses of atopic sensitisation to inhalant allergens with birth weight, season and floor type of dust sampling, presence of dung hill near the residence and heating with wood.

In the PASTURE study, asthma and wheezing during the first two years of life (III, Table 3) tended to be less common in children who lived in homes with higher amounts of dust in infancy. A similar result was seen with children during the first six years of life in the LUKAS study (Figure 3). Also, asthma and wheezing tended to be less common in children who lived in homes with higher loads of EPS and endotoxin in the PASTURE study (III, Table 3). In the LUKAS study, similar kind of association was found with EPS (Figure 4), but not with endotoxin (Figure 3).

In the PASTURE study, increased amounts of dust in the mother's mattress decreased the risk of asthma (III, Table 3). Endotoxin load in the living room floor sample showed a significant inverse association with asthma, as did EPS load on the living room floor with any wheezing (III, Table 3). No significant associations were observed between asthma or wheezing and concentrations of EPS or endotoxin (III, supporting information Table S3).

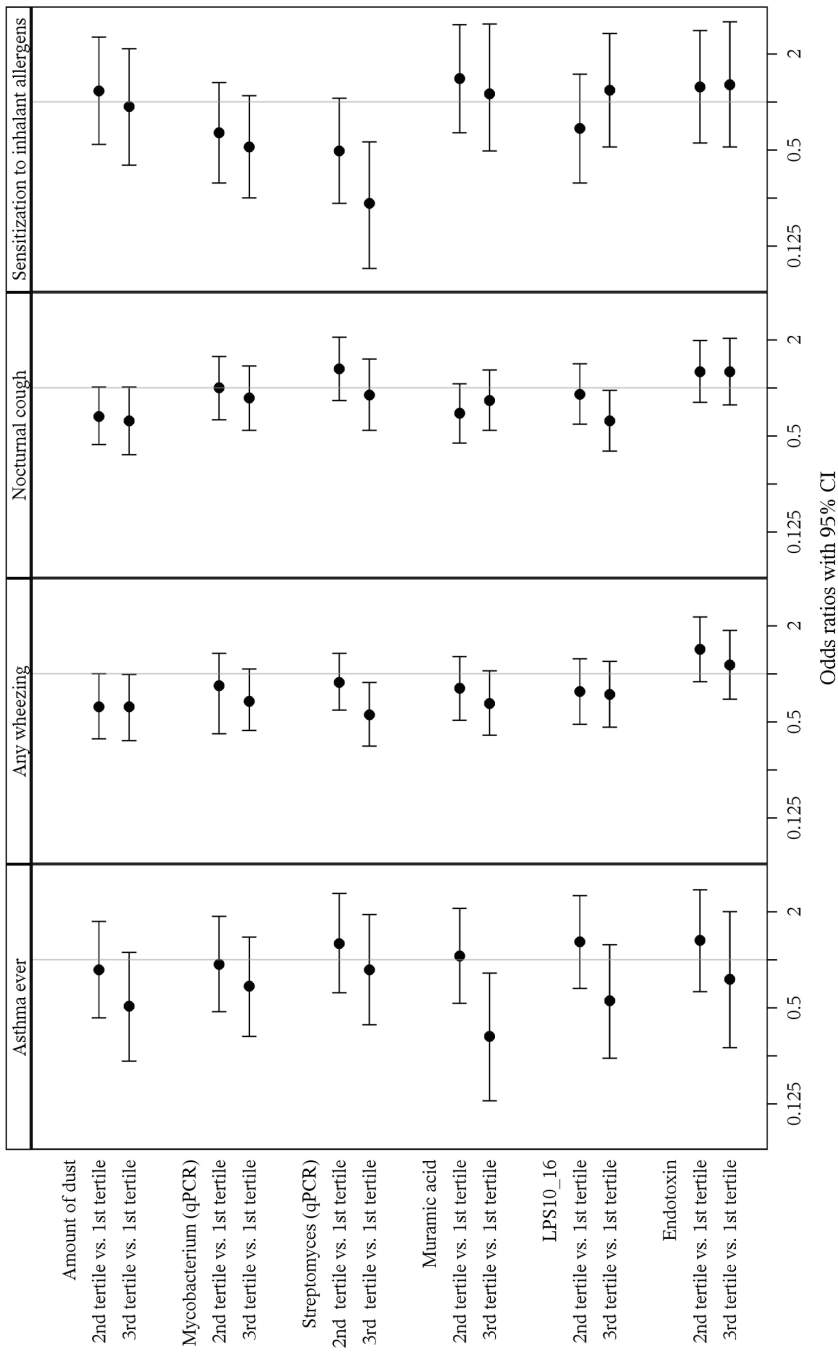

Figure 3. Adjusted associations between exposure to the amount of dust and load for bacterial markers at the age of two months and asthma, respiratory symptoms during the first six years of life and atopic sensitisation to inhalant allergen at the age of six years. (Adjusted for, see page 60.)

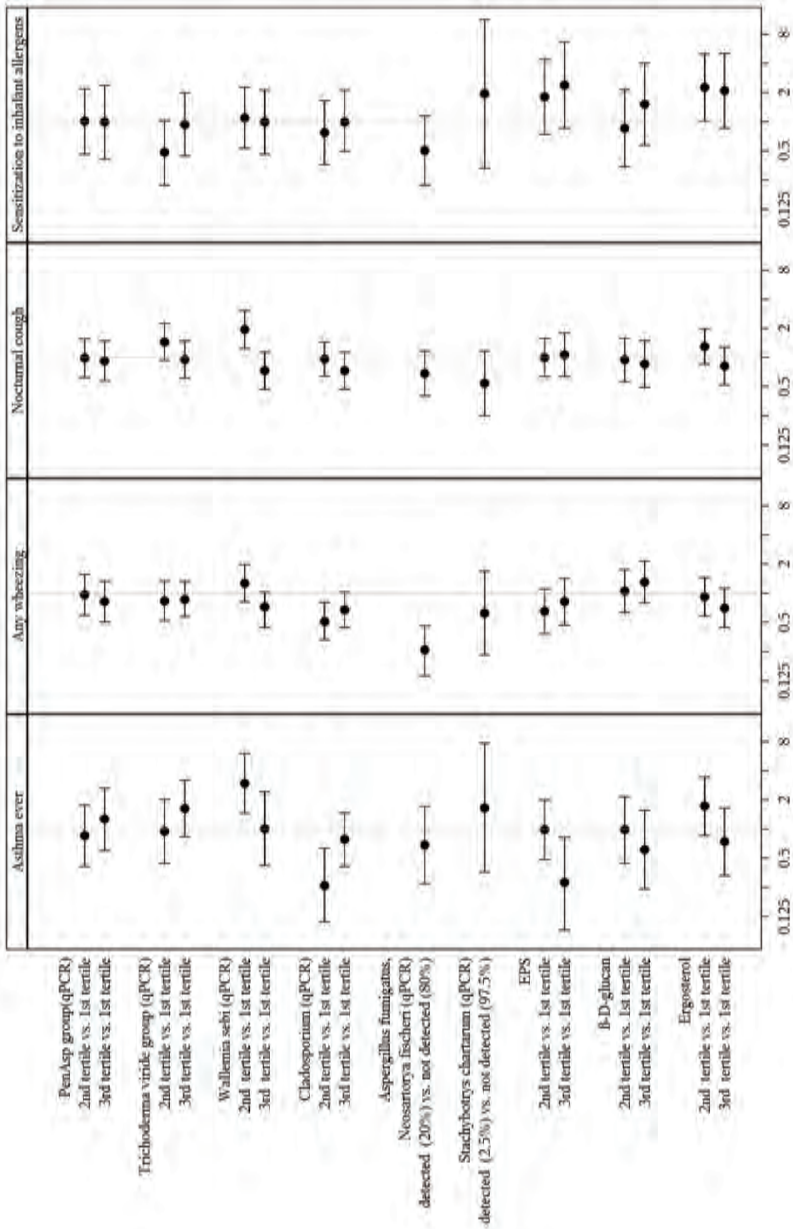

Figure 4. Adjusted associations between exposure to fungal loads at the age of two months and asthma, respiratory symptoms during the firstsix years of life and atopic sensitisation to inhalant allergen at the age of six years. (Adjusted for, see page 60.)

In further stratified analyses in the PASTURE study, inverse associations between three biological markers (amount of dust, EPS and endotoxin) and asthma or wheezing were seen especially among non-farmers' children (III, Table 5). In the PASTURE study (III), an effort to disentangle the roles of amounts of dust, EPS and endotoxin loads were made including all these three biological markers into the same model. Due to the high correlations between the markers, the direction and size of the observed associations for the three markers changed noticeably in general.

Microbial quantity and diversity score

The microbial quantity score was significantly (inverted U-shape, *p*-value for quadratic trend *<0.001*) associated with ever having asthma during the first six years of life independently of the diversity score in the LUKAS study (Figure 5): the highest risk for asthma was found with the medium level of microbial quantity score (aOR 3.45, 95%CI 1.07–11.15 for 3rd quintile) and the lowest risk at the highest quantity score (aOR 0.19, 95%CI 0.02–1.75 for 5th quintile) (Figiure 5). The *p*-values for the associations were quite similar to current asthma (IV, Table 3) and when using microbial markers other than ergosterol, endotoxin and muramic acid to create the quantity score (IV, Supplement Table E2). Quantity scores were inversely associated with any wheezing (IV, Table 3, *p*-value for linear trend *<0.001*). Adjusting for the amount of dust did not alter the results (data not shown).

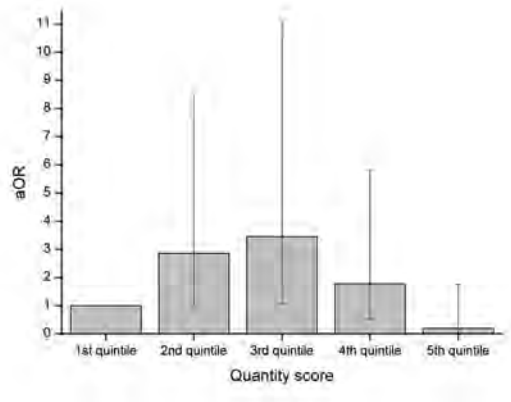

Figure 5. The risk of asthma during the first six years of life in relation to microbial quantity score in quintiles at the age of two months (sum of ergosterol, endotoxin and muramic acid). Model is adjusted for the same factors as listed on page 60, and additionally with diversity score.

In the LUKAS study, the number of detected microbial markers measured by qPCR (maximum of eight fungal or bacterial species, genera or groups) was associated with a decreased the risk of any wheezing and a similar tendency was seen for

asthma (IV, Table 3). The association with ever having asthma was, however, not robust to different adjustments, especially to the adjustment for farming. The association with any wheezing was similar among children living on farms and in rural or suburban areas as well as among children who were sensitized to at least one allergen at the age of six years, compared with those not sensitized (data not shown).

5.2.2 Atopic sensitisation to inhalant allergens and atopic dermatitis

Single microbial markers

In the PASTURE study, the risk of atopic sensitisation to inhalant allergens was increased at the age of one year with increasing endotoxin in the mother's mattress in infancy (III, Table 4 for loads and supporting information Table S3 for concentration) as was also seen with increasing endotoxin exposure in the mother's mattress and atopic sensitisation to any tested allergens (data not shown).

In the LUKAS study (IV), endotoxin exposure tended to increase the risk of atopic sensitisation to inhalant allergens at the age of one (IV, supplement Table E3) and at the age of six years (Figure 3). However, these results were not significant. No associations were found between any microbial markers and atopic sensitisation to food allergens in the PASTURE study or in the LUKAS study (data not shown). Furthermore, most of the associations between microbial markers and atopic sensitisation to inhalant allergens at the age of one year (IV, supplement Table E3) or six years (IV, Figure 2) were non-significant in the LUKAS study.

No associations were found between increasing EPS or endotoxin exposure and atopic dermatitis (III, Table 4 for loads and supporting information Table S3 for concentration) in the PASTURE study or in the LUKAS study (IV, supplement Table E3). In addition, most of the associations between microbial markers and atopic dermatitis were non-significant, however, the high level of amount of dust decreased the risk of atopic dermatitis and *Stachybotrys chartarum* was a risk factor for atopic dermatitis (IV, supplement Table E3).

Microbial quantity and diversity score

In the LUKAS study, the number of detected microbial markers measured by qPCR (maximum of eight fungal or bacterial species, genera or groups) were significantly (inverted U-shape) associated with atopic sensitisation to inhalant allergens at the age of six years (IV, Table 3), while a similar tendency, though non-significant, was seen with atopic sensitisation to inhalant allergen at one year (data not shown). No association was found between the number of detected qPCRs and atopic dermatitis (data not shown). Quantity scores were not associated with atopic dermatitis or atopic sensitisation to inhalant allergens (data not shown).

6 DISCUSSION

6.1 Observed moisture damage and mould or dampness in the home and health effects

The present study demonstrates that moisture damage, mould or dampness in the home are associated with wheezing and cough during the first six years of life. This supports the evidence from previous studies (Bornehag et al. 2001, Bornehag et al. 2004, Institute of Medicine 2004, Mendell et al. 2011, World Health Organization 2009). A recent systematic review confirmed that visible mould increases the risk of wheezing in children (Tischer et al. 2011a). Furthermore, most of the studies published after these recent reviews (Mendell et al. 2011, Tischer et al. 2011a) support the previous findings, although the articles other than the present study were mainly based on findings from cross-sectional studies with self-reported moisture damage, mould or dampness. Taken together, moisture damage and mould undisputably increase the risk of respiratory symptoms like wheezing in children.

The present prospective longitudinal study with home investigation in infancy shows that moisture damage, mould or dampness especially in the child's bedroom are associated with later asthma development is supported by the evidence of the recent reviews (Mendell et al. 2011, Tischer et al. 2011a, World Health Organization 2009). The systematic review by Tischer et al. (2011a) showed that visible mould increases 1.49-fold the risk of asthma in children. Following the recent reviews (Mendell et al. 2011, Tischer et al. 2011a), four prospective studies were published. In one of them (Hwang et al. 2011), the findings for the development of new asthma after exposure to observed moisture and moulds indoor are in line with the results of the present study, but the others have not found any association (Larsson et al. 2011, Reponen et al. 2011, Tischer et al. 2011c). These conflicting results might be partly explained by the fact that most studies have not assessed the location of the damage in the home. In addition, the reasons and mechanisms behind the observed associations are unclear.

In summary, moisture moisture damage and mould undisputably increase the risk of respiratory symptoms and the development of asthma. Visible moisture damage or mold growth, as well as the technical cause in the structure of the building, should be immediately renovated, without any need for microbial measurements. No microbial and other measurements are needed if such a condition is obvious.

Location of the damage

The results of this study demonstrate that the location of the moisture damage in the home is important as was suggested by Pekkanen et al. (2007) in a case-control study. The significant associations with asthma and wheezing during the first 18 months of life were mainly seen for moisture damage or mould in the kitchen and in the main living area, especially in the child's bedroom. These rooms are obviously the rooms where the children are expected to spend most of their time. Moisture damage and mould in the kitchen may be important for children during the first year of life, given that children of this age are usually taken care of at home (84% of the children in the LUKAS study) and they spend a lot of time in the kitchen with their parents. However, the kitchen as a place of damage seems to lose importance after the first years of life.

After the six-year follow-up, the most significant associations were found with the child's bedroom instead of the main living area. The main living area may include rooms where the children do not spend time if the houses are big enough, as was the case in the present study. Most of the families lived in detached single family houses with rather large (mean 120 m^2) living space and a quarter of houses were even larger farm houses. In the present study, the child's bedroom seemed to be the most important room for exposure during the whole childhood. Children spend approximately 10–12 hours per night in their bedroom in addition to significant periods during the daytime when playing.

In the present study, the child's bedroom was defined as the room where the child slept. In a prospective study with home inspection, the child's activity room was defined as the room where the child spent most of his/her time (Reponen et al. 2011). However, no results concerning observed moisture damage, mould or dampness in that room and asthma were reported. According to the findings of the present study, the location of the moisture damage, mould or dampness should be considered important when assessing the exposure and the rooms where the person spends time.

Weaker associations were observed with asthma and respiratory symptoms when moisture damage, mould and dampness for the whole house were studied. This was probably mostly related to the bathrooms, where moisture damage and visible mould were common, thus increasing the prevalence of the moisture damage, mould or dampness of the whole house. However, damage in these rooms was not associated with health outcomes, which was possibly due to the fact that bathrooms are well-ventilated and have usually negative air pressure, or the fact that characteristics of moisture damage, mould and dampness in the bathrooms is different, or the fact that time spent in the bathroom daily is limited.

In contrast to the findings of the present study, most of the earlier studies with self-reported moisture or mould damage and dampness found an association between such damage and asthma or wheezing, although the assessment of the moisture damage, mould or dampness was based on the whole house instead of the spe-

cific rooms or areas. These studies have mostly been cross-sectional studies with no objective assessment of moisture damage, mould or dampness as was shown in a recent review (Mendell et al. 2011). In addition, the estimates may have been biased due to cross-sectional designs with simultaneous reporting of home dampness, mould problems and adverse health effects (Strachan and Elton 1986).

On the other hand, many of these studies have included only small apartments, and the assessment of the moisture damage, mould or dampness in the whole house instead of specific rooms has not overestimated the exposure. Unlike the present study with rather large houses, the child is expected to spend time in all rooms more equally. In contrast, the recent prospective, longitudinal studies that have assessed the moisture and mould problems in the whole home have not revealed significant associations in longer follow-ups (Reponen et al. 2011, Tischer et al. 2011c). Thus, if the apartments are small, an overall assessment of the whole house with no room-specific assessments seems to be sufficient.

In summary, based on the present and previous studies, the location of the moisture damage, mould or dampness is important when assessing the exposure to moisture and mould problems. More weight in the exposure assessment should be given to rooms and areas of the house where the person spends most of his/her time. Specifically, moisture and mould damage in the bathrooms should be assessed separately from damage observed in other parts of the house. This is especially important in large, single-family houses.

Effect of length of the follow-up

The present study was not able to document changes over time in the effects between moisture damage, mould and dampness and asthma or respiratory symptoms. Moisture damage and mould in the child's bedroom in infancy were significantly associated with earlier as well as current asthma in the six-year follow-up, and there were no time interactions in respiratory symptoms with age when the children were classified into those under and those over three years of age. However, there was a tendency for associations between moisture damage or mould in the kitchen or in the main living area, but not in the child's bedroom, and respiratory symptoms may become weaker in a longer follow-up (three years or older).

In contrast, some recent prospective studies have found weaker associations over time (Cho et al. 2006, Reponen et al. 2011, Tischer et al. 2011c), which might be explained by characterizing the whole home instead of the specific rooms where the children usually spend their time. These studies have found, however, significant associations between moisture damage, mould or dampness in the whole home and wheezing in infancy but not later, which may be biased since the exposure has been assessed approximately at the same time or a few months before health outcomes have been enquired about (Cho et al. 2006, Tischer et al. 2011c).

In summary, the adverse health effects of early exposure to moisture damage, mould or dampness may be more likely to be persistent, if the damage is severe and if it is located where the child spends the most time.

Objective inspection versus self-reporting

In the present study, the exposure assessment was based on objective home investigation done by civil engineers, while most of the earlier studies relied on the parent-reported observations of moisture damage. As discussed above, self-reporting may cause information bias especially in cross-sectional studies when the parents with symptomatic children may be willing to over-estimate the damage, or parents occupying homes with damage may over-report the symptoms of the child (Strachan and Elton 1986).

The possibility of information bias has also ben suggested by two bith cohort studies in children. Self-reported indices of moisture damage were associated with doctor-diagnosed asthma in cross-sectional analyses in children at the ages of one to three years, but during follow-up, there were few significant associations with incident asthma (Larsson et al. 2011). A similar phenomenon was seen among older children (7–8 years old) in a cohort with a four-year follow-up (Bjerg and Ronmark 2008). Furthermore, the investigator should certainly be unaware of the health status of the residents; otherwise it might lead to artificial associations between home dampness and adverse health effects.

In the present study, when the results from the home investigation for moisture damage or mould were compared with parent-reported results, the investigator(s) observed more moisture damage or mould in the main living area than the parents reported in the one-year questionnaire. Most of the previous studies that have compared the results from objective and self-reported assessments, have found more signs of moisture damage, mould or dampness in the homes by investigator-based assessment, but found rather similar estimates with health measures with investigator-based and with occupants self-reporting (Emenius et al. 2004b, Verhoeff et al. 1995). However, not all studies have supported that finding (Hägerhed-Engman et al. 2009, Naydenov et al. 2008). Likewise, some studies have suggested that parent-reported moisture damage is more reliable than investigator-based, because the parents are aware of damage over a longer time period than investigators (Douwes et al. 1999). Although that study reported that there were no associations between EPS and observed moisture damage, mould or dampness in the home, the authors did not report results on the association between observed or reported moisture damage and respiratory symptoms (Douwes et al. 1999).

A home investigation requires manpower and other resources. Furthermore, the validity of results is dependent on the professional quality of investigator. In a nested case-control study, non-professional investigators reported less moisture damage, mould or dampness than the occupants, and the investigators reported

moisture damage, mould or dampness were not associated with health measures (Naydenov et al. 2008).

In summary, objectively observed moisture damage, mould and dampness are recommended due to reliable information of the exposure if the investigator is unaware of the health status of the occupants.

Atopic sensitisation, atopic eczema and allergic rhinitis

In the present study, there was some transient association between moisture and mould damage and dampness, and atopic sensitisation to cat dander at the age of one year, but not anymore at the age of six years. In addition, some (usually non-significant) associations were seen between atopic sensitisation to cat dander and several mould indices at the age of one year. However, the only significant association that was robust to adjustments was between atopic sensitisation to cat dander and mould damage in the bathroom, where the children spend less time, and where the character of microbial growth and the emission of harmful substances are obviously different than in the main living areas.

The recent reviews concluded that the evidence of the association between moisture problems and atopic sensitisation is insufficient (World Health Organization 2009) or only suggestive (Mendell et al. 2011). Likewise, a recent prospective meta-analysis did not find any association between moisture damage, mould or dampness and atopic sensitisation (Tischer et al. 2011c). However, the knowledge is limited concerning the early life exposure to moisture damage, mould or dampness and subsequent atopic sensitisation.

Some studies have suggested that mould damage may increase the risk of atopic sensitisation to inhalant allergens (Garrett et al. 1998, Jacob et al. 2002), but there are also conflicting findings (Cho et al. 2006, Rönmark et al. 2009). The IgE findings suggesting atopic sensitisation in infancy may merely reflect the natural maturation of the immune system, because no association between exposure to moisture and mould and dampness and atopic sensitisation were observed at the age of six years. Thus, the mechanisms behind the observed long-term adverse health effects seem rather to be due to irritation than IgE-mediated.

Although moisture damage, mould or dampness may not cause atopic sensitisation, they can be associated with asthma especially in atopic children, as documented in the present study, in a case-control study (Pekkanen et al. 2007), and in a meta-analysis of European birth cohorts (Tischer et al. 2011c). Most of the earlier studies (Iossifova et al. 2009, Jaakkola et al. 2005, Rydjord et al. 2007, Rönmark et al. 1999) have compared atopic asthma or atopic wheezing cases with all non-asthmatics or non-wheezers, which leads to biased estimates (Pekkanen et al. 2012), and thus, the results from different studies are poorly comparable. Children with atopic sensitisation may be more susceptible to react to environmental exposures such as microbes, which may lead to the development of asthma.

In the present study, no interaction between parental atopy and moisture damage or mould on asthma were observed, whereas in the meta-analysis, only 3-to-10-year old children with allergic parents had an increased risk of asthma (Tischer et al. 2011c). A similar interaction was seen in a cohort study when only children with maternal asthma had an increased asthma risk after exposure to moulds (Belanger et al. 2003).

In the present study, positive, but not significant, associations were found between moisture damage or mould and doctor-diagnosed atopic dermatitis, doctor-diagnosed allergic rhinitis or allergic rhinoconjunctivitis. In recent reviews, the evidence of an association between moisture damage, mould or dampness and allergic rhinitis was categorized as limited, suggestive (World Health Organization 2009) or sufficient (Mendell et al. 2011). Eczema was classified as having sufficient evidence, but no categorization was specifically done with atopic dermatitis (Mendell et al. 2011).

After the WHO review (2009), moisture damage, mould or dampness have been associated with allergic rhinitis or rhinoconjunctivitis in children in a systematic review (Tischer et al. 2011a), a meta-analysis (Tischer et al. 2011c) and in many observational studies (Civelek et al. 2010, Jaakkola et al. 2010, Shpakou et al. 2011, Sun and Sundell 2011), but not in all (Lam et al. 2011, Tischer et al. 2011b). In these recent studies, the children have mostly been approximately ten years old, thus older than the present study children. Regarding atopic dermatitis and moisture damage, mould or dampness, a cross-sectional study (Shpakou et al. 2011) and a study with a retrospective design (Garrido et al. 2010) have been published after the review (Mendell et al. 2011) and both of them found significant positive associations.

In summary, the evidence on the association between moisture damage, mould or dampness and atopic dermatitis is limited. Moisture damage, mould or dampness seem to be associated with allergic rhinitis, but the first six years of life is too short a time for an appearance in all cases. Findings concerning allergic rhinitis need to be confirmed in later follow-ups, and findings concerning atopic dermatitis need to be confirmed with prospective studies.

Respiratory infections

Moisture damage or mould in the home was not associated with respiratory infections during the first 18 months or at the six-year follow-up. Otitis media was an exception since visible mould in the child's bedroom and severe moisture and mould damage in the main living area increased the risk of otitis media. Thus, the present results of other respiratory infections are opposite to those of the WHO, which concluded that there is sufficient evidence that moisture damage, mould or dampness are associated with respiratory infections (World Health Organization 2009). Common cold, however, was classified as having limited or suggestive evidence for an association (Mendell et al. 2011).

During the first 18 months, the observed inverse association between visible mould in the whole house or kitchen and otitis media and common cold with and without fever was evidently not true, as all other respective odds ratios for different areas of the home were close to one. The same reason was probably behind the observation between minor moisture damage and laryngitis in the main living area and child's bedroom in the six-year follow-up, since no association was found with major moisture damage. In the case of major damage, the tendency was even protective. Thus, the results of the present birth cohort study do not support the conclusions presented in the report of WHO (2009).

In summary, findings concerning respiratory infections need to be confirmed with prospective studies.

Summary

The mechanisms behind the adverse health effects of moisture and mould problems have remained unknown. Evidence from epidemiological studies is mostly based on qualitatively assessed exposure data collected with questionnaires or home inspections. Such observed associations though strong are not sufficient to conclude any causal relationships.

In summary, the present results underline the importance of an assessment of moisture damage, mould and dampness in specific rooms of the home and support the earlier findings that moisture damage, mould or dampness in the home are significant risks for development of asthma in childhood. Multiple adverse health effects suggest that behind the observed effects, there are more than one causal agent and several mechanisms. Although the mechanisms and causal relationships are unclear thus far, it is highly recommended that moisture damage or mould growth and its cause are immediately renovated. No microbial measurements are needed if the occurrence of such conditions is obvious.

6.2 Microbial exposure and asthma and allergies

Asthma and respiratory symptoms

Epidemiological studies have mostly used endotoxin as a surrogate for microbial exposure to date. However, it is clear that such assessments provide an incomplete picture of the total quantity and quality of human microbial exposure (Heederik and von Mutius 2012). In the current study, two microbial markers were used to estimate the microbial exposure in the PASTURE study and a much wider set of microbial markers were analyzed in the LUKAS study. Due to the high correlation between detected microbial agents and the amount of dust, it is not possible to separate the individual effects of the microbial markers. Thus, there is a clear need to use indices of microbial exposure in the exposure assessment.

The results of the current study demonstrate that the microbial quantity score, consisting of the markers of gram-negative bacteria, gram-positive bacteria and fungi, in early life may be a better predictor of the later risk of asthma than single microbial markers. This is the first study using an index of microbial quantity score when studying the risk of asthma. Previously, an association between a microbial quantity score ($LPS_{10:0–16:0}$, ergosterol and muramic acid) and atopic sensitisation was evaluated in a cross-sectional study, which found a tendency for an inverse association (von Hertzen et al. 2010). In the present study, the microbial quantity score had an inverted U-shaped association with asthma, and the association was independent of the measured microbial diversity and amount of dust. In contrast, the single microbial markers were mostly non-significantly associated with asthma and the shape of the association varied. In the present birth cohort study, preliminary evidence was found that the microbial quantity score can be used to describe the inverted U-shaped association between the quantity of microbial exposure and asthma independently from the amount of dust and the diversity of the microbes.

The reason behind the observed association between microbial quantity and asthma is unclear. A potential, intriguing explanation is that different components of different microbial groups or genera share common antigen or molecular structures on their cell wall surface, which are detected by pattern recognition receptors presenting in innate immunity cells and have a profound influence on the immune system (Palm and Medzhitov 2009). If total exposure leading to a saturation of the recognition structures is the main causal factor explaining the association between the microbial exposure and lower risk of allergic diseases, then the microbial quantity score is likely to better reflect this exposure than single microbial markers. The varying correlations of single microbial markers with total microbial exposure would then explain the often weak and conflicting findings observed in different studies. In addition, some studies have shown synergistic interactions between different exposing agents (Huttunen et al. 2004) and an index of total microbial quantity may capture such synergistic effects. Data from the deep sequencing of microbial exposure (Margulies et al. 2005) will provide important information on microbial diversity and later create opportunities to further explore potential mechanisms related to that.

The present study demonstrates that the microbial quantity score has an inverted U-shaped association with asthma. Previous studies have suggested that the immune system may first enhance the response and subsequently achieve a tolerance (Liu 2002). The response-tolerance balance in children is age- and dose-dependent, and associations with a similar U-shape have been observed between allergen levels and risk of asthma or atopic sensitisation (Platts-Mills et al. 2001, Tovey et al. 2008). In addition, the shape of the association in the present study may be associated with some unknown attribute of the present study since some single microbial markers also showed an association with a similar shape. Further studies with larger cohorts

and in different settings are needed to better define the shape of the association between the microbial quantity and the risk of asthma in children.

The present study shows a similar inverse association between measured microbial diversity score and wheezing, as has been reported for asthma in two recent cross-sectional studies (Ege et al. 2011). The association with asthma was non-significant though in the same direction. Differences in the microbiological methods used may explain part of the difference in the results between the present study and previous studies. In the present study, the qPCR-method was used which quantitatively describes the presence of specific viable and non-viable micro-organisms (Haugland et al. 1999). Both are relevant due to their immunological properties (Hirvonen et al. 1997). However, the results obtained with qPCR depend on the prior decisions on the target microbes to be measured. The previous studies either cultivated viable microbes (GABRIELA study) or used single-strand conformation polymorphism analyses, which is a qualitative rather than quantitative method (PARSIFAL study) (Ege et al. 2011). The results of the present study are in line with previous studies (Ege et al. 2011) targeting a broader spectrum of microbes.

In summary, the total quantity of microbial exposure seems to offer relevant scope when assessing the total microbial exposure and its association with the development of asthma. Indicators summarizing the quantity and diversity of microbial exposure should be explored in future studies.

EPS or endotoxin and asthma or wheezing

The EPS load tended to be inversely associated with asthma and wheezing in early life, which is in line with earlier studies (Douwes et al. 2006b, Ege et al. 2007, Tischer et al. 2011b). In contrast to the protective EPS findings, three studies using air samples have found an association between the high levels of viable *Penicillium* spp. and increased risk of wheeze (Garrett et al. 1998, Gent et al. 2002, Rosenbaum et al. 2010) in one-year-old children at risk of asthma. A potential explanation for these contradictory findings might be the different exposure assessment methods. EPS is extracellular polysaccharide of the fungal species *Penicillium* and *Aspergillus*, and it was measured in house dust in the present study. In contrast, viable fungi were measured from air samples. Cultivation measures only viable, biologically active fungi, while EPS measures carbohydrate polymers excreted by mycelia of fungi representing better cumulative exposure to both viable and non-viable fungi. Accordingly to these findings, mould components may have a protective effect on asthma development though viable mould seems to increase the risk.

High endotoxin load in infancy decreased the risk of wheezing and asthma during the first two years of life, but no such association was seen in the six-year follow-up. These present results from the two-year follow-up are very similar to the findings of Douwes et al. (2006b), but are in disagreement with most cohort and cross-sectional studies. This was shown in a recent meta-analysis of 19 observational studies (Mendy et al. 2011), where endotoxin was associated with an in-

creased risk of wheezing in infants and toddlers, but had a protective effect on asthma in school-aged children. Likewise, high endotoxin exposure in adults at the workplaces may induce bronchial hyperresponsiveness (Smit et al. 2010), but is inversely associated with atopic sensitisation in both adults (Smit et al. 2010) and children (Simpson and Martinez 2010). The effect of endotoxin and asthma or wheezing seems to be dependent on age of immunological maturity.

One plausible explanation for this lack of consistency between the present study and previous studies on endotoxin may be the study setting. Most earlier birth cohort studies have been carried out in urban/ suburban areas, whereas the PASTURE study was carried out in rural areas with several different sources of microbes, such as stables and dung hills. In the LUKAS study, half of the children lived on a farm or other rural areas, and half lived in small towns or villages. In addition, the houses in the present studies were either single family and detached houses or farm houses. The microbial diversity is much higher in farm homes than in non-farming homes and significant differences in the prevalence of dominant microbes has been observed (Ege et al. 2011). In addition, microbial exposure coming from different sources, e.g. farm environment or moisture damage, may have different effects on health. Moreover, a measurement of endotoxin is only a crude marker of the total microbial exposure (Ege et al. 2011, Pakarinen et al. 2008, Pitkaranta et al. 2008) and in different environments may represent quite different microbial exposures.

In the present study, the inverse associations of EPS levels with respiratory outcomes were stronger among non-farmers than farmers. Farmers' children may be exposed to higher levels of microbes outside the home, e.g. in stables. Endotoxin and EPS levels in stable dust are five- to seven-fold higher than in dust from the living room floor in farm houses (Schram et al. 2005). Therefore, the misclassification of exposure among farm children may be greater than among non-farm children.

In summary, the amount and microbial content of house dust were inversely associated with asthma and wheezing, but due to the high correlation between microbial agents and the amount of dust, their individual effects could not be assessed separately. Better indices as evaluated in the present study need to be explored further in studies on the development of asthma.

Endotoxin and atopic sensitisation to inhalant allergens

The results of the present study show that high endotoxin exposure in dust in the mother's mattress increases the risk of atopic sensitisation to inhalant allergens at the age of one year. An association was found in another birth cohort in two-year-old children (Bolte et al. 2003). A tendency for an association between endotoxin measured from living room floor dust and atopic sensitisation was observed in the present study. In contrast, two other birth cohort studies examining dust samples from the bed, the mattress of the child or the carpet have found either an inverse association (Böttcher et al. 2003) or the suggestion of an inverse association (Wood

et al. 2011). No association was found in two other birth cohort studies among children aged below 15 months, when floor dust samples were analysed (Gillespie et al. 2006, Iossifova et al. 2007).

The tendency for an association between endotoxin measured from living-room-floor dust and atopic sensitisation to inhalant allergens was similar at six years of age to that observed at one year of age. Two other birth cohort studies have documented a tendency for either a positive (Chen et al. 2008) or an inverse association (Celedon et al. 2007). However, no association was found in three other birth cohort studies with longer follow-ups up to ten years (Bertelsen et al. 2009, Douwes et al. 2006a, Sordillo et al. 2010). In contrast, studies with a cross-sectional design have mostly confirmed an association or at least a tendency between higher endotoxin exposure from child's mattress or living room floor dust and a lower prevalence of atopy in school-aged children (Braun-Fahrlander et al. 2002, Carlsten et al. 2011, Ege et al. 2007, Gehring et al. 2008, Simpson et al. 2006, Sordillo et al. 2010).

Early atopic sensitisation in most children is transient and may be part of the normal maturation of the immune system (Matricardi et al. 2009). Most children with low levels of specific IgE (0.35–1.0 kU/L) at the age of two years lose their sensitisation up to the age of five years, whereas strong sensitisation with higher levels of specific IgE (above 3.5 kU/L) was more persistent (Matricardi et al. 2009). In the present studies, only 2% of the study children at the age of one year had such strong sensitisation, and accordingly, most sensitisation cases at that age are likely to be transient.

In summary, the results from earlier cross-sectional studies indicate that endotoxin may be inversely associated with the development of atopic sensitisation. However, the results form the present study and other cohort studies with exposure assessment in early age and longer follow-up does not support that finding. Endotoxin may increase the risk of atopic sensitisation at early age, which may be associated with the modulation of the normal immune system rather than with persistent atopic sensitisation.

EPS or endotoxin and atopic dermatitis

No associations were found between EPS or endotoxin exposure and atopic dermatitis during the first two years of life in the PASTURE study or during the first six years of life in the LUKAS study. Also, additional microbial markers or the indices of microbial exposure were not associated with atopic dermatitis in the LUKAS study during the six-year follow-up. Birth cohort studies among children around one year old have found inverse associations between endotoxin exposure and dermatitis or atopic dermatitis (Gehring et al. 2001, Perzanowski et al. 2006, Phipatanakul et al. 2004). Moreover, a birth cohort study found an inverse association only in children with the genotype CC of the CD14 gene (Simpson et al. 2006). No association between EPS and atopic dermatitis was observed in a cross-sectional study (Karadag et al. 2007).

In summary, the evidence from earlier studies and from the present study suggests that the role of microbial exposure in the development of atopic dermatitis is weaker than in case of asthma or atopic sensitisation or that the association may have been masked by genetic variation.

6.3 Methodological considerations

Study population, size and setting

The major strengths of the two studies in this thesis are the prospective study designs and the high participation rates. The number of included children was 1133 in the PASTURE study and 442 in the LUKAS study. The number of study children is sufficient to observe major health effects, but limited for stratified asthma models especially when stratified by farming. Though asthma is the most common chronic disease in Finnish children, the prevalence of doctor-diagnosed asthma in Finnish school age children is approximately 6% (Dunder and Pelkonen 2009) and 8–15% in the present study among preschool age children. In fact, a lower number of families attended and homes studied allowed for a detailed exposure assessment. Observational studies like the present study cannot reveal causal relationships, and the findings should be confirmed by doing experimental or randomized clinical studies.

In cohort studies, not all the children can be followed through the whole study. However, the proportion of drop-outs was not a problem in the PASTURE and LUKAS study. In the PASTURE study, 934 (82%) of the children took part in the six-year follow-up and in the LUKAS study, it was 373 (84%).

The PASTURE study is one of two birth cohort studies among children from rural areas with a high proportion of the children from farms. The farming environment, which is rich in microbes, offers a good opportunity to evaluate the importance of microbial exposures to the maturation of the immune system and children's respiratory health. However, there are differences between countries: the farming environment differs between countries (von Mutius and Radon 2008) which might be one reason for differences even in the direction of the association between exposure and health. Multi-center studies like the PASTURE study although done with similar protocols, different languages and with different concepts necessarily lead to some inconsistency in the data.

The LUKAS study was focused not only on the effects on confirmed moisture damage and mould, but also on farming and detailed microbial exposure. In the LUKAS study, 527 eligible mothers were not willing to participate. These eligible mothers when compared with participants lived less often on a farm (14.1% vs. 29.8%) and had a lower prevalence of maternal and/or paternal allergic rhinitis (30.0% vs. 41.7%) and atopic dermatitis (26.5% vs. 34.6%). However, the number of children, the prevalence of maternal and/or paternal asthma and the mother's educational level did not differ significantly between the groups. Due to differences

between the two Finnish cohorts as well as between countries in the PASTURE cohort and between farmers and non-farmers, the study cohort or study center and farming were always included in the statistical models as confounders.

In prospective studies, some of the children move out of their homes during the follow-up. In the present two studies, the exposure assessments were done during the first year of life, which has been suggested to be a crucial time window in the development of the immune system and the emergence of allergic diseases. Due to moving, the exposure data may be biased in both directions, either under-estimation or over-estimation of the exposure. However, the results were not changed when the children with early movers were excluded from the analysis.

The follow-up of the children, which continued only for the first 12–18 months of life, is too short a time to draw any definite conclusions concerning asthma in children. Likewise, even the first six years of life is too short a time when allergic rhinitis is the outcome. In the studies with short follow-up, the strongest and most significant associations were observed for doctor-diagnosed asthma, which was likely to indicate the severity of the disease. The association of moisture and mould problems in infancy with asthma at the six-year follow-up may be considered to be persistent.

The exposure assessments were made only once, which is one of the weaknesses of the studies. It would have been more reliable if the exposure assessments had been repeated during the follow-up. However, the exposures were assessed mostly during the first year of life, which is one of the crucial time windows for the development of the immune system (von Mutius and Vercelli 2010). In addition, depending on how much the living conditions have been changed, an exposure assessment at the age of two months or during the first year of life may reflect the exposure level which has occurred *in utero*. Thus, when assessing the exposure using a single measurement, the given time point might have been the most relevant. However, little is known about the other critical time points later in life while existing evidence on the timing of exposure is not clear (Douwes et al. 2009).

Home investigation for moisture and mould damage and dampness

The objective home inspection made by a trained civil engineer is one of the main strengths of the LUKAS study. During the inspection, moisture damage ormould were recorded and allocated in each room in infancy. The inspectors were blind to the health status of the study children.

Sensory assessments such as visual and smell-based assessments of moisture damage, mould or dampness may differ between inspectors (Haverinen-Shaughnessy et al. 2005, Hägerhed-Engman et al. 2009). The weakness of the study is that mould odour was detected in less than six percetage of the homes, whereas the prevalence has been slightly higher in another study with the same inspector (8–16%) (Pekkanen et al. 2007) and much higher in other studies with home inspection (≥30%) (Hägerhed-Engman et al. 2009; Rosenbaum et al. 2010). This is at least

partly due to the high number of farm homes included in the present study. The houses of farmers may have strong smell from stables that may interfere in the observation of the smell of mould. Another explanation can be the low sensitivity of the sense of smell of the inspector, whose ability has not been tested in the present study as was done in a cohort study (Reponen et al. 2010). In that study, five different investigators were used to assess mouldy odour though all home inspection team members of that cohort study had a sensitive sense of smell in a test on odour threshold.

In the present study, more than half of the homes had at least one instance of moisture damage somewhere in their house. This figure is high, even higher than has been observed in Finland earlier by objective assessments (Nevalainen et al. 1998). The method of home inspection for observing moisture damage, mould or dampness is comprehensive, even small signs of moisture damage and covers the whole house, including attics and basements if they were directly connected to the home living area. Also, the present study excluded apartments, and had a high proportion of farmers who live in older and bigger houses than people on average.

The parents were informed about the results of the home inspections, which may have affected the reporting of respiratory symptoms by parents, but would be expected to have less or no effect on doctor-diagnosed asthma and atopic sensitisation, and also on respiratory symptoms in the longer follow-up. However, there was a tendency that moisture damage in the kitchen or in the whole house had a dose-response with wheezing apart from cold in the shorter follow-up, but in the longer follow-up, it was much weaker, which might be due to willingness to report more symptoms in the home with moisture damage.

House dust samples and determined microbial markers

In the PASTURE study, house dust samples were collected by field workers and in the LUKAS2 study by parents using a standardized protocol. The parents were informed by phone and the written instructions with the photo protocol of the dust sampling were sent with sampling materials. A recent study showed a high correlation between occupant-collected and field-worker-collected dust samples in 43 residences (Van Dyke et al. 2012); however, some of the dust samples in the present study may have been taken differently than was advised.

The microbial exposure assessment was based on dust samples that have some limitations and advantages. Endotoxin studies have shown that endotoxin from house dust (floor or bed) samples correlate poorly with air samples (Hyvärinen et al. 2006a, Park et al. 2001b). Thus, not all the particles in the dust sample were breathable. However, house dust is thought to be a reservoir for microbes and reflects a more long-time exposure than air samples (Institute of Medicine 2004). Although the reproducibility of the floor dust samples was lower than of the bed dust samples, floor dust differentiated farm and non-farm homes (Hyvärinen et al. 2006a). Thus,

floor dust samples have been recommended for use in epidemiological studies involving hundreds of households given that floor dust samples are quite easy and inexpensive to collect.

In the PASTURE study, only two microbial markers, fungal EPS and bacterial endotoxin, were analysed in two dust samples from living room floors and from the mattresses of the mothers. Mother's mattress dust is only an indirect indicator of child's exposure, at least for the children who do not sleep with their mothers, but it reflects the exposure at home and also in the stables, as there is a fair correlation with the time that the mother and her baby spend in the stable. Theoretically, the mattress of the infant is more reliable in the exposure assessment, though other studies have shown that the mattresses of infants are often new and the microbial levels low (Gehring et al. 2004).

A large variety of different environmental microbial markers were analysed in the LUKAS study. However, no microbial secondary metabolites (e.g. microbial toxins and microbial volatile organic compounds (MVOC)) indicating metabolic activity of microbes were determined. Also, common indoor allergens, which have been associated with asthma in some studies (reviewed in Arshad 2010), were omitted from the analyses because the levels of house dust mites in Finland have been low due to low humidity indoors (Raunio et al. 1998) during the long heating period in winter time.

The calculated indices of microbial exposure condense information but are prone to errors. The microbial diversity score based on six fungal and two bacterial qPCR assays representing either microbial species or genera or groups provides only a suggestive and rough estimate for the overall microbial diversity. The microbial quantity score over-estimates the amount of gram-negative bacteria when using muramic acid together with endotoxin, because muramic acid is found also in gram-negative bacteria, although to a lesser extent (Brock et al. 1994). In addition, β-D-glucan originates mainly from fungi, but it has other sources such as some bacteria and higher plants. Also, the concentrations of β-D-glucan differ widely between fungal species (Iossifova et al. 2009). These circumstances may not appear to be a major problem, as the different indicators of overall microbial exposure used in the present study show very similar associations. Data from other techniques such as a deep sequencing of microbial exposure would give a better picture of the total diversity and help later in the assessment of the microbial exposure.

In summary, the results are based on birth cohort studies with a high response rate up to the age of six years, home inspections for moisture damage and mould in the home, and determinations of microbial exposure in early life. Although there are some disadvantages in the methods used in the present studies, the main results can be regarded as reliable.

6.4 Implications for future research

The present birth cohorts are ongoing studies and longer follow-ups will give information on the association between moisture or mould damage and allergic rhinitis as well as other health outcomes. Data on moisture or mould problems allow researchers to create indices of damage which can be used to characterise the buildings into those without major adverse health effects and those that are associated with major adverse health effects so that immediate renovation of the buildings can be enforced.

In future studies, the quantity score will be tested in the PASTURE study between farmers and non-farmers and a wider assessment of the exposure using secondary metabolites or microbial deep sequencing will give opportunities to explore causal effects in more detail. In addition, accumulated data from prospective studies would enable an investigation of the associations between different sources of microbial exposure and the effects of the maturation of the immune system and the development of allergic diseases.

7 CONCLUSIONS

Results from the present study indicate that different sources of environmental factors that may be related to microbial exposure may have both protective and adverse health effects. In this study, moisture damage and mould in the home had adverse effects on respiratory health while microbial exposure had a protective effect on asthma. Based on the present thesis, the following conclusions can be drawn:

1. Results from the present birth cohort study with objective home inspection for moisture damage or mould support earlier findings that moisture and mould damage are associated with respiratory symptoms and the development of new asthma. Thus, even though the reasons and mechanisms behind the observed associations are unclear to date, it is highly recommended that visible moisture damage or mould growth and its causes are immediately renovated.
2. When assessing the moisture damage, mould or dampness in the home, an estimate based on an assessment of the whole house is not sufficient, but room-based estimates are needed in the exposure assessment, especially when a house is a single family house. Also, the bathrooms should especially be considered separately and not recommended to include to the exposure estimate of the whole house.
3. The adverse health effects are more likely to be persistent if the moisture damage is severe or if it is located in the rooms where the occupants spend most of their time.
4. Although the presence of mould in the house may not induce atopic sensitisation, it may modulate the natural maturation of the immune system. Atopic children may be more susceptible to developing asthma when exposed to mould.
5. Single microbial markers characterising general exposure to environmental microbes are often highly correlated and it is not possible to disentangle their individual effects.
6. An index of microbial exposure, i.e. the quantity of environmental microbial exposure, may be associated with asthma with an inverted U-shape, with the highest risk found at medium levels and the lowest risk at the highest level. This index may predict asthma better than single microbial markers, independently of microbial diversity score and amount of dust. Indicators summarizing the quantity and diversity of microbial exposure should be explored in future studies.

The present results provide new insights on the causal pathways leading to the development of asthma and allergies and also suggest ways to prevent asthma. However, the present results suggest the importance of further exploration of

ways to better characterise moisture damage, mould and dampness and environmental microbial exposure. This information is needed for better understanding of the pathophysiology of asthma and allergies and thereby the prevention of asthma, but also for creating better tools and concepts to characterise and estimate the magnitude of the risks associated with living in a home with moisture and mould problems.

8 ACKNOWLEDGEMENTS

This study was carried out in the Department of Environmental Health, at the National Institute for Health and Welfare (formerly the National Public Health Institute), Kuopio during 2007–2012. I wish to thank Professors Terttu Vartianen, former director and Juha Pekkanen, the present head of the department, for providing the facilities for this study.

My principal supervisor, research Professor Juha Pekkanen, deserves my deepest and warmest thanks for guiding and supporting me through the study. His encouragement and trust has helped me over the difficult moments when I have faced health problems in my family or when I haven't believed in myself. I am also extremely grateful to my supervisors, Professor Matti Korppi and Docent Anne Hyvärinen, for their invaluable insight and dedication during this work. It has been my pleasure to work and to travel with you, 'Janne' and 'Hanne', and to have great memories that we can look back on and laugh about.

I sincerely thank the official reviewers of this thesis, Professors Jeroen Douwes PhD of Massey University, and Tiina Reponen, PhD of the University of Cincinnati, for their careful review and beneficial comments. I also thank Mark Phillips, BA for revising the language of this thesis and the articles.

I express my warm thanks to my Finnish co-authors Aino Nevalainen, Sami Remes, Marjut Roponen, Helena Rintala, Martin Tàubel, Jussi Lampi and all the co-authors from the PASTURE study group, especially Gert Doekes, Ulrike Gehring and the study leader, Professor Erika von Mutius, for sharing their expertise during this thesis. My special thanks go to my co-author and two-year roommate Docent Leea Keski-Nisula, who has supported me and shared a wide variety of feelings and moments.

I wish to thank all my colleagues in the Department of Environmental Health, especially the LUKAS –team members, group leaders Juha and Anne, study nurse Raija Juntunen, statistician Pekka Tiittanen and data manager Timo Kauppila, for their excellent co-operation and friendship. I was so lucky to have you in our team during these years. Also, laboratory assistants or researchers Reetta Tiihonen, Arja Rönkkö, Ulla Naukkarinen, Hanna Leppänen, Katja Saarnio, Heli Markkanen, Maria Valkonen, Susannne van den Brink, Timmoty Wigboldus, Mirian Boeve, Siegfried de Wind and Jack Spithoven, civil engineers Juho Halla-aho and Jari Koivisto, statistician Asko Vepsäläinen and study nurses Paula Tamminen, Merja Merikoski, Riikka Juola and Seija Antikainen are sincerely acknowledged for their hard work in the LUKAS study. Special thanks also go to work colleagues of YMEP for sharing thoughts and laughs during the lunch breaks: Anu, Jaana, Leea, Leena, Liisa, Mar-

jaleena, Raija, Taina and Tarja. Without you the days would have been miserable and lonely.

I owe my warm and hearfelt thanks to my parents, Maija-Liisa and Veikko, and my sisters, Minna and Hanna, for supporting me through my life. Also, I have been so lucky to have extremely lovely friends, Katsa, Kiles, Kossu, Regu, Riikka, Salsa, Satu, Päivi, Jutta and all the others who were not listed. No matter if we have not always met so frequently, our friendship has lasted. One more special thanks go to my friend Hertta for being my personal employment agent.

The most important thanks go to my family, my dear husband Aki, and our lovely sons, Eetu and Elias. My life has been full of happiness with you even through difficulties.

Financial support for this work was provided by the Graduate School in Environmental Health (SYTYKE), by the seventh framework programme of the European commission, EVO-funding, Farmers' Social Insurance Institution (Mela), the Academy of Finland, the Päivikki and Sakari Sohlberg Foundation and the Juho Vainio Foundation, which are sincerely acknowledged.

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