Changes in prevalence of asthma and allergy in Swedish school children over almost three decades and factors reducing risk of allergy

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What we know is a drop, what we do not know is an ocean

Isaac Newton

To my beloved parents and to my family: Donald, Paul and Thomas

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ABSTRACT

Background: Asthma and allergy are one of the most common chronic diseases among children. The prevalence of allergic diseases increased dramatically during the last decades of the twentieth century. The reason behind this increase is still not fully understood.

Aim: The aim of the thesis was to investigate changes over time in the prevalence of asthma and allergy in two Swedish towns, and to identify protective and risk factors for allergy development.

Methods: Data were obtained from two studies: a population-based cross-sectional study that was used in all four papers, and in paper III results from a birth cohort study was added. The cross-sectional study (N=1029) was performed in 2007 and prevalence data were compared with two previous cross-sectional studies conducted in 1979 and 1991. All three studies were based on questionnaires with the same set of questions on asthma and allergy, and the children were of the same age and from the same two Swedish towns: Mölndal/Gothenburg and Kiruna. Questions on dietary habits and food allergy were added in 2007 study. The birth cohort study (N=249), used in paper III, recruited children from Västra Götaland county. The children were recruited

at birth, between 1998 and 2005, and they were clinically assessed at the age of 8-9 years.

Results: Paper I: The prevalence of asthma were 2.5% (1979), 5.7% (1991) and 7.1% (2007), allergic rhino-conjunctivitis: 5.5% (1979), 8.1% (1991) and 11.1% (2007), eczema 7.1% (1979), 18.3% (1991) and 19.7% (2007) respectively. Allergic rhino-conjunctivitis continued to increase from 1979 to 2007, while asthma and eczema levelled off between 1991 and 2007. Having both parents born abroad was a protective factor for developing allergy.

Paper II: Hand-dishwashing decreased the risk of allergy (odds ratio 0.57; 95% confidence interval 0.37-0.85). The risk was reduced in a dose-response pattern if the child was also served fermented food, and if the family bought food directly from a farm.

Paper III: Keeping cats and dogs during the first year of life was associated with a decreased risk of allergy in a dose-dependent manner. Sensitisation to animals and pollen also decreased with an increasing number of cats and/or dogs kept indoors.

Paper IV: The total cumulative incidence of self-reported food allergy was 19.6%, and it was significantly higher in Kiruna (28.5%) than in Mölndal (15.7%). Introducing complementary food from 7 months of age or later, and a mother's history of allergy, were both independent risk factors for developing food allergy. Complementary food was introduced at a later age in Kiruna.

Conclusion and implications: In the latter years of the previous century, the rising trend of allergic diseases in children seemed to level off, with the exception of allergic rhino-conjunctivitis. Our results does not give any specific explanation for this break in the trend line for asthma and eczema, but one may speculate that climate change with longer pollen periods may have prevented a similar course for rhino-conjunctivitis. However, some important protective factors were found, such as lifestyle factors that were associated with a reduced risk of allergy development. Both dishwashing by hand, eating fermented food and buying food directly from farms were protective factors, as was the keeping of indoor pets during the first year of life. With pets, the risk of allergy was reduced in a dose-dependent pattern. Of the identified risk

factors, introducing complementary food late to an infant's diet increased the risk of food allergy, as did a maternal history of allergy.

Time trends describing changes in the prevalence of a disease is always important, not only for the organisation of the health care system, but also for our understanding of the underlying mechanisms. Of similar importance is the finding of factors that can protect children from allergy development, especially if they could be adopted to daily life and lifestyle habits. One such factor is when to introduce complementary food to a child's diet, and our results indicate that it should not be delayed. Pet keeping and dishwashing by hand may also be possible to implement by some families, but the main importance of their protective effects are mainly of mechanistic significance, with results supporting the hygiene hypothesis.

Keywords: allergy, children, epidemiology, risk factors, protective factors

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SAMMANFATTNING PÅ SVENSKA

Bakgrund: Astma och allergi har ökat markant i de industrialiserade länderna under 1900-talet och är de vanligaste kroniska sjukdomarna hos barn i Sverige samt i många delar av världen. Trots omfattande forskning är orsakerna till ökningen ännu inte helt klarlagda.

Metoder: Avhandlingen är baserad på data från två studier: en epidemiologisk tvärsnittsstudie, använd i alla fyra artiklar och en födelsekohortstudie använd i artikel III. Den frågeformulärsbaserade **tvärsnittsstudien** utfördes år 2007 (N= 1029) och jämfördes med två tidigare skolbarnstudier från 1979 och 1991. I alla tre studier användes samma frågor om förekomst av astma, allergisk rinokonjunktivit och eksem bland 7–8 år gamla barn i Mölndal och Kiruna. Enkäterna innehöll även frågor om ärftlighet för allergi och socioekonomiska förhållanden. Frågor om födoämnesallergi, eller födoämnesöverkänslighet, och kostvanor lades till i 2007 års enkät. I **födelsekohortstudien** värvades nyfödda barn (N=249) från Västra Götaland mellan 1998 och 2005. Barnen följdes till 8–9 års ålder.

Resultat: Artikel I: Förekomsten av astma var 2,5 % (1979), 5,7 % (1991) och 7,1 % (2007), allergisk rinokonjunktivit: 5,5 % (1979), 8,1 % (1991) och 11,1% (2007), eksem 7,1 % (1979), 18,3 % (1991) och 19,7 % (2007). Allergisk rinokonjunktivit fortsatte att öka i samma utsträckning som tidigare men ökningen av astma och eksem planade mellan 1991 och 2007. En skyddande faktor för allergi var om båda föräldrarna var födda utomlands.

Artikel II: Att handdiska minskade risken för allergi (odds ratio 0,57; 95 % konfidensintervall 0,37–0,85). Risken minskade på ett dos-effekt-liknande sätt om barnet också åt fermenterad mat eller om maten köptes direkt från en lantgård.

Artikel III: Att ha katter eller hundar inomhus under barnets första levnadsår var associerat med en allt minskande risk för allergi ju fler hundar och katter familjen hade. Sensibilisering mot djur och pollen minskade också i takt med stigande antal katter och/eller hundar inomhus.

Artikel IV: Förekomsten av självrapporterad födoämnesallergi/födoämnes-överkänslighet (någonsin) var 19,6 %, och den var signifikant högre i Kiruna

(28,5 %) än i Mölndal (15,7 %). Introduktion av fast föda från 7 månader eller senare och allergi hos mamman, var riskfaktorer för matallergi. Fast föda introducerades senare i Kiruna.

Slutsats: Det verkar som ökningen av allergiska sjukdomar bland barn planade ut mot slutet av 1900-talet, med undantag för allergisk rinokonjunktivit. Våra resultat ger inte någon specifik förklaring till detta trendbrott för astma och eksem men man kan spekulera om att en förändring till varmare klimat har medfört längre pollensäsonger vilket motverkat en liknande utveckling för rinokonjunktivit. Emellertid har viktiga förebyggande faktorer upptäckts, livsstilsfaktorer, som är associerade med minskad risk för allergiutveckling. Både handdisk, intag av fermenterad mat och inköp av mat direkt från bondgård var skyddande faktorer, liksom att ha husdjur inomhus under barnets första levnadsår. Risken för allergiutveckling minskade på ett dos-relaterat sätt med ökande antal husdjur. Sen introduktion av fast föda och allergi hos mor var riskfaktorer för allergiutveckling.

Förändring över tid i förekomsten av en sjukdom är alltid viktigt, inte bara för att planera sjukvårdens organisation, utan också för att öka förståelsen för mekanismerna bakom sjukdomen. Upptäckt av skyddande faktorer som hämmar utvecklingen av allergier är betydelsefullt, speciellt om vardagsliv och livsstil kan anpassas för att minska risken för allergiutveckling. En av dessa faktorer är tidpunkten när fast föda införs i barnets kost. Våra resultat visar att den inte bör fördröjas. Resultaten om handdisk och innehav av husdjur kan möjligen vara intressant för vissa familjer, men den viktigaste slutsatsen är dock att resultaten ger stöd för hygienhypotesen.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Hicke-Roberts A, Åberg N, Wennergren G, Hesselmar B. Allergic rhinoconjunctivitis continued to increase in Swedish children up to 2007, but asthma and eczema levelled off from 1991. Acta Paediatr. 2017 Jan; 106 (1):75-80.

II. Hesselmar B, Hicke-Roberts A, Wennergren G. Allergy in children in hand versus machine dishwashing. Pediatrics. 2015 Mar; 135 (3):e590-7.

III. Hesselmar B, Hicke-Roberts A, Lundell AC, Adlerberth I, Rudin A, Saalman R, Wennergren G, Wold AE. Pet-keeping in early life reduces the risk of allergy in a dose-dependent fashion. PLoS One. 2018 Dec; 19;13(12):e0208472.

IV. Hicke-Roberts A, Wennergren G, Hesselmar B. Late introduction of solids into infants' diets may increase the risk of food allergy development. BMC Pediatr. 2020 Jun 3; 20(1):273.

CONTENT

ABBREVIATIONS

ARC	Allergic rhino-conjunctivitis
CI	Confidence interval
FEV_1	Forced expiratory volume in one second
IBD	Inflammatory bowel diseases
IgE	Immunoglobulin E
IgG_4	Immunoglobulin G_4
ISAAC	International Study of Asthma and Allergies in Childhood
OPLS	Orthogonal partial least squares
OR	Odds ratio
PD_{20}	The provocative dose inducing a fall of $\geq$ 20% in FEV_1
PCA	Principal component analysis
PLS	Partial least square regression
SPT	Skin prick test
SRFA	Self-reported food allergy or intolerance

1 INTRODUCTION

Allergic diseases increased dramatically in the last decades of the twentieth century, especially in industrialised countries, affecting many individuals and causing a substantial burden for health care systems [1-5]. Asthma and allergic diseases are currently among the most common chronic diseases in children. The reasons for this increase are being investigated widely. Finding modifiable factors and mechanisms behind this increase could help reduce this negative course.

To meet this challenge, several hypotheses have been proposed. One early hypothesis stipulated that increased allergen exposure could cause allergy, and that allergen avoidance could prevent disease development [6]. Unfortunately, this was not the case. Other hypotheses, emerging in the later decades of the twentieth century, discussed the possibility that indoor environments with a high humidity [7-9], exposure to diesel exhaust and traffic air pollution [7,10, 11], or tobacco smoke exposure [7,12,13] could enhance a sensitising and disease-driving effect from allergen exposure, and hence cause allergic disease. These environmental effects are probably real, at least to some extent, but it is unlikely that they have caused the rise in allergic diseases seen in many countries. Hypotheses highlighting factors with a more tolerance-enhancing effect on the immune system have also been put forward. According to the "hygiene hypothesis" presented by Strachan in 1989, immunological tolerance is induced by immune stimulation from microbial exposure in early life, resulting in a decreased risk of allergy [14]. Hence, a decreased or less diverse microbial exposure in early childhood increases the risk of allergy development [14-17]. The allergen exposure dose, per se, or other factors associated with the keeping of cats and dogs, could also affect the risk of allergy. In contrast to previous recommendations, studies have shown that cat and dog keeping during early life may induce tolerance instead of allergy [18-20], and a similar effect has been seen in children growing up on dairy farms, with its multiple exposures to both allergens and microbes [21-25]. Children brought up on farms had a lower prevalence of asthma and allergy as well as a lower sensitisation to common airborne allergens [21-30].

It certainly appears that there is no single or simple explanation. The mechanisms behind the rise in the prevalences of allergic diseases are without doubt complex and multifactorial, and new hypotheses, based on the hygiene hypothesis, have more recently emerged (discussed in 1.1). Genetic, epigenetic and various environmental factors are probably playing an

interactive roll. Rapid industrialisation and technological developments have influenced our lifestyles and our biological environment during the last century, and combined could possibly contribute to the development of many diseases, among them allergic diseases. Unfortunately, similar processes are today observed in countries undergoing industrialisation, with similar lifestyle changes that westernised countries underwent decades ago [31,32], which could indicate that the burden of allergy will increase in a global perspective.

A possible model of this complex synergy between different factors affecting the risk of allergy development is presented later in the discussion section, integrating the recent findings from this research study. Even though we have seen a dramatic rise in allergic diseases in the past 50-70 years, several reports indicate that this increase in asthma and eczema has started to level off [24, 33-38]. But studies are conflicting, and some are still reporting an ongoing increase [4, 38, 39]. The apparent "slowing down" in the rise of asthma and allergy prevalences may be due to the proportion of susceptible individuals in the population having reached a high degree of saturation, at least in high-prevalence regions. Environmental factors inducing these diseases in genetically prone individuals seem to have reached a level of maximal effect on these individuals [38]. Another factor that might influence this time trend is the slightly decreased smoking habits seen in more affluent countries, at least the reduction of maternal smoking during pregnancy [37, 40]. In particular, maternal smoking during pregnancy is a widely accepted risk factor negatively influencing lung development and associated with a higher incidence of recurrent wheeze [7, 12, 41, 42].

The increase in food allergy currently seen came decades after the increase of the "first wave" of allergic diseases, and is considered to be the "second wave of the allergy epidemic" [43]. Although the true incidence of food allergy in the general population is difficult to estimate because of diagnostic challenges, the dramatic increase of anaphylactic reactions to food items during the last decades reflect a general increase of food allergy [44]. Approximately 3-10% of all children living in more affluent countries have food allergy according to challenge-proven studies, which is the gold standard in diagnosing food allergy [45-47], while 15-20% of children have a history of food allergy according to self-reported studies [48, 49]. Food allergy negatively affects many children and their families, influencing their quality of life as well as becoming a major public health burden [50].

The reasons behind this increase are still unknown. Similar to the other allergic diseases, genetic, epigenetic and various environmental factors have been proposed [51-53].

Rationale for study I

Asthma and allergic diseases have increased dramatically in the last decades but recent reports suggest that this increase might be levelling off, except for food allergy which is still increasing. The changes in the prevalences of allergic diseases are unlikely to be the same in different parts of the world. Estimating local changes in prevalences is not only interesting from an epidemiological point of view but, might also be of help in understanding the underlying mechanisms. The rationale for study I was to estimate the prevalence of asthma and allergic diseases in two Swedish towns, as well as to study potential changes in these prevalences over a long period of time. The results from this study are presented in article I.

1.1 ROLE OF MICROBIAL AND ALLERGEN EXPOSURE

Microbial exposure has decreased dramatically in the industrialised countries during the last decades due to environmental changes caused by industrialisation, changes in hygiene practice and lifestyle [54, 55]. According to the "hygiene hypothesis", a decreased microbial exposure leads to a decrease in the development of tolerance, which is considered to be one of the important reasons for the increase in allergic diseases [14-17].

1.1.1 Endogenous microbial exposure:

The individual's microbiome plays an essential role in the development of the immune system, especially in early life [15, 55- 60, 69]. Altering the child's microbiome early in life, during the maturation of the immune system, has been suggested as one of the important reasons of allergy development [15, 55, 57]. The microbiome is described by Lederberg as a "community of commensal, symbiotic, and pathogenic microorganisms within a body space or other environment" [61]. It is unique for every individual and consists of microorganisms habituating in the airway and gut as well as on the skin [57, 62]. The microbiome starts to establish itself already prenatally probably due to placental transmission [63, 64], develops and changes throughout the whole life but most significantly during early life [65]. There are many different factors which influence a child's intestinal microbiome. One of them is the mode of delivery. Gut microbiome of children born vaginally tend to have more *Lactobacillus* and *Prevotella* strains while the ones born by caesarean section more of Staphylococcus and Corynebacterium [66-69]. However, the association between the mode of delivery and asthma and allergy is inconclusive. Some studies have found a higher risk of developing asthma, ARC and food allergy in children born by caesarean section [70-72], whereas others have not [73-76]. These contradicting findings might be partly explained by the existence of other possible protective factors which compensate for the delivery by caesarean section. High environmental bacterial exposure at the time of delivery in developing countries might be one such explanation [54].

Breastfeeding plays an important role in developing the gut microbiome in infancy [58, 77]. Human milk contains oligosaccharides which are important as prebiotics in stimulating growth of a healthy intestinal microbiome, interacting with the epithelial cells of the intestine and influencing maturation of the immune system [78, 79]. The intestinal microbiome of children who are

breastfed has a higher microbial diversity [62, 80] than children receiving formula milk. Moreover, breastfed children's microbiome contains more of *Lactobacillus rhamnosus* and staphylococci than the microbiome of children fed with formula milk who have more strains of clostridia, enterococci, *Bacteroides*, *Enterobacteriaceae*, mostly *Klebisiella* and *Enterobacter* [57]. A lower diversity of the microbiome and colonisation by *E.coli* are associated with a higher risk of developing eczema [16, 57, 81] and colonisation with *Clostridia difficile* with asthma, eczema and sensitisation [16, 55, 57, 60, 81, 82].

Weaning and introducing solids to the infant's diet leads to changes in the intestinal microbiome, resulting in a decrease in *Lactobacillus* and *Bifidobacterium* strains whilst stimulating the growth of other bacterial strains which are important later in life [65]. Hence, the time of introduction of solids, plays an important role, both for microbiome growth stimulation and also possibly inducing food tolerance [83, 84] The intestinal microbiome changes during the life of the individual, and these changes depend on many factors, such as diet, use of antibiotics, aging, hormonal changes, smoking, environmental pollution and stress [58, 65, 85].

The changes over time of the intestinal microbiome occur not only over individuals' life spans, but have also occurred over the recent decades on a population level [57, 67]. Colonisation patterns and bacterial diversity in new-borns and infants have changed during this time period, probably due to improved hygiene standards and changes in the care policy in maternity wards, as well as the generally enhanced hygiene routines in everyday life [57]. The significance of these changes on the microbiome, and the effect they might have on the development and modulation of the immune system, is not yet well known [57]. But there are many studies indicating possible associations between the imbalance in the microbiome, its diversity and the development of immunomodulatory diseases such as inflammatory bowel disease (IBD), allergy and diabetes type I [57-59].

The early-life composition of the airways' microbiome, particularly on the nasopharyngeal and oropharyngeal sites, is reported to play an important role in developing respiratory tract diseases [55]. The colonisation with *Streptococcus pneumoniae, Moraxella catarrhalis and Haemophilus influenzae* during the first weeks of life, but not later, was significantly associated with a risk of respiratory tract infections, developing recurrent wheeze in childhood and asthma as well as being associated with an increase in number of eosinophils and the total immunoglobulin E (IgE) [86-88].

Microbial exposure in early life influences the host microbiome and, according to the hygiene hypothesis, stimulates the immune system leading to a decreased risk for allergy development.

In previous studies, a larger family has been associated with a decreased risk for developing asthma and allergy [14, 18, 89] while prenatal and early-in-life use of antibiotics [89-95] increases this risk. Having more siblings are presumed to increase microbial exposure through transmission between the children in the family. Use of antibiotics influences negatively the host microbiome, especially when used in early life which may influence the immune systems maturation.

Another example of influencing the individual's microbiome is the habit of sucking pacifier by parents as a cleaning method [96]. Children whose parents used to suck on the pacifier in order to clean it had less asthma and eczema at the age of three years than those whose parents cleaned pacifiers in other ways.

Rationale for study II

In affluent countries, microbial exposure decreased considerably during the last century. Furthermore, it is widely accepted that a decrease in microbial exposure leads to impaired tolerance development, which is one of the reasons for the increase of prevalence in allergic diseases. Therefore, finding daily-life factors, which increase microbial exposure in a safe way, is of great importance. New allergy-protective factors which have a potential for increasing microbial exposure have been investigated and are presented in article II.

1.1.2 Exogenous microbial and allergen exposure

Microbial presence in indoor and outdoor environments influences the human microbiome and indirectly determines the host's immune system [55].

Living close to forests and agricultural fields, or on farms, are inversely associated with sensitisation and the development of allergic diseases [21-27, 97].

Today, people living in industrialised countries spend 90% of their time indoors, and about 70% in their own housing [55, 98]. Therefore, the indoor environmental exposure plays a very important role. For instance, the children

living in inner-cities who were exposed to more diverse and richer microbial flora indoors during their first year of life did not develop atopy by the age of three [99].

The other aspect of an indoor environment is allergen exposure. The increased indoor exposure to cockroaches and mice was correlated with an increased risk of developing asthma and allergic diseases [62, 100]. Similarly, the increased exposure to house dust mite was correlated with a higher risk for asthma and allergy, [62, 101] but it has been suggested that this relationship is not linear. A very low and very high level of house dust mite was correlated with a lower risk of sensitisation, and lower risk for asthma and allergy while an intermediate level showed a high risk for these diseases [62, 102]. The indoor exposure to furred pets' allergen has also been studied thoroughly. Initially, exposure to the pets' allergen was presumed to promote allergic diseases [103, 104]. But these assumptions have been revised since early life exposure to pets and pets' allergen are found to have a protective factor. Hesselmar et al showed that children who had dog(s) or cat(s) during their first year of life had less asthma when they were 7-9 years old than children who were brought up without pets [18]. Other studies have later confirmed that early-in-life pet–keeping may protect children from developing allergy and atopy later in life [19, 20, 105-107]. Furthermore, keeping more than one dog or both a cat and a dog early in life leads to a decrease in IgE sensitisation to these animals later in life [20, 105]. Children who were exposed to a high dose of cat allergen [108] or were brought up with cats [109] had a cat-specific immunoglobulin G_4 (Ig G_4) but not IgE. This indicates that exposure to a high dose of pets' allergen induces immunological tolerance but the mechanism behind this process is still unknown. Developing tolerance may be induced by two different mechanisms. One of them could be that high exposure to the animal's allergen leading to development of tolerance. In this case, tolerance would be developed to the specific allergen, i.e. keeping a dog in early life would reduce the risk for developing allergy to dogs, and similarly keeping a cat in early life would reduce the risk for allergy to cats. However, there might also be another protective aspect of keeping pets indoors. The exposure to the animals' microbiota and endotoxins could also induce a protective effect by increasing the microbial exposure. In this case, the protective effect of keeping fur animals could influence a child's immune system, similar to living on a farm, and decrease the risk of allergy. If this is the case, keeping animals at home would result in decreasing sensitisation not only to the specific animal's allergen but also to other environmental allergens. With the keeping of pets, both these mechanisms may act simultaneously.

Rationale for article III

It is widely accepted that contact with environmental factors such as allergens and microbial exposure plays an important role in allergy development. There is much evidence that early microbial exposure influences the immunological response which leads to the development of tolerance to allergens and consequently decreases the risk for allergy. There are also studies which suggest that increased exposure to allergens might induce tolerance and decrease the risk of developing allergy.

According to the previous studies, keeping fur pets during early childhood has a protective effect on developing allergy later in life although the mechanism behind this phenomenon is not well understood. Therefore, further investigation was important. Previous studies have not fully investigated whether the number of kept animals during the first year of life influences the development of allergy in a dose–related fashion, or if the protective effect is specie-specific. If so, it would suggest that it is the greater allergen exposure, due to the increasing number of animals, which induces the development of tolerance. However, if the allergy-protective effect is not specie-specific, i.e. it also involves response to other environmental allergens, it could mean that pet-keeping during early childhood might also increase microbial exposure and, in this way, may have a more general allergy-protective effect. These two hypotheses were tested and presented in article III.

1.2 THE INCREASE IN FOOD ALLERGY

Many dietary factors have been discussed as possible reasons for the increase in food allergy. Both maternal diet during pregnancy and lactation, as well as the infant's diet, may play an important role in the development of food allergy [53]. Previously, antigen avoidance during pregnancy and lactation was proposed as enhancing the prevention of atopy in the offspring [110]. However, later studies did not support this hypothesis [111-113]. Contrarily, allergenic food intake such as tree nuts and peanuts during pregnancy have reduced the risk of allergy to tree-nuts or peanuts in the offspring [114]. Similarly, a high consumption of milk products at the end of pregnancy was associated with a lower risk for cow's milk allergy in children [115] and food allergy [116]. A diet rich in fish during pregnancy was associated with lower prevalence of asthma and eczema [117-119] as well as total allergy in the offspring [120, 121)]. Furthermore, a high consumption of butter and dairy products during pregnancy and lactation was negatively correlated with the prevalence of allergy in offspring while a maternal diet high in oils and margarine was positively associated with a prevalence of allergy in children [122].

Other aspects of dietary intervention during pregnancy have been investigated. Using n-3 long chain polyunsaturated fatty acid supplementation did not have any effect on food allergy development in the offspring but decreased the prevalence of eczema, asthma and sensitisation to eggs [123, 124] while other studies also found a protective effect on hay fever and food allergy [125]. Reports on supplementation of vitamin D or folic acid during pregnancy in relation to allergy development in the offspring have been inconclusive [126, 127].

The role of the infant's diet, such as breastfeeding and introduction of allergenic food items on the development of allergy, has been studied for many years. Exclusive breastfeeding in first 6 months of life is recommended by WHO [128, 129]. Breastfeeding is unquestionably the most beneficial diet for the infant's growth and development. Exclusive breastfeeding is considered very important in countries where the risk of inflicting gastrointestinal infection associated with using formulas or solids is high. The role of breastfeeding in establishing an adequate intestinal microbiome and its role in influencing the immune system has been already established [58, 78, 79]. Initially, breastfeeding was also considered protective against allergy [130-132]. However, later studies showed only some evidence for a protective effect on developing wheeze in early childhood, and even less for eczema and ARC [133] while other studies showed no evidence that breastfeeding is

protective for developing allergy [134,135] or eczema [136]. On the contrary, there are reports finding an increased risk for asthma and eczema with prolonged breastfeeding [129, 137, 138] although the risk of a reverse causation should be taken into account.

The increase in prevalence of food allergy led to the hypothesis that an early exposure to allergenic food might be one of the reasons for this increase. Therefore, delaying the introduction of allergenic food items such as milk, egg, fish, tree-nuts and peanuts was recommended, especially to children with an increased risk of atopy. Until the beginning of the 21st century, the introduction of milk products was recommended to be postponed until 12 months of age, egg until 24 months and nuts, peanuts, fish and seafood until 36 months, in case there was a risk of atopy [139-141]. In recent years this recommendation has been questioned as avoidance of allergenic food items did not lead to a decrease in the prevalence of food allergy [142-144], and as later studies did not confirm that avoidance of allergenic food in infancy would lead to a decrease in atopy and food allergy later in life [145]. On the contrary, many recent studies indicate that early exposure to allergenic food items may help in developing tolerance to these food items [142,146]. The early and regular consumption of peanut's protein, from the age of four months until 11 months, decreased dramatically the risk for developing peanut allergy in high-risk children [147, 148]. Regular consumption of peanut's or egg's protein from 3 months of age in the general population significantly decreased the risk of developing food allergy to these allergens [149]. Daily intake of certain amount of cow's milk formula between one and two months of age decreased significantly the risk of developing allergy to cow's milk [150]. Moreover, delaying exposure to allergenic food can lead to an increased risk of developing sensitisation and other manifestations of allergy [151, 152]. Early exposure to fish reduced the risk for eczema in infancy [136, 153], and ARC and asthma at a later age [91,154,155]. Moreover, eating fish regularly at the age of 12 months had a protective effect on developing ARC at the age of 12 years [156]. Introduction of egg before the age of 11 months was associated with a decreased risk for asthma development at the age of five years [155].

The time of introduction of complementary food has been discussed, but the results are still disputable [53, 83, 129, 157]. Complementary food includes all foods that are not breast- or formula milk. The first studies mostly analysed risks with early introduction of different food items, mostly allergenic. Very early exposure to complementary food, before the age of 4 months, seemed to be associated with a risk of allergic sensitisation and eczema [83, 158]. Later studies did not find associations between introduction of different food items

during 4-6 months of age and an increased risk of sensitisation or allergy [159, 160]. Moreover, there are studies suggesting that introducing complementary food, both allergenic and non-allergenic, beyond 6 months of age resulted in a higher risk of developing eczema, recurrent wheeze and atopic sensitisation [151, 155].

Rationale for article IV

The mechanisms of a possible influence of complementary food on the development of allergy is not well known. Evolving tolerance to food proteins requires regular exposure and proper immunological maturation. Exposure to food proteins in the "critical early window", most likely between four and six months of age, is important in developing tolerance [84]. However, adequate maturation of the intestinal immune system is also essential in developing this tolerance. Therefore, introducing food proteins very early, before a certain stage of immunological maturation, may impair tolerance development and lead to allergy. As already discussed, the intestinal microbiome plays an essential role in the maturation of the gut immune system [56, 58, 59, 78]. Consequently, all factors that alter the adequate development of the intestinal microbiome in early life might lead to a delay of the immunological maturation and decrease the ability of developing tolerance. Hence, the time of introduction of non-allergenic complementary food to infants' diets may play an important role in developing food allergy.

There are many studies evaluating the time of allergenic food introduction to an infant's diet in regard to the development of food allergy. The association between the time of introduction of non-allergenic complementary food and the development of food allergy has not been investigated in depth, so that universal recommendations can be formulated. The role the timing of introducing non-allergenic complementary food plays may have in the development of food allergy, as well as the analyses of other possible risk or protective factors for food allergy, have been studied in article IV.

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2 AIM

1. To analyse the prevalence of asthma, allergic rhino-conjunctivitis and eczema in 2007 and the change in prevalence between 1979 and 2007 in seven to eight year-old children from the two towns in Sweden, Mölndal in the southwest and Kiruna in the north of Sweden (Paper I).

2. To investigate dietary and lifestyle factors, which are supposed to increase microbial exposure that may be protective for developing asthma and allergic diseases (Paper II).

3. To investigate whether pet-keeping during early life influences the development of asthma and allergy, and if so, whether this association is correlated to the number of pets kept indoors (Paper III).

4. To analyse the cumulative incidence of self-reported food allergy or intolerance (SRFA) in seven to eight year-old children in Mölndal and Kiruna in 2007 and to estimate the risk factors for developing food allergy (Paper IV).

3 METHODS

Data were obtained from two population studies: a cross –sectional study, used in all four papers and a birth cohort study in paper III.

3.1 THE CROSS-SECTIONAL STUDY (PAPER I-IV)

3.1.1 Population and study design

The cross-sectional study was performed in 2007. In paper I, this study was compared with two other cross-sectional studies conducted in 1979 and 1991[2,161]. In all three studies, a questionnaire on asthma and allergic diseases was distributed to children aged 7-9 year old in two different Swedish towns: Mölndal and/or Gothenburg in south-west of Sweden and Kiruna, in the north of Sweden. Mölndal is a town with 69,000 inhabitants (December 2019). The town is an integrated part of the Gothenburg area, with a population of over a million, located on the west coast of Sweden. Gothenburg is the biggest city in the Western region with almost 600 000 inhabitants (November 2019). Mölndal and Gothenburg have similar urban and socio-economics structures. Kiruna is an inland mining town with 23,000 inhabitants, (September 2019) located north of the Arctic Circle.

Figure 1. A map of Sweden. Lantmäteriet.

The questionnaire was distributed and collected by school nurses or teachers and filled in by parents or legal guardians. Written informed consent was obtained. The 1979 study included all seven year old children in the Gothenburg area (number of answered questionnaire n= 4255, response rate 95%) and Kiruna (n=427, response rate 97%) [161]. In 1991, the questionnaire was distributed to seven year olds in a sample of schools in all parts of Gothenburg (n=1115, response rate 83%) and to all seven year-old children in

Mölndal (n= 534, response rate 93%). In order to get an adequate sample size, the questionnaire was sent to all seven to nine year old children in Kiruna (n=832, response rate 91%) [2]. In 2007, all seven and eight year old children in Mölndal (n= 717, response rate 53%) and Kiruna (n=312, response rate 64%) were included.

The same set of questions on asthma and allergy were used in all three studies. These questions were validated [2]. Questions on heredity, familial, environmental and socio-economic background were also included.

In 2007, in the cross-sectional study, the Swedish version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was added.

In addition, questions on food allergy or intolerance as well as on dietary and eating habits were added. The Swedish version of the 2007 questionnaire is included in Appendix I and a translated version in English in Appendix II.

3.1.2 The diagnostic criteria.

The child was diagnosed to have or have had:

- **asthma ever**: if there was positive response to the question *'Has your child had asthma or asthmatic bronchitis?'*
- **current asthma**: if there was a positive response to *'Has your child had asthma or asthmatic bronchitis in the previous year?'*
- **allergic rhino-conjunctivitis (ARC) ever:** if the answer was positive to the question *'Has your child had allergic rhinitis or allergic conjunctivitis?'*
- **current ARC**: if there was a positive response to *'Has your child had allergic rhinitis or allergic conjunctivitis in the previous year?'*
- **eczema ever**: if the answer was positive to the question *'Has your child ever had eczema?'*
- **current eczema**: if there was a positive response to *'Has your child had eczema in the previous year?'*

- **allergy ever or allergy last year:** if the child had any of asthma, ARC or eczema ever or the last year, respectively.
- **food allergy or intolerance**: if there was a positive answer to the question *'Has your child reacted with allergy or intolerance to any foodstuff*?*'*
- **specific food allergies or intolerances**: if one or more positive answers to the questions: *'Has your child reacted with allergy or intolerance to:*
    a. *-milk?*
    b. *-eggs?*
    c. *-fish?*
    d. *-peanuts?*
    e. *-tree nuts or almonds?*
    f. *-cereals?'*

Furthermore, there were questions on age at onset and possible cessation of symptoms of specific food allergy or intolerance as well as the type of symptoms such as: oral symptoms, diarrhoea, rash, oedema, respiratory symptoms, vomiting, stomach ache, eczema, urticaria, and rhino-conjunctivitis.

3.1.3 The other variables analysed and presented in papers I-IV:

- **breastfeeding**: implemented or not and the duration in months
- time of introduction of **formula and gruel** in the child's diet (age in months)
- time of introduction of **solids** to the child's diet (age in months)
- whether **hand or machine dishwashing** was mainly used

- whether or not **fermented food** was included in the child's diet

- whether or not **food from farm** was included in the child's diet

- whether or not and frequency of **home cooking** to the child during its first year of life

- **traditional cooking**: included dishwashing by hand and/or use of fermented food and/or farm food

- **number of cats and dogs** in the household during pregnancy and child's first year of life

- **heredity for allergic diseases**: if the father or mother have or have had any of asthma, ARC or eczema

- **heredity for food allergy**: if the father or mother have or have had food allergy or intolerance.

- mother's and father's **country of birth**

- mother's and father's **education level in years** (nine-year schooling, twelve-year schooling, university degree 3 years or shorter, university degree more than 3 years)

- **prenatal smoking exposure** (- whether mother smoked during pregnancy) and **exposure for smoking during the child's first year of life**

- whether the child attended **day care**

- **current residence** (flat, house or farm)

3.2 THE BIRTH COHORT STUDY (PAPER III)

3.2.1 Population and study design

The birth cohort study contained data from two birth cohorts: "**Allergyflora**" and "**Farmflora**". Both studies were designed to investigate the effect of early gut colonisation and other early life events on later development of allergy. Both studies were conducted in the same way. The main difference between cohorts were the populations included: urban in "Allergyflora" and rural in "Farmflora".

Allergyflora was a cohort of 184 children recruited from Mölndal between 1998 and 2003 [68,153].

Farmflora was a cohort from a rural area of the Skaraborg county, northeast of Gothenburg, of 28 children living on dairy farms and 37 children living in the same rural area but not on farms. Children were recruited between 2005 and 2007 [120,122, 152].

The parents were contacted before the birth of the children. Children who were born at the gestational age of 38 weeks or above were included in the study when they were 0-3 days old. Interviews with the parents were conducted by the study nurse when the children were recruited, and at 6 and 12 months of age. Paediatric allergologists examined the children at the age of 18 months, and at 3 and 8-9 years. The diagnostic criteria for asthma and allergy were based on symptoms and results from clinical examinations and laboratory tests.

3.2.2 Examinations

In paper III, mainly outcome data from the 8-9-year follow-up were used, and the investigations performed at that age were: lung function test, flow-volume curves with reversibility test and methacholine challenge as well as blood test and skin-prick test (SPT). Data on parental history of allergy were collected during recruitment, and the number of cats and dogs at the 6-month interview.

Criteria for allergic diagnoses of allergic diseases at 18 months and 3 years are specified in Appendix III.

Lung function tests

Flow-volume curves and reversibility tests were performed in accordance with American Thoracic Society and European Respiratory Society guidelines [162] using Spida 5 spirometry software (Micro Medical Limited, Rochester, UK). If FEV_1 increased by >12% from the baseline, bronchodilator response was considered positive.

Direct methacholine challenge was used to determine airway hyperresponsiveness [163]. Using tidal volume-triggered dosimetric method (Spira Elektro 2 jet nebulizer; Spira Respiratory Care Centre Ltd, Hämeenlinna, Finland). Baseline FEV_1 was determined after inhalation of isotonic saline. Methacholine was subsequently inhaled in increasing doses at intervals of at least 1 minute until FEV_1 had decreased by ≥ 20%, or a cumulative dose of 6.1875 mg had been given. At the end of the challenge, all subjects received an inhalation of salbutamol and FEV_1 was measured to ensure recovery (FEV_1>90% of the baseline value). The provocative dose inducing a fall of ≥ 20% in FEV_1 (PD_{20}) was determined by interpolating the dose-response curve. Airway hyperresponsiveness was defined as PD_{20} <0.6 mg. The slope was calculated from the maximum fall in FEV_1 divided by the cumulative dose.

Blood tests

Blood eosinophil cells, specific immunoglobulin E (IgE), and total IgE were all analysed at the Sahlgrenska University Hospital. All analyses were accredited by the Swedish Board for Accreditation and Conformity Assessment. For specific IgE and total IgE, Phadiatop and ImmunoCAP tests were used (Thermo Fisher Scientific, Uppsala, Sweden).

Skin prick tests

Skin prick tests (SPTs) were carried out for common airborne allergens (cat, dog, horse, rabbit, birch, grass, mugwort, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Cladosporium herbarum*) according to the

standards of the Subcommittee on Skin Tests of the European Academy of Allergy and Clinical Immunology [164]. Allergen extracts were all manufactured by ALK (Hørsholm, Denmark). A positive SPT corresponds to a weal with a diameter exceeding the negative control by ≥ 3 mm. More detailed information on the investigations is provided in the paper III.

3.2.3 The diagnostic criteria

- **asthma:** diagnosed at the age 8-9 years if the child, during the last 12 months had symptoms of wheeze/heavy breathing and FEV_1 reversibility >12%, or bronchial hyper-responsiveness to methacholine (PD_{20} <0.6 mg), or ongoing medication with inhaled corticosteroids or leukotriene antagonist.

- **allergic rhino-conjunctivitis (ARC)**: diagnosed at the age 8-9 years if the child during the last 12 months, had eye or nose symptoms of allergic disease and a positive skin-prick test or specific IgE to the relevant allergen.

- **eczema:** diagnosed at the age 8-9 years if the child, during the last 12 months, had a skin condition fulfilling Williams criteria [165], or an itching dermatitis that had been chronic or relapsing for at least 6 months.

- **number of cats and dogs in the household during the first year of life**: data was obtained from the 6-month telephone interview.

- **heredity for asthma and allergy:** if father and mother had a doctor´s diagnosed asthma, ARC or eczema (data collected at recruitment).

- **allergy ever**: any of asthma, ARC or eczema diagnosed at any of the follows up at the age 18 months, 3 years or 8-9 years (Appendix IV).

- **allergy last year:** included any of asthma, ARC or eczema diagnosed at the age 8-9 years.

3.3 STATISTICAL ANALYSIS

The data from the 2007 questionnaire study were transferred manually into Microsoft Access databases, doubled checked by a second person, and then transformed to an SPSS-database.
Analyses were performed with SPSS statistical software (version 22 paper I, II, IV and version 24 in paper III; IBM Corp., Armonk, NY, USA).

Paper I, II, III and IV.

Chi-square (χ^2) tests were used to compare differences between proportions and multiple logistic regression tests to investigate the influence of different factors on the outcome variable, and to adjust for possible confounders.

A two-sided *P*-value < 0.05 was considered statistically significant.

Paper II

The SIMCA software (version 13.0.3; Umetrics AB, Umeå, Sweden) was applied for the principal component analysis (PCA) and orthogonal partial least squares (OPLS) analyses. Variables were centred and scaled to unit variance. SPSS statistical software (version 22) was used for cross-tabulations and multiple logistic regressions.

A five step analysis approach was used:

1. A principal component analysis (PCA) to explore data and identify variables with the strongest negative correlation with the outcome variable.
2. Univariate analyses with χ^2 and Fisher's exact test were used for selected patterns. Odds ratio (OR) with 95% confidence interval (CI) presented the differences.
3. Identifying possible confounding variables. In 3 orthogonal partial least squares (OPLS) analyses possible associations between all independent variables and outcome variable were tested. Variables that showed a significant association with both total allergy and hand dishwashing and total allergy and traditional cooking were regarded as possible confounders.

4. Analysing the confounders in two models. The confounders were entered to two backward multiple logistic regression-models. Parental history of allergy, mother or father born in Sweden, mother's education, town postcode, pet keeping during infancy, day care attendance, crowding (square metres/number of persons at home) mother smoking during pregnancy and hand dishwashing (model 1) or traditional cooking (model 2).
5. Using the variables from the last steps in model 1 and model 2 in hierarchical multiple logistic regression models. The hierarchical model (model 3) analysed the association between hand dishwashing and the different allergic diseases (total allergy, asthma, allergic rhino-conjunctivitis, eczema) adjusted for parental history of allergy, day care attendance, and pet keeping during infancy. The hierarchical model (model 4) analysed the association between traditional cooking and total allergy adjusted for parental history of allergy, day care attendance, pet keeping during infancy, and father born in Sweden.

Paper III

The SIMCA-P+ software (version 14.1; MKS Umetrics AB, Umeå, Sweden) was used for multivariate analyses.

Linear-by-linear association and exact tests were used for trend analysis and backward logistic regression models for control of covariates and possible confounders. A two-sided P-value < 0.05 was considered statistically significant.

In the birth-cohort study the orthogonal projection to latent structures (OPLS), was used to analyse the relationship between the number of pets at 6 months of age, parental history of allergy, and 12 independent outcomes from the follow-up at 8–9 years.

Beta-coefficients on scaled and centred data were calculated with 95% confidence intervals.

3.4 ETHICAL APPROVAL (PAPER I-IV)

Written, informed consent was obtained from all parents or legal guardians. The study was approved by the Ethics Committee of the University of Gothenburg, Sweden (R448-97 and Ö 446–00) and the Human Research Ethics Committee of the Medical Faculty, University of Gothenburg, Sweden (Dnr. 321–05, 363–05, 105–07 and 674–14).

The questionnaires were coded. Only data and code numbers were transferred to the database to avoid the risk of identification of individual participant's answers. All the questionnaires were stored in the research archives of Sahlgrenska University Hospital.

4 RESULTS

4.1 POPULATION

4.1.1 The Cross-Sectional Study

In 2007, a total of 1838 questionnaires were distributed in Mölndal and Kiruna of which 1029 were returned (response rate 56%). In Mölndal 717 out of 1354 (53%) and in Kiruna 312 out of 484 (64%) were returned.

The gender ratio was almost equal, with marginally more girls (53%, 546 out of 1029) than boys responding to the questionnaire but there was no significant difference between the towns. Almost all children (92%) in both Mölndal and Kiruna had siblings.

Heredity for asthma and allergy was high and similar in both towns, 48% of mothers and 39% of fathers had a history of allergic diseases. Most parents in Kiruna and Mölndal were born in Sweden, 93% and 84% respectively. Educational level with a university degree was equally common in mothers and fathers in Mölndal while it was less common in Kiruna, especially among fathers.

A history of smoking exposure was low, 6% of mothers smoked during pregnancy and 4% of children were exposed to smoking inside the house during the first year of life. The majority of all children, 91%, attended a day care centre and 76% lived in houses (detached, semidetached or terrace house).

4.1.2 The Birth Cohort Study

The total number of participants was 249, 50% boys (125) and girls (124).

In the "**Allergyflora**" study 187 children were recruited, of whom 184 were followed up at 18 months, 174 at 3 years and 162 at 8 years. In the "**Farmflora**" study 65 children were recruited and all were followed up at 18 months, 63 at 3 years and 48 were followed up at 8 years.

Heredity for asthma and allergic diseases was slightly lower than in the cross-sectional study but similarly more common among mothers (44%) than fathers (36%). A doctor´s diagnosis of asthma and allergy among parents was required.

4.2 PREVALENCE OF ASTHMA AND ALLERGY

4.2.1 Total prevalence of allergy (Paper I and III)

In the cross-sectional study, almost 47% of all children (481 out of 1029) have had any of the following diagnosis during their life time: asthma, ARC or eczema ("allergy ever**"**). The prevalence of "allergy last year" i.e. any of asthma, ARC or eczema during the previous year was 30.5% (314 out of 1029). There was no significant difference between Mölndal and Kiruna.

In the birth cohort study, 38% of all children (95 out of 249) were diagnosed to have had "allergy ever**"**, i.e. any of asthma or ARC or eczema. "Allergy last year**"** was diagnosed in 29% of the children (73 out of 249).

4.2.2 Prevalence of asthma, ARC, eczema and food allergy (Paper I, IV)

In the cross-sectional study, the prevalences of "asthma ever", "ARC ever" and "eczema ever" were 13.2%, 12.9% and 35.8% respectively. There were no significant differences between Mölndal and Kiruna. The prevalence of current asthma, ARC and eczema are presented in Table 1.

The prevalence of "current asthma" was 7.1% and was significantly more common in Kiruna than in Mölndal, especially among boys. The prevalence of "current ARC" was 11.1% and significantly more common among boys in Mölndal than in Kiruna. There was no difference among girls. Almost 20% of children had "current eczema", which was significantly more common among boys in Kiruna than in Mölndal. Geographical differences in the total prevalence of allergic diseases or current allergic diseases (any of asthma, ARC or eczema) were not observed. The differences in current allergic diseases were mostly observed among boys. Regarding self–reported food allergy or intolerance, the cumulative incidence was significantly higher in Kiruna than in Mölndal.

Table 1. The prevalence of current asthma and allergy in 2007. Significant p-values are marked with bold. Hicke-Roberts A, Acta Paediatrica 2016 (Paper I).

	Total N=1029	**Mölndal** N=717	**Kiruna** N=312	***P*-value** Mölndal vs Kiruna
Asthma				
Total	73 (**7.1%**)	43 (**6%**)	30 (**9.6%**)	**0.038**
Boys	40 (8.3%)	20 (6.2%)	20 (12.7%)	**0.015**
Girls	33 (6.0%)	23 (5.9%)	10 (6.5%)	0.774
P-value				
Boys vs girls	0.168	0.879	0.068	
ARC				
Total	114 (**11.1%**)	86 (**12%**)	28 (**9%**)	0.156
Boys	65 (13.5%)	52 (16%)	13 (8.2%)	**0.019**
Girls	49 (9%)	34 (8.7%)	15 (9.7%)	0.685
P-value				
Boys vs girls	**0.023**	**0.003**	0.627	
Eczema				
Total	203 (**19.7%**)	136 (**19%**)	67 (**21.5%**)	0.353
Boys	80 (16.6%)	43 (13.2%)	37 (23.4%)	**0.005**
Girls	123 (22.5%)	93 (23.7%)	30 (19.5%)	0.295
P-value				
Boys vs girls	**0.015**	**<0.001**	0.414	

The cumulative incidence of self-reported food allergy or intolerance (SRFA) was 19.6 % (201 out of 1029). Approximately one of five children had reported immediate or late symptoms due to food intake any time during the first 7-8 years of life. There was a significant difference between Mölndal 15.7% (112 out of 717) and Kiruna 28.5% (89 out of 312), $p < 0.001$.

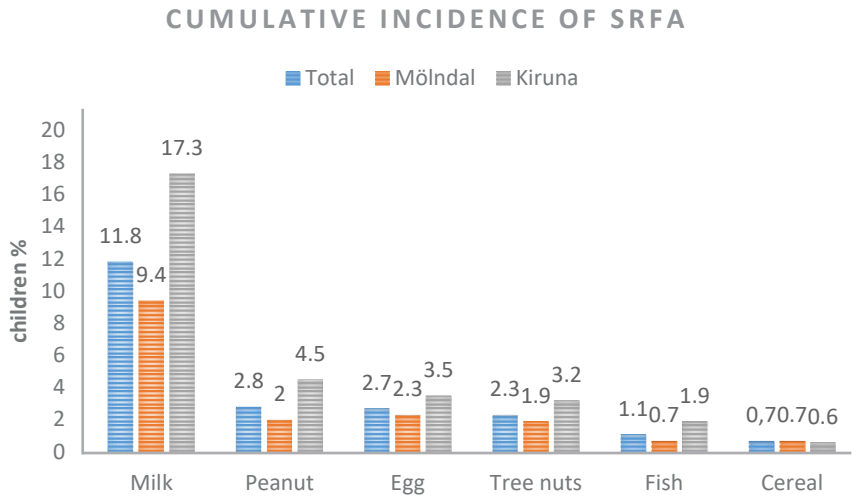


Figure 2. The cumulative incidence of self-reported food allergy or intolerance (SRFA) to different food items.

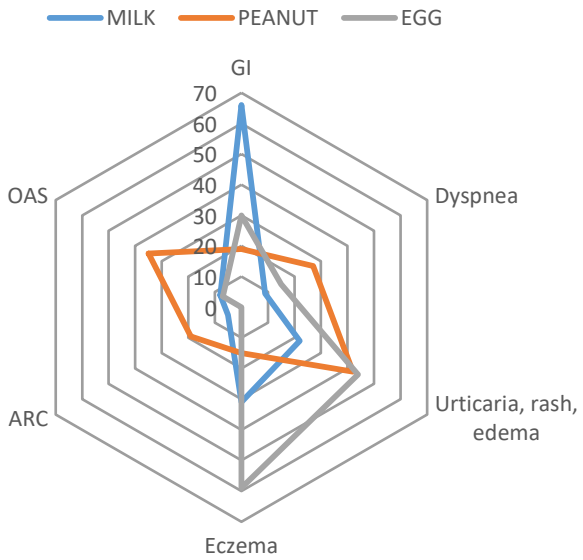
The most common SRFA was to cow's milk (11.8%). An early onset of symptoms to cow's milk before the age of three years was reported in 7.3% of all children. SFRA to peanut, egg, tree nut, fish and cereal were less common (range 2.8-0.7%) (Figure 2). Children with reported peanut allergy and symptoms other than oral allergy syndrome (OAS) were 2.3% and to tree nut 1.4%. There was significantly more children in Kiruna than in Mölndal who had SRFA to milk (P=0.001), peanut (P =0.039), peanut with symptoms other than OAS (P=0.008), tree nut with symptoms other than OAS (P=0.006).

Detailed information about cumulative incidence of SRFA is available in article IV, Table 1.

4.2.3 Symptoms of SRFA (Paper IV)

There were different adverse reactions to different food items (Figure 3). Gastrointestinal symptoms such as abdominal pain, vomiting and diarrhoea as well as eczema were the most common adverse reactions to milk. These symptoms usually represent a "late-type onset reaction" without immunoglobulin E (IgE) sensitization. Urticaria/rash/angioedema were less common. The adverse reactions to peanuts and tree nuts were similar, most commonly presenting as urticaria/rash/angioedema, dyspnoea or oral allergy syndrome. These are usually immediate type of reactions, which are IgE-mediated. Eczema and urticaria/rash/angioedema were the most common symptoms of egg allergy. Reactions to fish were mostly urticaria/rash/angioedema, whereas symptoms to cereal were mainly gastrointestinal.

Oral allergy syndrome (OAS) as the only symptom was reported by 27 of 201(2.6%) children with SRFA.



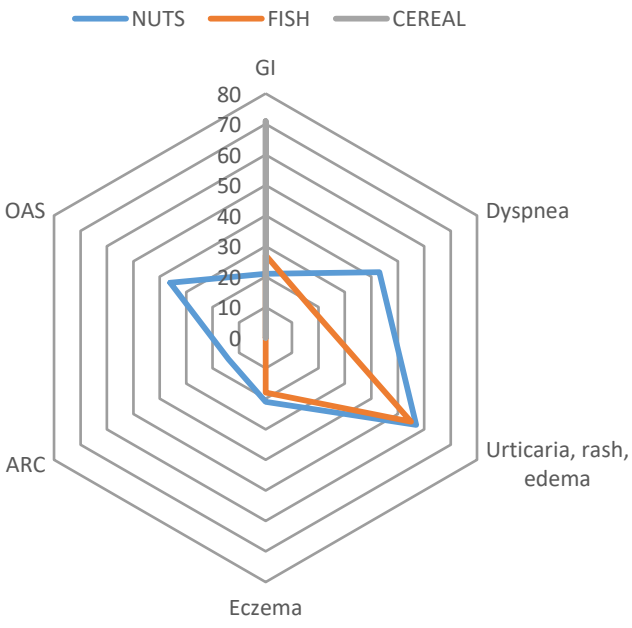


Figure 3. Symptoms of SRFA. GI-gastrointestinal symptoms, ARC-allergic rhino-conjunctivitis, OAS-oral allergy syndrome.

4.3 THE CHANGES IN PREVALENCE OF ASTHMA, ARC, ECZEMA BETWEEN 1979 AND 2007 (PAPER I)

The results from the cross-sectional study in 2007, as well as from the previous studies from 1979 and 1991, revealed the change in the prevalences of allergic diseases and asthma during these periods of time [2,161, paper I].

The prevalence of asthma, ARC and eczema, as well as total allergic diseases (any of asthma, ARC, eczema) increased significantly (p <0.001) between 1979 and 1991[2,161]. From 1991 to 2007 the increase had levelled off for total allergic diseases (P=0.327), asthma (P=0.123) and eczema (P=0.337). However, ARC continued to increase at the same rate (about 0.2% per year) from 1979 to 2007. The increase of ARC was significant even in the period 1991-2007 (p=0.004) (Figure 4).

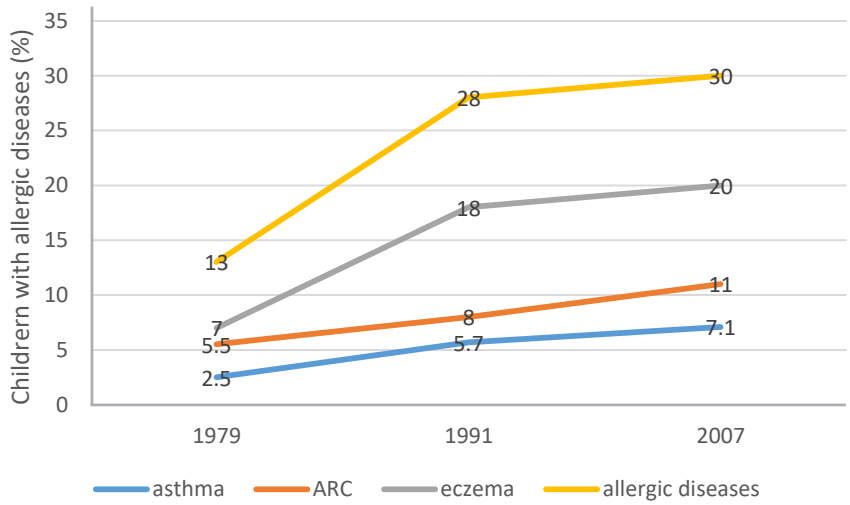


Figure 4. Prevalence of current allergic diseases (any of asthma, ARC or eczema), asthma, ARC and eczema. ARC-allergic rhino-conjunctivitis.

When comparing the two towns, the increase was significant in all allergic diseases and asthma in both Kiruna and Gothenburg/Mölndal between 1979

and 1991 [2,161]. The increase in the prevalence of ARC was significant only in Mölndal between 1991 and 2007. [Table S3 in paper I].

4.4 RISK FACTORS FOR ASTHMA AND ALLERGY (PAPER I, IV)

4.4.1 Heredity as a risk factor for asthma, ARC and eczema

The various family factors which were previously associated with a risk of developing allergy such as heredity for allergy, parents' educational level, parents' country of birth and the number of siblings were statistically analysed in a multiple regression test, where total "allergy ever" (any of asthma, ARC or eczema) was the dependent variable.

Only the parental history of allergy was noted as a risk factor for developing any form of allergy. Thus, the risk associated with a mother with a history of allergy resulted in an adjusted OR of 1.65, (95%. CI 1.27-2.16), while the risk with a father with a history of allergy revealed an adjusted OR of 1.72, (95% CI 1.32-2.26).

In another multiple regression model, the influence of parental allergy on specific current allergy or asthma was tested. Both mother's and father's history of allergy were independent risk factors for all types of allergy and asthma. However, maternal history of allergy was not a risk factor for eczema (Figure 5).

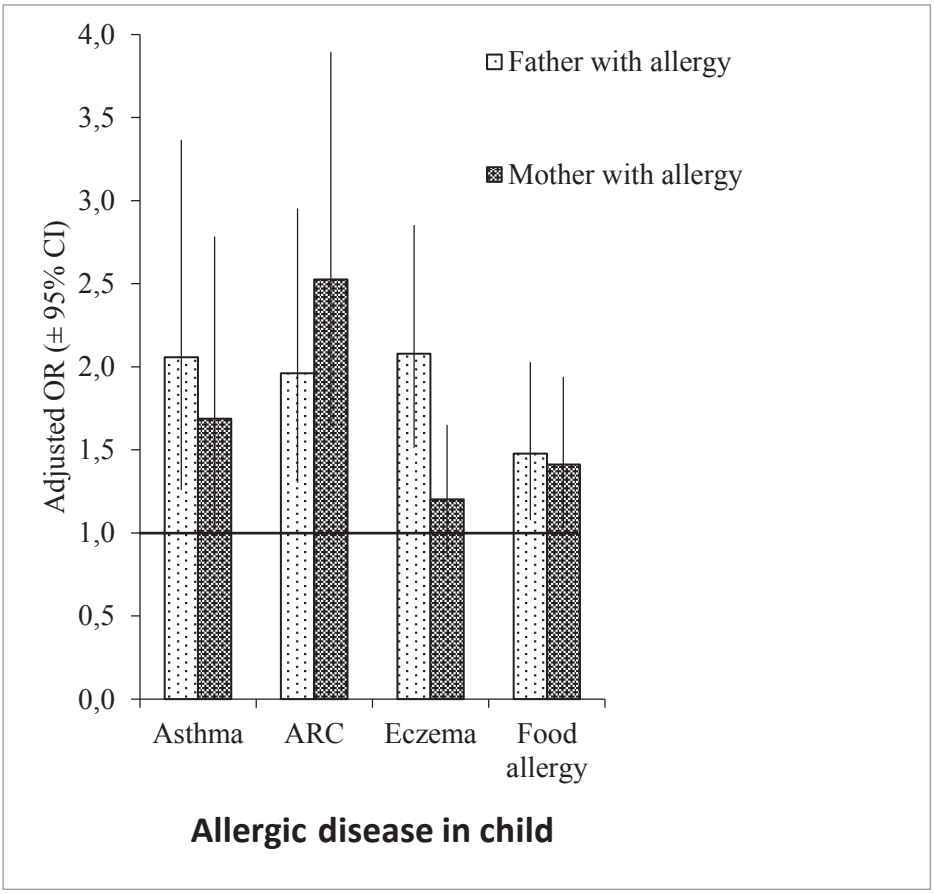


Figure 5. The influence of parental history of allergic diseases on the occurrence of current asthma, current ARC, current eczema and incidence of food allergy/intolerance in children. Multiple logistic regression with maternal and paternal history of allergy as independent variables. ARC-allergic rhino-conjunctivitis, CI-confidence interval, OR-odds ratio. Hicke-Roberts A. Published in Acta Pediatrica.2016. (Paper I).

4.4.2 Late introduction of solids and mother's history of allergy increased SRFA risk

Infant's diet:

Majority of infants were breastfed

Nearly all children were breastfed (95%), with no difference between the towns (Mölndal 94.6%, Kiruna 94.8%). However, children in Kiruna were breastfed longer (mean duration 10 months) than in Mölndal (8.7 months) and this was statistically significant (*P*=0.004). The difference in duration of breastfeeding between Kiruna and Mölndal was significant even with regard to children without milk allergy (10.1 respectively 8.6 months, *P*<0.001). The duration of breastfeeding was similar for the children with (9.6 months) and without milk allergy (9.1 months) (P=0.594). Furthermore, the duration of breastfeeding was not a risk factor for the cumulative incidence of SRFA (Table 2).

The majority of infants were introduced to formula/ gruel and solids in the first six months of life in both towns, but a later introduction of formula/gruel and solids was more common in Kiruna

Formula and gruel (a mixture of formula and cereal) constituted the major part of almost all the infants' diets, most often introduced to children at the age of 4-6 months. Significantly more children in Mölndal (n=351/677, 52%) than in Kiruna (125/292, 43%, *P*=0.009) started with formula and gruel at this age. Hence, more children in Kiruna (102/292, 35%) than in Mölndal (183/677, 27%) started with formula and gruel at the age of 7 months or later. This difference was significant (*P*=0.013). The time of introduction of formula or gruel, before or after the age of 7 months was not a risk factor for the cumulative incidence of SRFA (Table 2) and, thus, the difference between this early and late introduction was not significant (*P*=0.18).

The solid foods first given to infants were usually porridge (mixture of formula and oat or wheat) and purees made from different vegetables, fruit, rice, meat or fish. The majority of children started with solids during the first 6 months of life (871/988, 88.2%), in Mölndal (619/693, 89%) and Kiruna (252/295, 85%), with no significant difference between the towns (*P*=0.0826).

However, significantly more children in Kiruna (43/295, 15%) than in Mölndal (74/693, 11%, *P*=0.002) started with solids at the age of 7 months or later. There was a significant difference in the cumulative incidence of SRFA between children who received solids before 7 months of age (161/870) and after this age (32/116), *P*=0.001. The multiple logistic regression test was used to investigate eight presumptive risk factors for food allergy or intolerance (Table 2). Late introduction of solids (at the age of 7 months or later) and mother's history of allergy were independent risk factors for SRFA.

Table 2. Multiple logistic regression analysis of cumulative incidence of SRFA in relation to confounding factors. Hicke-Roberts A, BMC Pediatrics 2020.

Variable	**Adjusted OR**	**95% CI**	***P***
Male	1.179	0.834-1.667	0.35
Duration of breastfeeding	1.014	0.980-1.048	0.429
Father with a history of allergy[1]	1.318	0.932-1.864	0.119
Mother with a history of allergy[1]	1.599	1.126-2.270	0.009
Father's education, university degree	0.827	0.536-1.276	0.392
Mother's education, university degree	1.155	0.768-1.737	0.489
Introduction of formula/gruel before 7 months of age	0.715	0.462-1.102	0.129
Late introduction of solids[2]	2.290	1.395-3.761	0.001

[1]A history of asthma, rhino-conjunctivitis, eczema

[2]Introduction of solids at 7 months of age or later

OR-Odds ratio

CI-Confidence Interval

The late introduction of solids to infants' diets was not connected to parental history of food allergy

The multiple logistic regression test was used in order to evaluate if parental history of food allergy could explain the late introduction of solids to the child's diet. SRFA was used as the dependent variable while the independent variables were: duration of breastfeeding, mother with a history of food allergy, father with a history of food allergy and late introduction of solids. This revealed that the late introduction of solids still remained as an independent risk factor for SRFA, OR 1.8 (95% CI 1.15-3.02).

The late introduction of solids was the risk factor and not the consequence of early allergy symptoms such as asthma and eczema in infancy or in their siblings

The late introduction of solid foods to the children's diet could have been caused by them already suffering from allergy. Asthma and eczema are the early manifestations of allergy, usually beginning in infancy. The association between the late introduction of solids and asthma and eczema were analysed in order to evaluate possible reverse causality (Figure 6). Solids were often introduced late in children with SRFA but not in children with asthma or eczema. Therefore, the late introduction of solid foods can be considered a risk factor for SRFA and not a consequence of early allergy symptoms. To further investigate if the late introduction of solids is the risk factor for food allergy or the consequences of food allergy an analysis (linear regression with duration of breastfeeding and late onset of solids as independent variables) of the child's siblings' allergy profile was performed. A late introduction of solids to the child was associated with a higher ratio of siblings with food allergy (p=0.026), but there was no increased ratio of siblings with asthma (p=0.523) or eczema (p=0.63). This increase in ratio of food allergy in siblings but not in asthma or eczema could indicate that late introduction of solids is the consequence of habit or recommendations and simultaneously a risk for food allergy.

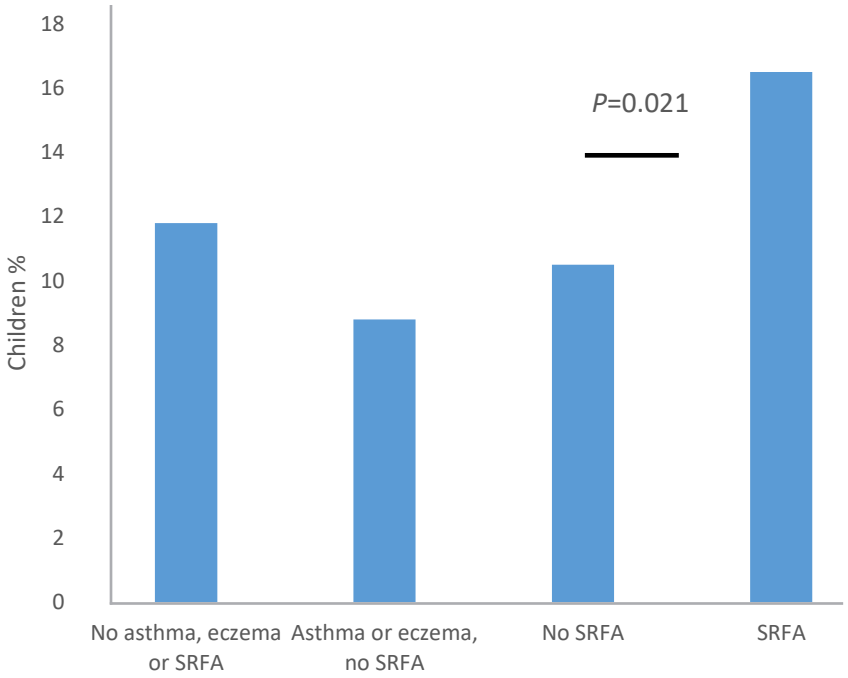


Figure 6. Percentage of children with late introduction of solids and manifestations of allergic disease. While solids were often introduced late in children with SRFA, no such pattern was seen for children with asthma or eczema, indicating that late introduction of solids is a risk factor and not consequence of food allergy. SRFA-self reported food allergy. Hicke-Roberts A, BMC Pediatrics 2020 (Paper IV).

Higher cumulative incidence of SRFA in Kiruna cannot be explained by the parental history of allergy but later introduction of solids may be a factor

Heredity for food allergy (Kiruna: 144/610; 23%, Mölndal: 312/1395; 22%), as well as for asthma, ARC or eczema (Kiruna 275/609; 45%, Mölndal 622/1393; 45%) were similar in Mölndal and Kiruna. Self-reported food allergy was more common in children in Kiruna than in Mölndal (Table 2). Late introduction of solids was more common in Kiruna compared to Mölndal and this may be one explanation for the higher cumulative incidence of self-reported food allergy in Kiruna (28.5%).

Other putative risk factors for allergy such as number of siblings, parental smoking, keeping pets at home, use of antibiotics, pre-school attendance, number of respiratory tract infections were evaluated in univariate analyses. No statistically significant correlation with SRFA was found.

4.5 THE PROTECTIVE FACTORS FOR ASTHMA AND ALLERGY (PAPER I, II, III)

4.5.1 Parental birth country (Paper I)

A reduced risk for allergy was found if both parents were born abroad: OR 0.44; 95% CI 0.22-0.87 (multiple logistic regression test with "total allergy ever" as the dependent variable).

4.5.2 Lifestyle-related habits (Paper II)

Hand dishwashing, eating fermented food and food bought directly from farms were protective factors for total allergy

Hand dishwashing was identified having the strongest negative correlation with allergic diseases, "total allergy" (any of asthma, ARC or eczema) (PCA analysis). An association between hand dishwashing, breastfeeding, eating fermented food, buying food directly from farms, home cooking and allergic diseases were tested (univariate analyses). Dishwashing by hand (OR 0.51; 95% CI 0.34-0.77), eating fermented food (OR 0.53; 95% CI 0.32-0.87) and buying food directly from farms (OR 0.67; 95% CI 0.46-0.98) were statistically significant and decreased the risk for total allergy. The association between eating fermented food or buying food directly from farms, and protection from specific allergy did not reach statistical significance (Table 2, paper II). Only dishwashing by hand revealed a decreased risk for eczema (OR 0.49; 95% CI 0.32-0.77) and asthma (OR 0.21; 95% CI 0.05-0.85) but not for ARC (OR 0.78; 95% CI 0.42-1.42). After adjusting for covariates and confounders (multiple logistic regressions models: 1 and 2, described in Methods) dishwashing by hand was significantly protective only for eczema and total allergy but did not reach statistical significance for asthma (Figure 7).

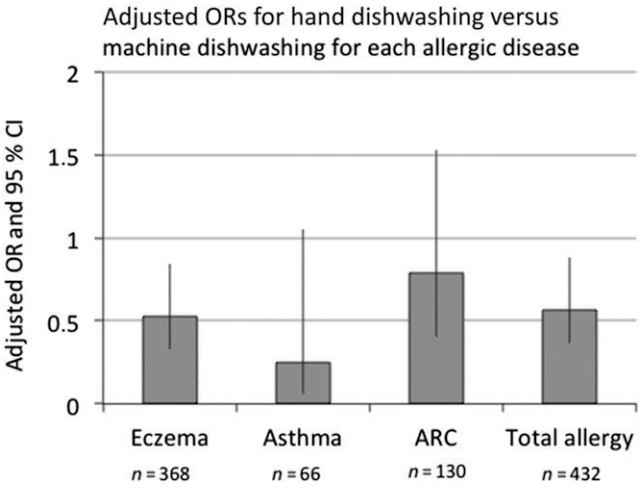


Figure 7. Adjusted ORs and 95% CIs for eczema, asthma, ARC, and total allergy (any of the 3 diseases) when analysed with respect to dishwashing method used by the family. The prevalences of eczema and total allergy were significantly lower in children from families who use hand dishwashing (n = 126) instead of machine dishwashing (n = 868), whereas asthma revealed a borderline significance. Confounders were adjusted for in a hierarchical multiple logistic regression model, with parental history of allergy, day care attendance, and pet keeping during infancy as independent variables. Hesselmar B et al. Pediatrics 2015(Paper II).

Traditional cooking is a protective factor for total allergy in a dose-related pattern

The association between traditional cooking (any of: hand dishwashing, eating fermented food or food bought directly from farms) and allergic diseases "total allergy" was evaluated. In the families where traditional cooking was practiced, 33% of children were reported to have had some type of allergic disease while among those who did not use traditional cooking it was 46% (*P*=0.000). Traditional cooking can be associated with a certain lifestyle. Multiple logistic regression model (model 2 described in Methods) was used to analyse possible confounders and covariates (father born in Sweden, day care attendance, parental history of allergy, pet keeping during infancy). Traditional cooking was still the significant protective factor for total allergy (adjusted OR 0.56 95% CI: 0.41-0.77).

The possible effect of the number of protective factors, a dose-related pattern, was investigated in multiple logistic regression model (model 2). "Total allergy" as the outcome variable was adjusted for confounders (model 2). The total allergy was reported in 46% of children in the families which did not use food from farms, used machine dishwasher and child did not eat fermented food. If one of the protective factors was applied the total allergy decreased to 35% and if two or three were applied it further fell to 19% (Figure 8). The prevalence of allergic diseases decreased with increased numbers of factors in traditional cooking.

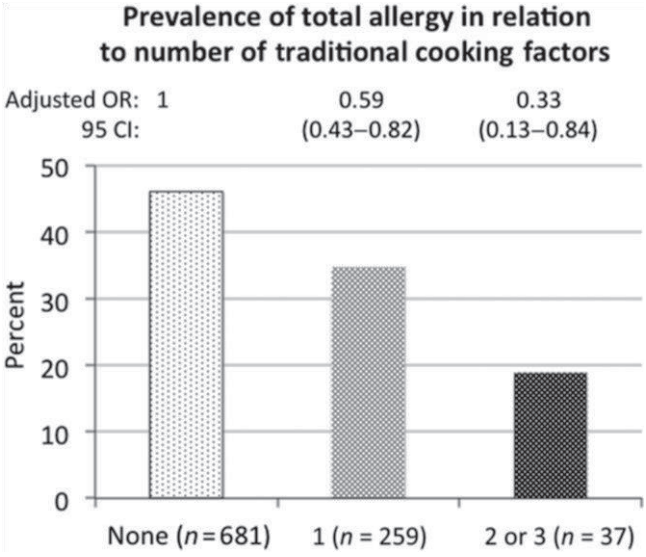


Figure 8.The prevalence of total allergy (eczema, asthma, or ARC) according to the number of protective factors reported for the child's family: that is, the number of factors included in the umbrella term traditional cooking (hand dishwashing, use of fermented food, or buying food from farm). Differences are expressed as adjusted ORs and 95% CIs, and confounders were adjusted for in a hierarchical multiple logistic regression model with parental history of allergy, day care attendance, pet keeping during infancy, and father born in Sweden, as independent variables. Hesselmar B et al., Pediatrics 2015 (Paper II).

4.5.3 Pet keeping in early life (Paper III)

Almost one third of children had pets (dogs and cats) during the first year of life, in the Cross-Sectional Study 28% and the Birth Cohort Study 27%.

Table 3. Number of household pets during the first year of life, range from zero to five, in the Cross-Sectional Study and zero to two or more in the Birth Cohort Study.

Number of pets	Cross-Sectional Cohort	Birth Cohort
0	767	181
1	165	40
2	64	
≥ 2		28
3	21	
4	7	
≥ 5	2	

Pet keeping during the first year of life was a protective factor for allergy in a dose-related pattern

The cumulative incidence ("allergy ever") and prevalence ("allergy last year") of allergic diseases decreased with the number of household pets both in the cross-sectional study (Figure 9, *P*-value for trend with exact test: 0.006 for "allergy ever" and 0.038 for "allergy last year") and in the birth cohort study (Figure 10, *P*-value for trend with exact test: 0.007 for "allergy ever" and 0.008 for "allergy last year").

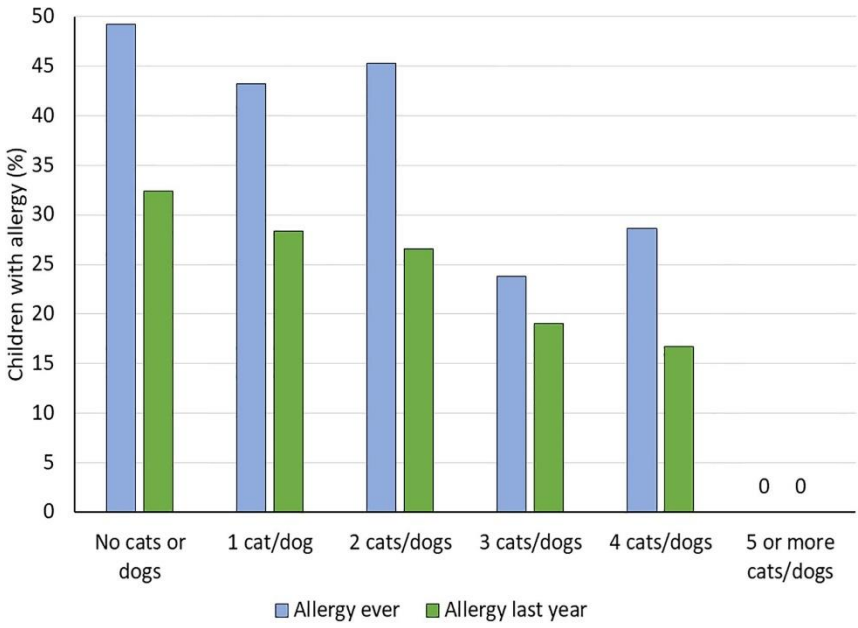


Figure 9. ***Data from the cross-sectional study.*** *Allergy (any of asthma, allergic rhino-conjunctivitis, or eczema) in relation to the number of household cats and dogs during the child's first year of life. Allergy last year required current symptoms, i.e. symptoms in the last 12 months. Hesselmar B et al. PLoS ONE 2018 (Paper III).*

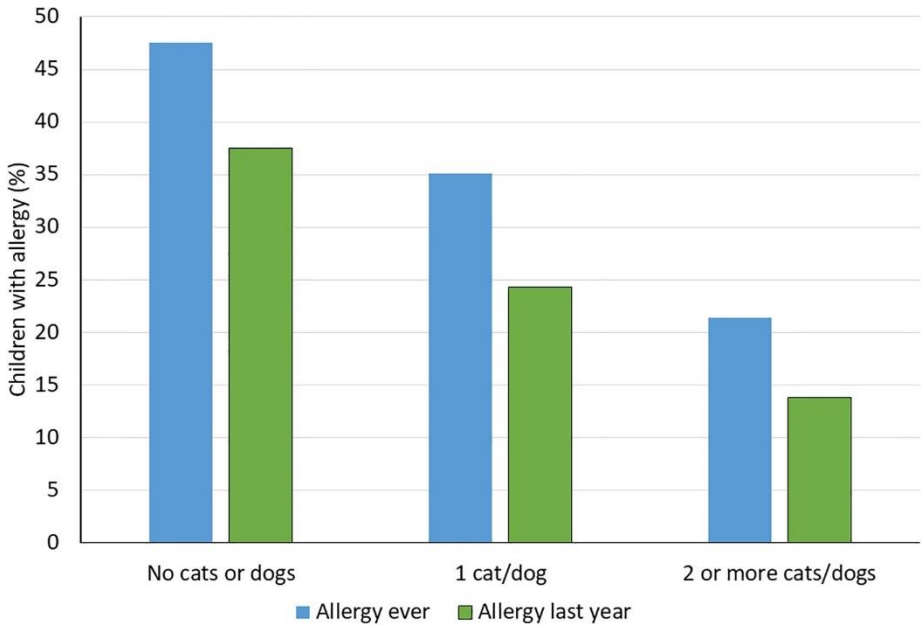


Figure 10. ***Data from the Birth Cohort****. Allergy (any of asthma, allergic rhino-conjunctivitis, or eczema) in relation to the number of household cats and dogs when the child was 6 months old. Allergy last year required current symptoms, i.e. symptoms in the last 12 months. Hesselmar B et al. PLoS ONE 2018 (Paper III)*

The decreased risk of developing allergy with increasing number of pets remained the same even after adjusting for confounders in a backward multiple logistic regression analysis. The "allergy ever" was a dependent variable and number of pets, gender, parental history of allergy, number of siblings were independent variables. In both cohorts, only parental history of allergy and number of pets remained in the last step. In the Cross-Sectional Study the OR of 0.8 for every additional pet (P=0.012) and in the Birth Cohort OR of 0.65 for each additional animal (P=0.058) was found.

The choice of having pets was not influenced by parental sensitisation to pets

To evaluate if parental allergic diseases influenced the choice of having pets, the sensitisation among parents was tested in the Birth Cohort Study. Blood

samples from 290 parents (149 mothers and 141 fathers) were obtained and Phadiatop tests for sensitisation were analysed. There was no significant difference in sensitisation (positive Phadiatop test) between parents who did not have pets and those who had pets when their child was 6 months old (*P* value for trend: mothers 0.590, fathers 0.425).

The degree of sensitisation in children were decreasing with increasing number of pets

In the Birth Cohort Study the skin prick test (SPT) was performed when the children were 7-8 years old. In the OPLS analysis (Figure 11), the relation between the number of pets when the child was 6 months and degree of sensitisation is shown. The diameter of SPT, which is the sign of sensitisation, decreased with increasing number of household animals in the infancy. This association was not only seen for the sensitisation to dogs or cats but even to pollen (birch and grass).

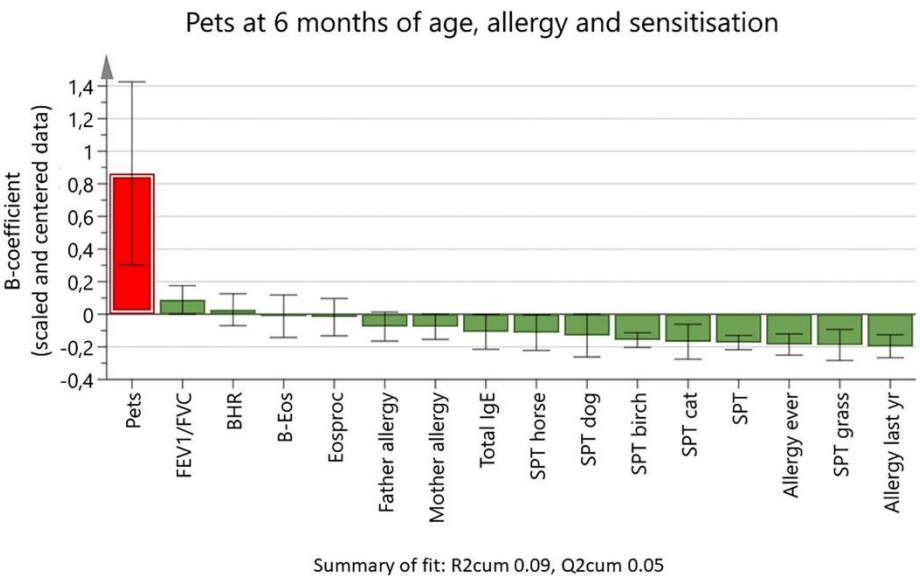


Figure 11. ***Orthogonal projection to latent structures loading plot showing associations between the number of household cats and dogs when the child was 6 months old (Y variable), and a set of 15 X variables.*** *The outcome variables for lung function (forced expiratory volume in 1 s [FEV_1]/forced vital capacity [FVC]), bronchial hyperresponsiveness (BHR), blood eosinophil count (B-Eos), percentage of*

blood eosinophils (Eosproc), total immunoglobulin (IgE), and skin-prick tests (SPTs) were from the age 8–9 years follow-up. .SPTs are given as weal diameter. X variable bars pointing in the same direction as the Y variable are positively associated with the Y variable, and bars pointing in the opposite direction are negatively associated. The height of the bars shows the B-coefficients for scaled and centred data, with 95% confidence intervals. Hesselmar B et al. PLoS ONE 2018 (Paper III).

5 DISCUSSION

5.1 PREVALENCE AND CHANGES OVER TIME

The prevalence of ARC alone continued to increase at the same rate between 1979 and 2007 while the increase in asthma and eczema levelled off between 1991 and 2007. The geographical differences, with a higher prevalence of allergic diseases in the north, which was reported in 1979 and 1991 [2, 161], was not a general pattern in the 2007 study. However, in 2007 food allergy was more common in the north, and boys in Kiruna had more often asthma and eczema than boys in Mölndal, whereas boys in Mölndal more often reported ARC. These geographical differences, only seen in boys, are difficult to interpret. However, they may reflect a minor geographical difference, that can be only be visualised in boys, since allergic diseases are more commonly seen in boys at this age [166, 167].

Bjerg and co-workers have also performed epidemiological studies on asthma and allergy among children of the same age in the north of Sweden, and how the prevalence of these diseases has changed over a ten-year period from 1996 to 2006 (33). The prevalence figure for asthma in our 2007 study was similar to the one found by Bjerg et al, but we found slightly higher figures for ARC and eczema. Neither Bjerg, nor we, found a general increase in allergic disease between the last follow-up measurements, even though we found an ongoing increase in ARC, and Bjerg et al. found an increase in sensitisation. This ongoing increase in ARC is further supported by the World-wide ISAC-study (4).

The reported prevalence of asthma, ARC and eczema, and the changes over time in prevalences, varies globally [4, 38, 39]. The majority of countries reported an ongoing increase in allergic diseases among 6-7 year old children, according to ISAAC phase one to three studies, during the period 1994-2003 [4]. In several European countries, such as UK, Germany and Spain, increasing prevalences of asthma, ARC and eczema were still seen, while in Austria the prevalence of asthma decreased but ARC and eczema increased [4]. This diverging and discordant pattern of changing prevalences over time has also been seen in other studies. There are several reports, with similar findings to our study, indicating that the increase in asthma and eczema has started to level off [24, 33-38]. A possible explanation for this phenomenon could be that the proportion of susceptible individuals in the population of some countries has already reached a high degree of saturation, while this may not be the case in other countries.

The cumulative incidence of self-reported food allergy (SRFA) in our study was also in accordance with other studies [48, 49]. In the study conducted by Strinnholm and co-workers in 2006 in the north of Sweden, 21% of all seven to eight year old children reported food hypersensitivity, which is similar to our findings [49]. The exact prevalence of food allergy is, however, not easy to estimate as it should be based on oral food challenge tests measuring both acute- and late-onset reactions, and such tests are both costly and time consuming. Most reports on food allergy are based on self-reported data which probably overestimate the true prevalence of food allergy [50]. Only about 10% of self-reported food allergy can be confirmed by double-blind food challenge tests [46, 50, 168], but such tests are limited by the fact that they usually only measure acute reactions, and not late-onset reactions that may be visible after a few days. Furthermore, they only measure current allergy, and not the cumulative incidence of disease during an individual's life. The global prevalence of food allergy based on the oral-challenge test varies between 1-10% [50].

The significant difference in the cumulative incidence of SRFA between Kiruna and Mölndal is unlikely to be explained by genetic factors. The majority of the population are of similar ethnicity and have a similar lifestyle in both towns. Moreover, the prevalence of asthma and other allergic diseases was similar in both towns, as was the parental history of total allergy and food allergy. The only significant "lifestyle" difference between the towns, found in our study, was the time of introduction of solids to the diet of infants as a late introduction of solids was more common in Kiruna than in Mölndal.

In contrast to changes in the prevalences of asthma and other allergic diseases which vary in different countries, the prevalence of food allergy is reported to be rising globally [50, 52, 53]. The change over time in food allergy could not be estimated in this thesis research study due to a lack of available data on the incidence of food allergy in the previous studies from 1979 and 1991.

The main limitation of this thesis research study was the response rate of approximately 60% in the cross-sectional study of 2007, which is not uncommon nowadays, but was lower than in the previous studies from 1979 and 1991. It could have affected the power of the study and the risk of type two errors. Nonetheless, the risk of selection bias is low as the results from the study are similar to the results from other studies performed in the same time periods in northern Sweden [33, 49]. Moreover, the increase of the prevalence of asthma and eczema levelled off, which should not be the case if there was a problem with selection bias, meaning the opposite would be expected since

mostly parents to allergic children were presumed to respond to the questionnaire if selection bias was an issue.

5.2 RISK FACTORS FOR DEVELOPING ALLERGY

Similar to previous reports [8,161], we found that a parental history of allergy was a risk factor for allergic disease. In contrast to this, the number of siblings or the parental level of education, which were considered risk factors in earlier reports [14, 18, 169], were not found to be risk factors in this study. A probable explanation could be that almost all children attended day-care centres, most commonly from the age of one or two years. Therefore, almost all of them have been exposed to microbes and infections regardless of having siblings. Due to the Swedish educational and welfare system, lifestyle in Sweden has become more homogenous for the majority of the Swedish population in recent years, a fact that may explain why parental education did not seem to influence the development of allergy. However, a maternal history of allergy and food allergy were risk factors for children developing food allergy. The prevalence of allergy and food allergy among parents were similar in both towns. Hence, the difference in incidence of SRFA between Mölndal and Kiruna could not be explained by this factor.

The role of the child's diet in the development of food allergy has been discussed intensively in recent decades, especially the time of weaning. Exclusive breastfeeding during the first 6 months of age is recommended by WHO [128], and it is especially important in developing countries where the risk of gastrointestinal infection is high [170]. Although there are no common recommendations, consensus suggests that delaying the introduction of complementary food beyond 6 months in industrialised countries is not recommended [171-173].

Neither duration of breastfeeding nor the time of introduction of formula/gruel were associated with the risk for SRFA in this thesis research study.

The role of solids, and the timing of introduction to the infants' diets, on the development of allergic diseases has been discussed recently [83, 84]. There are reports indicating that very early introduction, before 4 months of age, might increase the risk of allergy [158]. Late introduction of allergenic food was previously recommended, especially to children with a high risk of developing allergy [139-141,145]. However, recent studies did not support this recommendation. Late introduction of solids, both allergenic and non-allergenic, beyond the age of six months, did not protect the child from developing allergy [159]. Moreover, late introduction of allergenic food, such as cow's milk, was associated with a higher risk of eczema, and delaying other

solids in infants' diets resulted in an increased risk of recurrent wheeze and higher sensitisation to inhalant allergens and food [151,152,155].

The timing of presenting food proteins to a child's immune system in order to develop tolerance is important as it should occur at a certain level of maturity of the immune system [84]. This immunological "early window" is most likely to be between the fourth and sixth month of age. [84, 174]. Missing this time period with the introduction of food proteins might result in dysregulation of the immune system, leading to food allergy and/or autoimmune diseases [175,176]. There are many studies on the timing of introducing allergenic food [136, 147-156] but timing of introduction of non-allergenic food may also be important. The possible effect of complementary food on developing food tolerance might play out both in presenting food proteins to the immune system and influencing the immune system through the impact on intestinal microbiome, which plays an important role in the development of the immune system [56, 58, 59, 78]. Therefore, all factors affecting the microbiome development will consequently influence the immune system, especially in early age. Breastfeeding and weaning affect the intestinal microbiome [77, 79]. First solids, most often cereals, vegetables and fruits contain inulin and fructooligosaccharides, which act as prebiotics for many bacteria in the gut microbiome [58, 177]. Hence, the time of introducing solids ought to play an important role in stimulating the development of the gut microbiome and consequently influencing the immune system and developing tolerance to food proteins. This thesis research study shows that the timing of the introduction of complementary foods might be important for developing tolerance or food allergy.

We found that the late introduction of solids to the infant's diet was associated with a higher risk of SRFA, but not of asthma or eczema for the child or for the child's siblings. Food allergy, asthma and eczema are considered to be early signs of atopy, usually developing in the first year or years of life. There is always a risk of reverse causation, meaning that parents to a child with already existing allergic disease delayed the introduction of solids, as compared to parents of a healthy child. In order to minimise the risk of reverse causation, analyses of the association between late introduction of solids and the risk of developing asthma, eczema and SFRA were performed not only in the index child, but also among the siblings. The results indicate that the late introduction of solids was caused not by an already existing allergic disease in the index child or siblings, but rather was a result of family habits or recommendations. Moreover, further analyses revealed the fact that the late introduction of solids remained a risk factor for SFRA even after adjusting for the intake of formula/gruels introduced before 7 months of age, meaning that

the late introduction of solids was not caused by an already existing food (milk) allergy.

The questions on food allergy in the questionnaire were not validated, which was a limitation of the study. Other limitations were that the data was collected retrospectively and that the incidence of food allergy was self-reported. This might involve a recall bias, and the latter aspects may raise the incidence of food allergy. However, the cumulative incidence of self -reported food allergy from this research study was in accordance with a similar study from the north of Sweden [49], which could indicate that the cumulative incidence of SRFA might be on this level. Ideally, the prevalence of food allergy should be proven in double-blind oral food challenges, which definitively would give lower prevalence figures than the self-reported cumulative incidence. However, the difference between the two towns would probably remain, even if recall bias occurred, since such a bias would have a similar effect in the two towns. Therefore, neither the difference in SRFA between the towns, nor the time of the introduction of solids, can be explained by recall bias. The substantial difficulties with analyses and studies investigating the influence of a feeding mode and the introduction of different food items on the development of allergy, run the risk of selection bias and reverse causation. Therefore, prospective randomised intervention-studies are of choice, but they are arduous, not always possible to perform, and sometimes ethically challenging. As a consequence, and limitation, many studies investigating this subject are often based on questionnaires, as this research study was. Taking into consideration the difficulties associated with these types of epidemiological studies, several statistical methods and analyses, described above, were used in order to decrease the risk involved with selection and reverse causation.

5.3 PROTECTIVE FACTORS FOR DEVELOPING ALLERGY

Hand dishwashing, eating fermented food and food bought directly from farms were protective factors for total allergy

Dietary and lifestyle factors, which may increase microbial exposure have been shown, in our study, to be protective for the development of asthma and allergy. Dishwashing by hand, instead of machine dishwashing, was a strong protective factor against allergy, and the protective effect increased, in a dose-related pattern, if the child also ate fermented food and food bought directly from a farm.

Hand dishwashing is not as efficient as machine dishwashing with regard to bacterial content. The efficiency of machine dishwashing was shown to be superior to hand dishwashing already in the 1940's, while testing 1000 dishwashers in New York [178]. This finding was later confirmed in a German study, where even different hand dishwashing techniques were tested and compared to both each other and to machine dishwashing [179]. The German study showed that bacterial survival on the dishes after hand dishwashing depends on many factors such as the type of dishes, their shape, use of cleaning devices, sanitising agents, the water temperature and especially the remnants of milk products which were difficult to be utilised [180]. Therefore, it is most probable that hand dishwashing increases microbial exposure.

Fermentation is a process where different bacteria such as *Lactobacillus, Streptococcus, Bacillus*, and *Pseudomonas*, and yeasts and fungi play an important role. Therefore, fermented food can increase bacterial exposure, especially fermented vegetables, which can act both as prebiotics and probiotics. The role of pre and probiotics in the development of the gut microbiome, as well as its possible role in preventing and influencing many diseases, has been intensively discussed [181,182], especially in relationship to the development of allergy [183-185]. Buying food directly from farms, often milk products, could also increase microbial exposure. Although there are early reports indicating that ingesting unpasteurised milk products had an allergy-protective effect among people living on farms [186] it is difficult to determine if consumption of unprocessed food or other environmental factors were protective. This study suggests that eating fermented or unprocessed food could have a protective effect on allergy development.

Microbial exposure in affluent countries has decreased due to improved hygiene standards. This has led to a decreased morbidity and mortality from infectious diseases. However, according to the hygiene hypothesis [14], microbial exposure in early life stimulates the immune system, and as such, induces tolerance. Consequently, a decrease in microbial exposure could enhance allergy development. There are many studies which discuss the role of microbial exposure on developing allergy, such as living on a farm [21-30], the presence of a high diversity of intestinal microbiome [16, 57, 81], the microbial content in drinking water [187], the family size [14, 18, 89], attending a day-care facility [188], infections in early life [189] or the parental habit of sucking the child's pacifier as a cleaning method [96].

Many of these exposures are difficult or potentially dangerous for individuals to actively implement. Therefore, identifying new, easily implementable and harmless daily living factors which increase the microbial exposure in affluent countries might be a way of counteracting the rise of allergy.

The main difficulties in interpreting the findings in this part of the study were the risk that confounding factors may influence the results. A lower prevalence of allergic diseases among children whose parents mostly washed dishes manually might be associated to living conditions bound by socioeconomic status. There are other studies showing that a lower socioeconomic status, and an overcrowded housing environment, are protective against developing allergy [190]. These factors could be related to hand dishwashing. Therefore, complex statistical analyses adjusting for the different confounding factors have been applied (Paper II). Recall bias could also be a problem in this part of the thesis research, as parents answered the questions when the children were 7-9 years old. Furthermore, it was not specified if the dish-washing practice recorded in the questionnaire was always applied, also during the child's first year of life. The question concerning fermented food included examples of fermented vegetables, but still, such a question could be misinterpreted. These were expected limitations in the cross-sectional studies and should be taken into consideration while interpreting the results.

Pet keeping during the first year of life was a protective factor for allergy in a dose-related pattern.

Pet keeping during the first year of life was found to be a protective factor for allergy in this study, and the result supports earlier findings [18-20, 105-107]. However, there are other studies which did not find the same association [191]. Moreover, our study showed that the protective effect of keeping animals increased with the number of kept pets. The other interesting finding

was that the degree of sensitisation, not only to animals but also to pollen, decreased with the number of pets kept.

In this part of the thesis (Paper III), two different population studies have been used to increase the quality of research methodology and decrease the risk of spurious results and false interpretations. Both population studies (the birth cohort and the cross-sectional study) showed similar results, namely, a decreased risk of allergy with an increasing number of kept pets, which reduces the risk of selection bias and a "false positive" type 1 error. Although cross-sectional studies have many advantages, such as large populations, problems with recall bias and validity should be addressed. In this part of the research, the results from the cross-sectional study were confirmed with the results from the birth cohort study. In this way the risk of recall bias and reverse causation were minimised. The problem with the validity of diagnosis in the cross-sectional study could also be addressed by this study approach. The birth cohort study, with the strict diagnostic criteria, showed the same results as the cross-sectional study. A second approach to address selection bias, beside the dose-response approach, was in the cross-sectional study to ask the parents for the reasons for not having animals, and in the birth cohort study parents were asked about a history of allergy already during the enrolment and then tested when the child was 6 months old. Both these approaches, as well as the finding that there was no difference in sensitisation between parents who had or did not have animals, support our conclusion that the observed allergy-protective effect from early animal exposure was not explained by selection bias.

The result of the pattern of sensitisation, the decreased sensitisation to both animals and pollen with an increased number of animals kept indoors, indicates that the effect is not species-specific. This supports the hypothesis that early exposure to animals might have a protective effect not only via high allergen exposure, but also via an increase in microbial exposure, i.e. in a farm-like manner.

The tolerance development is most likely to be induced by direct contact with the animal kept indoors [192], via exposure to the allergens, as well as the animals' microbes and endotoxin. If the animal is not kept indoors then the child will be exposed only to the allergen and not to its endotoxins and microbes. According to the hygiene hypothesis [14], microbial exposure is required for proper immune stimulation in order to develop tolerance. Therefore, an increase in microbial exposure is a probable explanation for the protective effect of keeping cats and/or dogs indoors during early life.

Parental birth country

Having both parents born abroad was also a protective factor found in this study. The lower prevalence of allergic diseases among migrants and their children, especially at the early phase of their life in a new country, has previously been reported [194, 195]. However, the protective effect of migration seems to decrease with time [194, 196], indicating that environmental factors in the new country may have an important influence on the risk of allergy development.

A theoretical model of allergy development

A possible model of the complex synergy between different factors affecting the risk of allergy development, integrating the findings from this research, is presented in the figure 12. In this model, the risk of allergy development depends on three different processes. The first is the individual genetic propensity, presented in the left bar. This determines the overall risk of developing allergy, but it can be adjusted by: 1) general tolerance mechanisms presented in the second bar influencing the development of tolerance by altering the immune defence and, 2) selective mechanisms acting as allergy switches presented in the third bar. The genetic propensity cannot explain either the difference in the prevalence of asthma and allergic disease between Kiruna and Mölndal recorded in 1979 and 1991, or in the prevalence of food allergy in 2007. The ethnic population in both towns, and the heredity for allergic diseases, were similar. The general mechanisms influencing development of tolerance, presented in the second bar, are probably subject to individual influence. Therefore, increasing microbial exposure, especially during early childhood such as hand dishwashing, eating fermented food or food directly bought from a farm, and keeping animals at home, could influence the immune system and stimulate development of tolerance. Introducing solids during the early immunological window could also increase tolerance development, e.g. through the influence on the intestinal microbiome. Furthermore, eating fish early in life and consumption of butter may influence tolerance development through immunomodulatory agents [122, 153]. The third bar in the "allergy development model" acts through selective processes which are supposed to be of environmental and epigenetic origin. Climate changes may influence the duration and intensity of the pollen season, and act as a switch-on factor leading to the development of pollen allergy but no other type of allergy. Keeping animals indoors, especially more than one, may act as a switch-off factor through high continuous allergen exposure and decrease the risk of developing allergy to the specific animal. If the time trends for asthma, eczema and allergic rhino-conjunctivitis are

applied to our three-dimensional model for allergy development, it seems that positive changes in some of the general mechanisms are responsible for the break in the trend line for asthma and eczema, while selective mechanisms (such as a longer pollen period) may explain why no such break in the time line is seen for rhino-conjunctivitis.

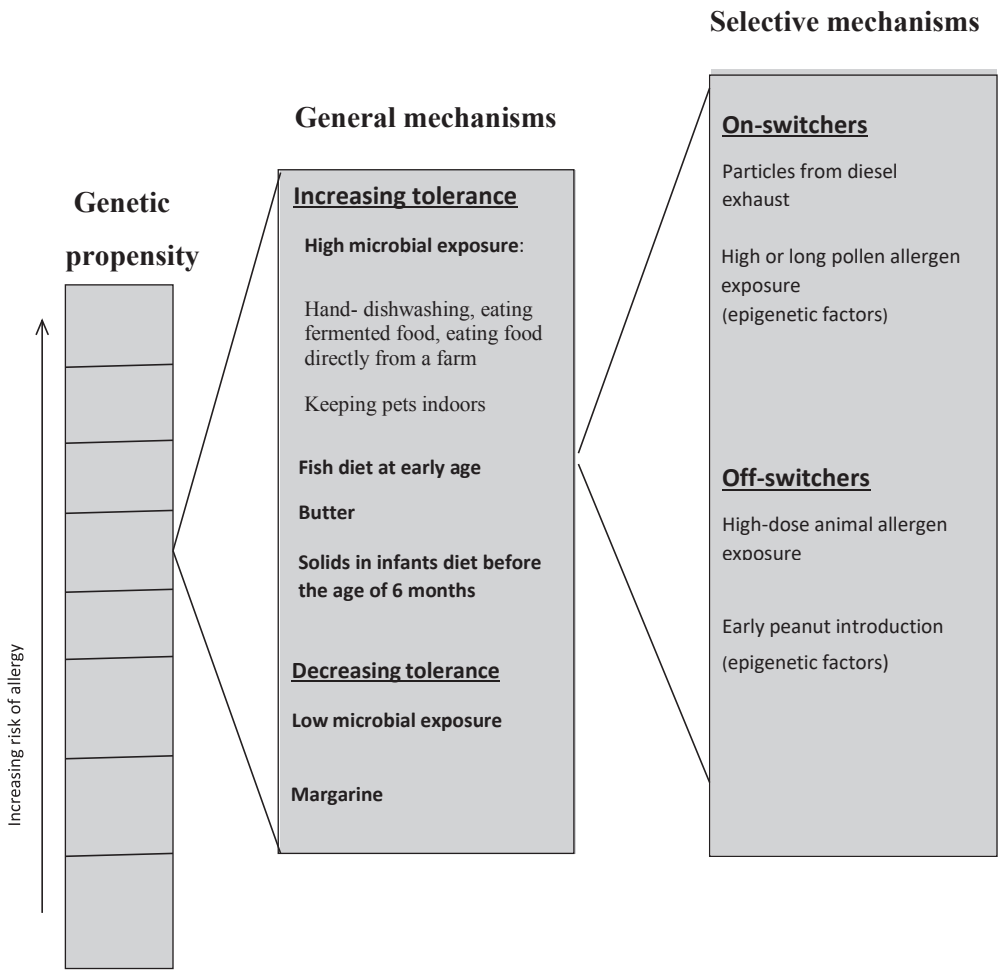


Figure 12. A model integrating genetic propensity, the hygiene hypothesis and specific exposures in the explanation of allergy development. The left-hand bar symbolises genetic propensity. A low genetic propensity results in a relatively low risk of developing allergies independent of other factors. A high genetic propensity, on the other hand, results in a relatively high risk even during the most favourable circumstances. On every genetic propensity level, the risk may be adjusted by general tolerance mechanisms, such as degree of microbial exposure, as shown in the middle bar. However, these first two steps only act on

a global level that is adjusting the risk of becoming allergic. In actual fact becoming sensitised or allergic to a specific allergen is mainly related to specific allergen characteristics and adjuvants, as shown in the right-hand bar. Hicke-Roberts et al. Acta Paediatrica 2016. Paper I. The text in the figure is partly based on the results of this thesis.

5.4 STRENGTHS AND LIMITATIONS

This study was mainly based on the cross-sectional study performed in 2007, and the results were compared with two previous studies from 1979 and 1991. The same set of questions, in the same geographical regions among the same age group of children, were used. The questions on asthma, ARC and eczema were validated in 1992 [2]. The population of the study and the amount of collected data were also strengths of this survey.

In one part of this research (paper III), a prospective birth cohort was used to complement the results from the retrospective cross-sectional study. With this approach, the validity of the diagnosis used in the cross-sectional study was confirmed, and it reduced the risk of selection bias, recall bias and reverse causation.

Different statistical methods were used to decrease the risks associated with the cross-sectional study-methodology.

A limitation was the lower response rate in the 2007 cross-sectional study, as compared to the studies in 1979 and 1991. It could affect the power of the study and cause a risk of type two errors. Nonetheless, the risk of selection bias is low because the results from our study are similar to the results from other studies in similar time periods from northern Sweden [33, 49]. The increase in the prevalence of asthma and eczema levelled off, which suggests the absence of selection bias because the opposite would have been expected if selection bias was an issue since, in such a case, mostly parents to allergic children would respond to the questionnaire.

Other limitations were that questions on food allergy were not validated and that the data was collected retrospectively. Furthermore, the incidence of food allergy was self-reported. This may involve recall bias, as well as diagnostic uncertainty, raising the incidence of food allergy. However, self -reported food allergy from this study was similar to another study from the north of Sweden [49], which indicates that the cumulative incidence of SRFA might be at this level. The prevalence of food allergy should be proven in double-blind oral food challenges for accurate results. Probably, the difference between the two towns would still remain, even if recall bias occurred and would result in a similar effect in both towns. Therefore, neither the difference in SRFA between the towns, nor the time of introduction of solids, can be explained by recall bias. Taking into consideration the difficulties associated

with risk for selection bias and reverse causation in these types of epidemiological studies, several statistical methods and analyses, described above, were used in order to decrease the risk involved with selection and reverse causation.

The risk of confounding factors which might have influenced the results was another issue. A lower prevalence of allergic diseases among children whose parents mostly washed dishes manually might have been associated to living conditions bound by a socioeconomic status. Therefore, complex statistical analyses adjusting for the different confounding factors were applied (Paper II). Recall bias could also have been a problem as parents answered the questions when the children were 7-9 years old. Furthermore, it was not specified if the dish-washing practice recorded in the questionnaire was always applied, in particular during the child's first year of life. The question about fermented food could have been misinterpreted although it included examples of fermented vegetables. These were expected limitations in the cross-sectional studies and should be taking into consideration while interpreting the results.

The problem with recall bias and validity was also of concern in Paper III. Therefore, the results from the cross-sectional study were confirmed with the results from the birth cohort study. In this way, the risks of recall bias, reverse causation and the problem of diagnostic validity in the cross-sectional study were decreased. To address selection bias, besides the dose-response approach, parents were asked for the reasons for not having animals in the cross-sectional study, and, in the birth cohort study parents were asked about a history of allergy already during the enrolment and then tested when the child was 6 months old. These approaches, as well as the finding that there was no difference in sensitisation between parents who had or did not have animals, support our conclusion that the observed allergy-protective effect from early animal exposure was not explained by selection bias.

6 CONCLUSION

The prevalence of asthma and eczema levelled off from 1991 to 2007, while allergic rhino-conjunctivitis continued to increase. While regional differences in the prevalence of asthma, ARC and eczema were noticed only among boys, the cumulative incidence of food allergy was significantly higher in Kiruna than in Mölndal in both sexes. Heredity for allergic diseases was a risk factor for the development of allergies. Our results do not give any specific explanation for this break in the trend line for asthma and eczema, but one may speculate that climate change with longer pollen periods may have prevented a similar course for rhino-conjunctivitis.

Late introduction of solids to infants' diets increased the risk for the development of food allergy. While there was no difference in the history of allergy among parents in Kiruna and Mölndal, it was found that solids were introduced later in Kiruna. Therefore, late introduction of solids to infants' diets may, per se, be one explanation for the higher cumulative incidence of food allergy in Kiruna.

Hand dishwashing instead of using machine dishwashing was a strong protective factor against allergy. The protective effect increased, in a dose-related pattern, if the child also ate fermented food and food bought directly from a farm. These factors might increase microbial exposure and, in this way, stimulate the immune system to develop tolerance.

Pet-keeping during the first year of life was also found to be protective against development of allergy. This protective effect increased with the number of pets. The degree of sensitisation, not only to animals but even to pollen, decreased with the number of pets, suggesting that indoor pets may reduce the risk of allergy development in a "farm-like" manner.

7 FUTURE PERSPECTIVES

It is important to follow up how the prevalence of allergic diseases among children develop over time, both regionally and nationally. Will ARC and food allergy continue to increase, or will they start to level of as has been seen for asthma and eczema? Analysing the time trend in relation to possible protective factors may also help in understanding the mechanism behind allergy development, and it will help in planning and estimating the burden for the health system.

It would be also interesting to follow up the cumulative incidence of food allergy and if the regional difference between Kiruna and Mölndal will remain the same as recorded in 2007. In this research study the changes over time could not be analysed for food allergy, since we had no such data in 1979 nor in 1991. There are, however, many reports showing that food allergy is increasing globally, but the knowledge of local changes would be of benefit to the health services in caring for children suffering from allergy.

Finding new protective factors against allergy, preferably by increasing microbial exposure in a safe way, is desirable in affluent countries. This should ideally be investigated in prospective- or interventional studies to avoid or minimise methodological pitfalls. Some of the allergy-protective factors found in this study ought to be confirm in prospective studies before official recommendations can be encouraged in parental lifestyles.

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APPENDIX

I- The questionnaire in Swedish

II-The translated questionnaire in English

III- Diagnostic criteria birth cohort

Appendix I- The questionnaire in Swedish

Frågeformulär

Förekomst av astma och allergi bland skolbarn i Sverige och Polen

Datum då formuläret fylls i……

Barnets namn……

Barnets
personnummer…….

Barnets adress……

Postnummer……

Postadress……

Tel dagtid ……Mammans mobiltel……

Tel kvällstid……Pappans mobiltel……

Kön: Flicka ☐

Pojke ☐

Informerat samtycke

Jag har läst och fått en kopia av föräldrainformationen. Jag har haft möjlighet att ställa frågor och mina frågor har besvarats tillfredställande. Jag är medveten om att deltagandet är frivilligt och att vi föräldrar kan avbryta vårt barns medverkan när vi önskar utan närmare motivering, utan att det kommer att påverka vårt barns möjligheter till framtida behandling.

Jag ger också min tillåtelse till att insamlade uppgifter bevaras i dataregister.

Datum……..

Förälders namnteckning……

Textat namn……

Andningsbesvär – frågor baserade på ISAAC-formuläret

	Ja	**Nej**
2) Har Ert barn ***någonsin*** haft väsande eller pipande andningsljud i bröstet?	☐	☐

Om ni svarat **"NEJ",** gå direkt till fråga 7

---

3) Har Ert barn haft väsande eller pipande andningsljud i bröstet någon gång under de ***senaste 12 månaderna***? ☐ ☐

Om ni svarat **"NEJ",** gå direkt till fråga 7

---

4) Hur många episoder med väsande eller pipande andningsljud i bröstet har Ert barn haft de ***senaste 12 månaderna***?

- Ingen ☐
- 1 – 3 gånger ☐
- 4 – 12 gånger ☐
- Fler än 12 gånger ☐

5) Under de ***senaste 12 månaderna*** hur ofta har i genomsnitt

Ja **Nej**

Ert barns sömn störts av andningsbesvär enligt ovan?

Aldrig vaknat med besvär ☐
Mindre än en natt/vecka ☐
En – flera nätter/vecka ☐

6) Under de ***senaste 12 månaderna*** har Ert barns andningsbesvär någon gång varit så svår att det endast kunnat säga ett-två ord mellan andetagen?

☐ ☐

---

7) Har Ert barn ***någonsin*** haft ***astma***?

☐ ☐

8) Har Ert barn de ***senaste 12 månaderna*** haft pip i bröstet eller väsande andning under eller efter ansträngning? ☐ ☐

9) Har barnet under de ***senaste 12 månaderna*** haft nattlig torrhosta utan att ha varit förkyld eller haft en luftrörskattar eller lunginflammation

☐ ☐

Andningsbesvär – frågor baserade på tidigare Svenska undersökningar

	Ja	**Nej**
10) Har barnet haft ***astma*** eller ***astmatisk luftrörskatarr***?	☐	☐

Om ni svarat **"NEJ",** gå direkt till fråga 22

---

11) Vid vilken ålder märktes

besvären första gången?…….. års ålder

12) Vid vilken ålder hade

barnet senast besvär?……års ålder

13) Har barnet haft astma eller astmatisk luftrörskatarr

senaste året? ☐ ☐

14) Antal gånger med besvär senaste året…… antal ggr

15) Hur många dagar varar besvären varje gång? ☐ 1-3 dagar

☐ 4-fler

Ja **Nej**

16) Har barnet varit inlagt på sjukhus för astma eller astmatisk luftrörskatarr? ☐ ☐

17) Om ja, hur många gånger har barnet varit inlagt på sjukhus för astma eller astmatisk luftrörskatarr? …… antal ggr

18) När får/fick barnet astma eller astmatisk luftrörskatarr?

☐ Vid förkylning

☐ Vid ansträngning

☐ Av kyla

☐ Vid kontakt med djur

☐ Vid lövsprickning (utomhus i maj)

☐ Utomhusvistelse i juni-juli

☐ Vid kontakt med damm

☐ Av födoämnen

19) Har barnet ***senaste 12 månaderna*** behövt vara hemma från skolan pga astma eller astmatisk luftrörskatarr? ☐ ☐

Ja **Nej**

20) Hur många dagar har barnet senaste 12 månaderna behövt vara hemma från skolan pga astma eller astmatisk luftrörskatarr?……antal dagar

21) Har barnet senaste 12 månaderna tagit medicin mot astma eller astmatisk luftrörskatarr? ☐ ☐

Om ja, skriv vilka mediciner barnet tar, vilken styrka och hur ofta medicinen tas:

Medicinens namn	***Styrka***	***Hur ofta tas medicinen***

22) Brukar barnet få hosta vid ansträngning? ☐ ☐

Näs- och ögonbesvär - frågor baserade på ISAAC formuläret

	Ja	Nej
23) Har Ert barn ***någonsin*** varit besvärad av nysningar, rinnsnuva eller nästäppa utan att ha varit förkylt?	☐	☐

Om ni svarat **"NEJ",** gå direkt till fråga 28

---

24) Har Ert barn under de ***senaste 12 månaderna*** varit besvärad av nysningar**,** rinnsnuva eller nästäppa utan att ha varit förkylt? ☐ ☐

Om ni svarat **"NEJ",** gå direkt till fråga 28

---

25) Har under de ***senaste 12 månaderna*** dessa näsbesvär åtföljts av kliande, rinnande ögon? ☐ ☐

26) I vilken/vilka månader hade Ert barn näsbesvär (sätt "x" i lämpliga rutor)?

☐ januari	☐ maj	☐ september
☐ februari	☐ juni	☐ oktober
☐ mars	☐ juli	☐ november
☐ april	☐ augusti	☐ december

27 Under de ***senaste 12 månaderna***, hur mycket påverkade näsbesvären Ert barns dagliga aktiviteter?

☐ Inte alls
☐ Något lite
☐ Måttligt
☐ Ganska mycket

28) Har Ert barn ***någonsin*** haft "hösnuva"? ☐ ☐

Näs- och ögonbesvär – frågor baserade på tidigare Svenska undersökningar

	Ja	Nej
29) Har barnet haft ***allergisk snuva/ögonkatarr?***	☐	☐

Om ni svarat **"NEJ",** gå direkt till fråga 36

30) Vid vilken ålder märktes besvären första gången?……års ålder

31) Vid vilken ålder hade barnet senast besvär……. .års ålder

32) Har barnet haft allergisk snuva/ögonkatarr senaste året? ☐ ☐

33) När får barnet allergisk snuva/ögonkatarr?

☐ Vid kontakt med djur
☐ Vid lövsprickning (maj)
☐ Under juni-juli månad
☐ Vid kontakt med damm

34) Har barnet senaste året fått behandling för allergisk snuva/ögonkatarr? ☐ ☐

35) Om ja, hur ofta?

☐ Enstaka gånger
☐ Vår och/eller sommar
☐ Året runt

Hudbesvär – frågor baserade på ISAAC-formuläret

	Ja	Nej
36) Har Ert barn ***någonsin*** haft ett kliande utslag som kommit och gått under minst 6 månader?	☐	☐

Om ni svarat **”NEJ”,** gå direkt till fråga 42

---

37) Har Ert barn haft detta kliande utslag någon gång under de senaste 12 månaderna?	☐	☐

Om ni svarat **”NEJ”,** gå direkt till fråga 42

---

38) Har detta kliande utslag vid något tillfälle förekommit på något av följande ställen: *armveck, knäveck, fotleder, på lårens baksidor eller på halsen, kring ögonen eller öronen*? ☐ ☐

39) Vid vilken ålder sågs utslagen första gången?

☐ Före 2 års ålder
☐ mellan 2 och 5 års ålder
☐ Från fyllda 5 års ålder

40) Har detta utslag försvunnit helt vid något tillfälle under de ***senaste 12 månaderna***? ☐ ☐

41) Under de **senaste 12 månaderna**, hur ofta, i genomsnitt, har det kliande utslaget hållit Ert barn vaket nattetid?

☐ Aldrig
☐ Inte så ofta som en natt/vecka
☐ En eller flera nätter/vecka

42) Har Ert barn ***någonsin*** haft eksem? ☐ ☐

Hudbesvär – frågor baserade på tidigare Svenska undersökningar

	Ja	Nej
43) Har barnet haft ***eksem***?	☐	☐

Om ni svarat **"NEJ",** gå direkt till fråga 50

---

44) Vid vilken ålder märktes eksemet första gången?…….. års ålder

45) Vid vilken ålder hade barnet senast besvär…….. års ålder

46) Har barnet haft eksem senaste året? ☐ ☐

47) Behöver barnet daglig smörjning pga eksem? ☐ ☐

48) Vilka salvor brukar barnet använda?

Mjukgörande salva/kräm	Kortisonsalva/kräm
☐ Aldrig	☐ Aldrig
☐ Ibland	☐ Ibland
☐ Nästan varje dag	☐ Nästan varje dag
☐ Varje dag	☐ Varje dag

Ange namn på annan salva eller kräm som barnet använder mot eksem?…….

49) Får barnet klåda av viss mat? ☐ ☐

---

50) Har barnet haft ***nässelutslag/allergisk svullnad***? ☐ ☐

	Ja	**Nej**
51) Vid vilken ålder märktes besvären första gången?……. års ålder		
52) Vid vilken ålder hade barnet senast besvär?……års ålder		
53) Har barnet haft nässelutslag/allergisk svullnad senaste året?	☐	☐
54) Har barnet fått utslagen av viss mat?	☐	☐

Födoämnesallergi/överkänslighet

55) Har barnet reagerat med allergi eller överkänslighet mot något födoämne? ☐ ☐

Om ”**NEJ**”, gå till fråga 56

Ange nedan för varje födoämne vid vilken ålder besvären började, vid vilken ålder de märktes senast, och vilka symtom/besvär barnet hade

☐ Mjölk
besvären började vid…….års ålder. Besvär senast vid……års ålder

Symtom/typ av besvär

☐ Klåda i munnen	☐ Kräkningar
☐ Diarré	☐ Magvärk
☐ Hudrodnad	☐ Eksem
☐ Svullnad i ansikte	☐ Nässelutslag
☐ Andningsbesvär	☐ Ögon/näsbesvär

☐ Ägg
besvären började vid……. års ålder. Besvär senast vid……års ålder

Symtom/typ av besvär

☐ Klåda i munnen	☐ Kräkningar
☐ Diarré	☐ Magvärk
☐ Hudrodnad	☐ Eksem
☐ Svullnad i ansikte	☐ Nässelutslag

☐ Andningsbesvär ☐ Ögon/näsbesvär

☐ Fisk
besvären började vid……. års ålder. Besvär senast vid……års ålder

Symtom/typ av besvär
☐ Klåda i munnen ☐ Kräkningar
☐ Diarré ☐ Magvärk
☐ Hudrodnad ☐ Eksem
☐ Svullnad i ansikte ☐ Nässelutslag
☐ Andningsbesvär ☐ Ögon/näsbesvär

☐ Jordnötter
besvären började vid……. års ålder Besvär senast vid……års ålder

Symtom/typ av besvär
☐ Klåda i munnen ☐ Kräkningar
☐ Diarré ☐ Magvärk
☐ Hudrodnad ☐ Eksem
☐ Svullnad i ansikte ☐ Nässelutslag
☐ Andningsbesvär ☐ Ögon/näsbesvär

☐ Andra nötter/mandel
besvären började vid……. års ålder. Besvär senast vid……års ålder

Symtom/typ av besvär
☐ Klåda i munnen ☐ Kräkningar
☐ Diarré ☐ Magvärk
☐ Hudrodnad ☐ Eksem
☐ Svullnad i ansikte ☐ Nässelutslag
☐ Andningsbesvär ☐ Ögon/näsbesvär

☐ Mjöl (vete, havre, korn eller råg)
besvären började vid…….års ålder . Besvär senast vid……års ålder

Symtom/typ av besvär
☐ Klåda i munnen ☐ Kräkningar
☐ Diarré ☐ Magvärk
☐ Hudrodnad ☐ Eksem
☐ Svullnad i ansikte ☐ Nässelutslag
☐ Andningsbesvär ☐ Ögon/näsbesvär

☐ annat födoämne (ange vilket)
……

besvären började vid…….års ålder Besvär senast vid……års ålder

Symtom/typ av besvär

- ☐ Klåda i munnen
- ☐ Diarré
- ☐ Hudrodnad
- ☐ Svullnad i ansikte
- ☐ Andningsbesvär
- ☐ Kräkningar
- ☐ Magvärk
- ☐ Eksem
- ☐ Nässelutslag
- ☐ Ögon/näsbesvär

☐ annat födoämne (ange vilket)
……

besvären började vid…….års ålder Besvär senast vid……års ålder

Symtom/typ av besvär

- ☐ Klåda i munnen
- ☐ Diarré
- ☐ Hudrodnad
- ☐ Svullnad i ansikte
- ☐ Andningsbesvär
- ☐ Kräkningar
- ☐ Magvärk
- ☐ Eksem
- ☐ Nässelutslag
- ☐ Ögon/näsbesvär

Barnets pappa

	Ja	**Nej**
56**)** Har pappan haft ***astma eller astmatisk luftrörskatarr?***	☐	☐

Om **"NEJ",** gå direkt till fråga 59

---

57) Vid vilken ålder märktes besvären första gången? …… års ålder

58) Vid vilken ålder hade pappan senast besvär? …….. års ålder

---

	Ja	**Nej**
59) Har pappan haft ***allergisk snuva/ögonkatarr?***	☐	☐

Om **"NEJ",** gå direkt till fråga 62

---

60) Vid vilken ålder märktes besvären första gången? ……års ålder

61)Vid vilken ålder hade pappan senast besvär? …… .års ålder

---

62) Har pappan haft ***eksem?*** ☐ ☐

Om **"NEJ",** gå direkt till fråga 66

63**)** Vid vilken ålder märktes besvären första gången? …… års ålder

64)Vid vilken ålder hade pappan senast besvär? ……års ålder

65) Har eksemet varit kontaktallergi? ☐ ☐

---

66) Har pappan haft ***nässelutslag/allergisk svullnad****?* ☐ ☐

Om **"NEJ",** gå direkt till fråga 69

67**)** Vid vilken ålder märktes besvären första gången? ……års ålder

68) Vid vilken ålder hade pappan senast besvär? ……års ålder

---

69) Har pappan reagerat med allergi eller överkänslighet mot **något födoämne?** ☐ ☐

70) Har pappan reagerat med allergi eller överkänslighet mot **något födoämne sista året**? ☐ ☐

Om **"NEJ",** gå direkt till fråga 72

---

71) Om ja, mot vilka födoämnen?

☐ Mjölk ☐ Ägg ☐ Fisk ☐ Vete (och andra mjölsorter)
☐ Soja ☐ Äpple ☐ Jordnöt ☐ Andra nötter
☐ Annat födoämne, ange vilket/vilka
……

Barnets mamma

	Ja	Nej
72) Har mamman haft ***astma eller astmatisk luftrörskatarr?***	☐	☐

Om **"NEJ",** gå direkt till fråga 75

---

73) Vid vilken ålder märktes besvären första gången? ……års ålder

74) Vid vilken ålder hade mamman senast besvär? ……års ålder

---

75) Har mamman haft ***allergisk snuva/ögonkatarr?*** ☐ ☐

Om **"NEJ",** gå direkt till fråga 78

---

76) Vid vilken ålder märktes besvären första gången? ……års ålder

77)Vid vilken ålder hade mamman senast besvär? …….års ålder

---

78) Har mamman haft ***eksem?*** ☐ ☐

Om **"NEJ",** gå direkt till fråga 82

79**)** Vid vilken ålder märktes besvären första gången? …….års ålder

	Ja	**Nej**
80)Vid vilken ålder hade mamman senast besvär? …….	års ålder	
81) Har eksemet varit kontaktallergi?	☐	☐
82) Har mamman haft ***nässelutslag/allergisk svullnad?***	☐	☐

Om **"NEJ",** gå direkt till fråga 85

83) Vid vilken ålder märktes besvären första gången? ……års ålder

84) Vid vilken ålder hade mamman senast besvär? ……års ålder

85) Har mamman reagerat med allergi eller överkänslighet mot **något födoämne?**	☐	☐
86) Har mamman reagerat med allergi eller överkänslighet mot **något födoämne sista året**?	☐	☐

Om **"NEJ",** gå direkt till fråga 88

87) Om ja, mot vilka födoämnen?

☐ Mjölk ☐ Ägg ☐ Fisk ☐ Vete (och andra mjölsorter)
☐ Soja ☐ Äpple ☐ Jordnöt ☐ Andra nötter
☐ Annat födoämne, ange vilket/vilka
……..

Frågor om familj och miljö

	Ja	Nej
88) I vilken graviditetsveckan föddes barnet?.....vecka		
89) Föddes barnet i Sverige?	☐	☐
Om ”Nej” i vilken land …….		
90) Föddes pappan i Sverige?	☐	☐
Om ”Nej” i vilken land …….		
91) Vilket år föddes pappa? 19……		
92)Vilket yrke ha pappa ?.....		
93)Vilken utbildning har pappa?(sätta en kryss i motsvarande rutan)		

Pappans utbildning		
		Grundskola
		Gymnasium 2-4år el motsvarande
		Högskola eller universitet, max 120 poäng
		Högskola el universitet > 120 poäng

94) Föddes mamma i Sverige?	☐	☐
Om ”Nej” i vilken land …….		
95) Vilket år föddes mamma? 19……		
96) Vilket yrke har mamma?.....		
97) Vilken utbildning har mamma? (sätta en krys i motsvarande rutan)		

Mammans utbildning		
		Grundskola

		Gymnasium 2-4år el motsvarande
		Högskola eller universitet, max 120 poäng
		Högskola el universitet > 120 poäng

	Ja	**Nej**
98) Har barnet syskon?	☐	☐

Om ”Ja”, fyll i tabellen nedan

Ange syskonens:			**Har syskonen:**			
Förnamn	**kön**	**födelseår**	**astma**	**hösnuva**	**eksem**	**Födoämnesallergi**

99) Rökte mamman under graviditeten?	☐	☐
100) Har pappan eller någon annan i hushållet rökt regelbundet inomhus medan mamman var gravid med barnet?	☐	☐
101) Har någon rökt inomhus under barnets första levnadsår?	☐	☐
102) Har Ni någon gång under graviditeten haft hundar, katter eller andra djur som bott eller i huvudsak vistats inomhus?	☐	☐

Om ”Ja”, ange:
Antal hundar:……..
Antal katter…….
Andra djur……..

	Ja	**Nej**
103) Hade Ni någon gång under barnets första levnadsår hundar, katter eller andra djur som bott eller i huvudsak vistats inomhus?	☐	☐

Om ”Ja”, ange:
Antal hundar:……..
Antal katter …….
Andra djur……..

Om ”Nej”, ange varför:
☐ vi/jag ville inte ha djur
☐ ville, men kunde ej pga allergibesvär hos någon i familjen
☐ vi/jag ville undvika att barnet blev allergiskt
☐ annat skäl……..

104)Har ni haft hund, katt eller annat djur under senaste året? ☐ ☐

105)Vistas något av djuren regelbundet inomhus? ☐ ☐

Om ”Ja”, ange vilket/vilka:…….

106) Ungefär hur många luftvägsinfektioner (förkylningar) har barnet haft?

Under första levnadsåret?

☐0-5 ☐ 6-10 ☐ mer än 10 ☐ minns ej

under senaste året ?

☐0-5 ☐ 6-10 ☐ mer än 10

107) Hur många gånger behövde barnet ta antibiotika?

Under det första levnadsåret…….. ……

Totalt under hela livet? ungefär……..

	Ja	**Nej**
108) Har barnet varit på daghem/hos dagmamma?	☐	☐

Om ”**JA**”:
I vilket ålder började barnet på daghem?.....års ålder

109)Bor barnet tillsammans med

☐ båda föräldrarna
☐ en förälder och styvförälder
☐ ensamstående mamma
☐ ensamstående pappa

110) Bor familjen i:

☐ villa
☐ rad- eller kedjehus
☐ lägenhet
☐ bondgård

Bostad storlek……m2
Antal boende……personer

111)Har Ni fukt eller mögelskada i bostaden? ☐ ☐

Barnets kost

	Ja	Nej
112)Ammades barnet?	☐	☐

Om ”**JA**” hur många månader?.....

113) Vid vilken ålder började barnet få tillägg eller välling ?
……..månaders ålder

114) Vid vilken ålder började barnet få pure och annan mat?…….månaders ålder?

115) Brukade ni lagat mat själv till barnet under första levnadsåret?

☐ aldrig eller nästan aldrig
☐ ibland
☐ ung. hälften
☐ oftast
☐ alltid

116) Hur ofta äter barnet frukt?
☐ aldrig eller nästan aldrig
☐ 1-2 frukter per vecka
☐ 3-6 frukter per vecka
☐ Minst 1 frukt per dag
☐ Flera frukter per dag

117) Hur ofta äter barnet grönsaker?
☐ aldrig eller nästan aldrig
☐ 1-2 ggr per vecka
☐ 3-6 ggr per vecka
☐ Minst 1 gång per dag
☐ Flera ggr per dag

118) Hur ofta äter barnet yoghurt?
☐ Aldrig
☐ 1-3 ggr per vecka
☐ mer än 4 ggr per vecka

119) Hur ofta äter barnet fisk?
☐ Aldrig eller nästan aldrig
☐ 1 -2 ggr per månad
☐ 1 ggr per vecka
☐ flera ggr per vecka

120) Äter barnet mat som innehåller fermenterade (surgjorda) grönsaker(t.ex. surkål, sur gurka – inte inlagd gurka) eller annat mat som är fermenterad?
☐ Aldrig eller nästan aldrig
☐ 1 -2 ggr per månad
☐ 1 ggr per vecka
☐ flera ggr per vecka

121) Hur ofta brukar Ni använda halvfabrikat när Ni lagar mat åt barnet (t.ex. fiskpinnar, köttbullar, korv)?
☐ Aldrig eller nästan aldrig
☐ 1-3 ggr per månad
☐ 1-3 ggr per vecka
☐ varje dag eller nästan varje dag

122) Hur ofta brukar barnet äta ”snabbmat ” (t.ex. hamburgare, pizza)?
☐ Aldrig eller nästan aldrig
☐ 1-3 ggr per månad
☐ 1-2 ggr per vecka
☐ mer än 3 ggr per vecka

123) Hur ofta brukar barnet äta jordnötter?
☐ Aldrig
☐ 1-2 ggr per månad
☐ 1-2 ggr per vecka
☐ mer än 3 ggr per vecka

124) Hur ofta brukar barnet äta andra nötter (tex mandel, hasselnöt etc)?
☐ Aldrig
☐ 1-2 ggr per månad
☐ 1-2 ggr per vecka
☐ mer än 3 ggr per vecka

125) Hur ofta brukar barnet dricka läsk?
☐ Aldrig
☐ 1-2 ggr per månad

Ja **Nej**

☐ 1-2 ggr per vecka
☐ varje dag eller nästan varje dag

126) Hur mycket mjölk dricker barnet?
☐ Aldrig
☐ 1-2 glas per vecka
☐ 1-2 glas dagligen
☐ mer än 3 glas dagligen

127)Hur ofta äter barnet viltkött, älg, ren, hjort, rådjur eller vildsvin?
☐ Aldrig eller nästan aldrig
☐ 1-5 ggr per år
☐ 1-2 ggr per månad
☐ en gång per vecka eller oftare

128)Köper ni ibland ägg, kött eller opastöriserad mjölk direkt från bondgården? ☐ ☐

129) Hur brukar ni diska?
☐ oftast handdisk
☐ oftast i diskmaskin

Tack för er medverkan.
Frågeformuläret lämnas till skolan. Använd gärna bifogat kuvert.

Appendix II

Questionnaire
Occurrence of asthma and allergy among school children in Sweden and Poland

Date when the questionnaire is answered ……

1) Name of the child
……

Personal identification number
……

Address……..

Postal code…… Postal address……

Phone day time …… Mother's mobile phone ……

Phone evening…… Father's mobile phone ……

Gender: Girl ☐
Boy ☐

Informed consent

I have received and read the copy of the information regarding the study. I have had the right to ask questions regarding the study and my questions have been answered. I am aware that taking part in the study is voluntary and we can withdraw participation of our child at any time during the study without offering a motivation and without influencing our child's possibility of any future treatment.

Furthermore, I agree to allow any information that is given in the study to be kept in a computer bank.

Date…….

Parent's
signature……

Name in block letters ……..

Wheezing module – questions from the ISAAC questionnaire

	Yes	No
2) Has your child ever had wheezing or whistling in the chest at any time in the past?	☐	☐

IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 7

---

3) Has your child had wheezing or whistling in the chest in the last 12 months? ☐ ☐

IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 7

---

4) How many attacks of wheezing has your child had in the last 12 months?

None ☐
1 to 3 ☐
4 to 12 ☐
More than 12 ☐

5) In the last 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?

Never woken with wheezing ☐
Less than one night per week ☐
One or more nights per week ☐

6) In the last 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths? ☐ ☐

---

7) Has your child ever had asthma? ☐ ☐

8) In the last 12 months, has your child's chest sounded wheezy during or after exercise? ☐ ☐

Yes No

9) In the last 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or a chest infection? ☐ ☐

Breathing difficulties – questions based on previous Swedish investigations

10) Has the child had ***asthma*** or ***wheezing bronchitis***? ☐ ☐

If you answered **"NO",** go directly to question 22

---

11) At what age were the symptoms first seen? ……..
year of age

12) At what age did the child last time have the symptoms? ……
year of age

13) Has the child had asthma or wheezing bronchitis during the last year? ☐ ☐

14) Number of times with symptoms during the last year ……
times

15) How many days do the symptoms last each time?
☐ 1-3 days
☐ 4-more

16) Has the child been hospitalised due to asthma or wheezing bronchitis? ☐ ☐

17) If yes, how many times has the child been hospitalised due to asthma or wheezing bronchitis?…… times

Yes No

18) When does/did the child get asthma or wheezing bronchitis?

☐ with colds
☐ At exercise
☐ By cold weather
☐ On contact with animals
☐ At leafing (outdoors in May)
☐ Outdoors in June-July
☐ On contact with dust
☐ By foodstuffs

19) Has the child during the ***last 12 months*** needed to stay home from school due to asthma or wheezing bronchitis? ☐ ☐

20) How many days during the ***last 12 months*** has the child needed to stay at home from school due to asthma or wheezing bronchitis?……number of days

21) Has the child during the ***last 12 months*** taken any medication due to asthma or wheezing bronchitis? ☐ ☐

If yes, write down what medicines the child takes, what strength and how often the medicine is taken:

Name of the drug	***Strength***	***How often is it taken***

	Yes	No
22) Does the child usually cough during exercise?	☐	☐

Rhinitis module – questions from the ISAAC questionnaire

23**)** Has you child ever had a problem with sneezing, or a runny, or a blocked nose when he/she DID NOT have a cold or the flu? ☐ ☐

IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 28

---

24) In the past 12 months, has your child had a problem with sneezing, or a runny, or a blocked nose when he/she DID NOT have a cold or the flu? ☐ ☐

IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 28

---

25) In the past 12 months, has this nose problem been accompanied by itchy-watery eyes? ☐ ☐

26) In which of the past 12 months did this nose problem occur?
(please tick any which apply)

☐ January	☐ May	☐ September
☐ February	☐ June	☐ October
☐ March	☐ July	☐ November
☐ April	☐ August	☐ December

27) In the past 12 months, how much did this nose problem interfere with your child's daily activities?

☐ Not at all

Yes **No**

☐ A little
☐ A moderate amount
☐ A lot

28) Has your child ever had hay fever? ☐ ☐

Nose and eye troubles – questions based on previous Swedish investigations

29) Has the child had ***allergic problems from the nose/eyes?*** ☐ ☐

If you answered **"NO",** go directly to question 36

---

30) At what age were the troubles first seen?……years of age

31) At what age did the child last have troubles?…… years of age

32) Has the child had allergic problems from the nose/eyes during the last year? ☐ ☐

33) When does the child get allergic problems from the nose/eyes?

☐ On contact with animals
☐ At leafing (May)
☐ During June-July
☐ On contact with dust

34) Has the child during the last year got treatment for allergic problems from the nose/eyes? ☐ ☐

35) If yes, how often?

☐ Occasionally
☐ Spring and/or summer
☐ All-the-year-round

Eczema module – questions from the ISAAC questionnaire

	Yes	No
36) Has your child ever had an itchy rash which was coming and going for at least 6 months?	☐	☐

IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 42

---

37) Has your child had this itchy rash at any time in the last 12 months?	☐	☐

IF YOU ANSWERED "NO" PLEASE SKIP TO QUESTION 42

---

38) Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?	☐	☐

39) At what age did this itchy rash first occur?

☐ Under 2 years
☐ Age 2–4
☐ Age 5 years or more

40) Has this rash cleared completely at any time during the last 12 months?	☐	☐

41) In the last 12 months, how often, on average, has your child been kept awake at night by this itchy rash?

☐ Never in the last 12 months
☐ Less than one night per week
☐ One or more nights per week

42) Has your child ever had eczema?	☐	☐

Skin problems – questions based on previous Swedish investigations

Yes **No**

43) Has the child had ***eczema***? ☐ ☐

If you answered **"NO",** go directly to question 50

---

44) At what age was the eczema first seen?……..years of age

45) At what age did the child last have problems……. years of age

46) Has the child had eczema during the last year? ☐ ☐

47) Does the child need daily application of ointments due to eczema? ☐ ☐

48) Which ointments does the child usually use?

Softening ointment/cream

☐ Never
☐ Sometimes
☐ Almost every day
☐ Every day

Cortisone ointment/cream

☐ Never
☐ Sometimes
☐ Almost every day
☐ Every day

Name of any other ointment or cream that the child uses against eczema
……..

49) Does the child get an itch from certain foods? ☐ ☐

---

50) Has the child had ***urticaria (hives)/allergic oedema***? ☐ ☐

Yes **No**

51) At what age were the problems first seen?…… years of age

52) At what age did the child last have problems?……years of age

53) Has the child had urticaria (hives)/allergic oedema during the last year? ☐ ☐

54) Has the child got a rash from certain food? ☐ ☐

Food allergy/intolerance

55) Has the child reacted with allergy or intolerance to any foodstuff? ☐ ☐

If "**NO**", go to question 56

State below for each foodstuff at what age the problems started, at what age they were last seen, and what symptoms/problems the child had.

☐ Milk. The problems started at…….years of age. Problems last time experienced at……years of age.

Symptom/type of problem

☐ Itch in the mouth
☐ Diarrhoea
☐ Red flush (skin)
☐ Swollen face
☐ Breathing problems
☐ Vomiting
☐ Stomach pain
☐ Eczema
☐ Urticaria/hives
☐ Eye/nose troubles

☐ Egg. The problems started at…….years of age. Problems last time experienced at……years of age.

Symptom/type of problem

☐ Itch in the mouth
☐ Diarrhoea
☐ Red flush (skin)
☐ Swollen face
☐ Vomiting
☐ Stomach pain
☐ Eczema
☐ Urticaria/hives

☐ Breathing problems ☐ Eye/nose troubles

☐ Fish. The problems started at…….years of age. Problems last time experienced at……years of age.

Symptom/type of problem
☐ Itch in the mouth ☐ Vomiting
☐ Diarrhoea ☐ Stomach pain
☐ Red flush (skin) ☐ Eczema
☐ Swollen face ☐ Urticaria/hives
☐ Breathing problems ☐ Eye/nose troubles

☐ Peanuts. The problems started at….years of age. Problems last time experienced at……years of age.

Symptom/type of problem
☐ Itch in the mouth ☐ Vomiting
☐ Diarrhoea ☐ Stomach pain
☐ Red flush (skin) ☐ Eczema
☐ Swollen face ☐ Urticaria/hives
☐ Breathing problems ☐ Eye/nose troubles

☐ Other nuts/almonds. The problems started at…….years of age. Problems last time experienced at……years of age.

Symptom/type of problem
☐ Itch in the mouth ☐ Vomiting
☐ Diarrhoea ☐ Stomach pain
☐ Red flush (skin) ☐ Eczema
☐ Swollen face ☐ Urticaria/hives
☐ Breathing problems ☐ Eye/nose troubles

☐ Flour (wheat, oats, barley or rye).The problems started at…….years of age. Problems last time experienced at……years of age.

Symptom/type of problem
☐ Itch in the mouth ☐ Vomiting
☐ Diarrhoea ☐ Stomach pain
☐ Red flush (skin) ☐ Eczema
☐ Swollen face ☐ Urticaria/hives
☐ Breathing problems ☐ Eye/nose troubles

☐ Other foodstuff (state which)……
The problems started at…….years of age. Problems last time experienced at……years of age.

Symptom/type of problem

- ☐ Itch in the mouth
- ☐ Diarrhoea
- ☐ Red flush (skin)
- ☐ Swollen face
- ☐ Breathing problems
- ☐ Vomiting
- ☐ Stomach pain
- ☐ Eczema
- ☐ Urticaria/hives
- ☐ Eye/nose troubles

☐ Other foodstuff (state which)……
The problems started at…….years of age. Problems last time experienced at……years of age.

Symptom/type of problem

- ☐ Itch in the mouth
- ☐ Diarrhoea
- ☐ Red flush (skin)
- ☐ Swollen face
- ☐ Breathing problems
- ☐ Vomiting
- ☐ Stomach pain
- ☐ Eczema
- ☐ Urticaria/hives
- ☐ Eye/nose troubles

The child's father

	Yes	**No**
56**)** Has the father had ***asthma or asthmatic bronchitis?***	☐	☐

If **"NO",** go directly to question 59

---

57) At what age were the problems first seen? …… years of age

58) At what age did the father last have the problems?…….. years of age

---

59) Has the father had ***allergic problems from the nose or eyes?*** ☐ ☐

If **"NO",** go directly to question 62

---

60) At what age were the problems first seen? **……** **y**ears of age

Yes **No**

61) At what age did the father last have the problems? **……..** years of age

---

62) Has the father had ***eczema?*** ☐ ☐

If **"NO",** go directly to question 66

63**)** At what age were the problems first seen? **……**years of age

64) At what age did the father last have the problems? **……**years of age

65) Has the eczema been contact allergy? ☐ ☐

---

66) Has the father had ***urticaria (hives)/allergic oedema****?* ☐ ☐

If **"NO",** go directly to question 69

67**)** At what age were the problems first seen? **……** years of age

68) At what age did the father last have the problems? **……**years of age

69) Has the father reacted with allergy or intolerance to **any foodstuff?** ☐ ☐

70) Has the father reacted with allergy or intolerance to **any foodstuff last year**? ☐ ☐

If **"NO",** go directly to question 72

---

71) If yes, to which foodstuffs?

☐ Milk ☐ Egg ☐ Fish ☐ Wheat (and other types of flour)
☐ Soy ☐ Apple ☐ Peanuts ☐ Other nuts

☐ Other foodstuffs, state what/which……

……

The child's mother

	Yes	No
72) Has the mother had ***asthma or asthmatic bronchitis****?*	☐	☐

If **"NO",** go directly to question 75

---

73) At what age were the problems first seen? …… years of age

74) At what age did the mother last have the problems? …….. years of age

---

75) Has the mother had ***allergic problems from the nose or eyes?*** ☐ ☐

If **"NO"**, go directly to question 78

---

76) At what age were the problems first seen? **……**years of age

77) At what age did the mother last have the problems? **……** years of age

---

78) Has the mother had ***eczema?*** ☐ ☐

If **"NO"**, go directly to question 82

79**)** At what age were the problems first seen? **……**years of age

Yes **No**

80) At what age did the mother last have the problems? **…….** years of age

81) Has the eczema been contact allergy? ☐ ☐

82) Has the mother had ***urticaria (hives)/allergic oedema****?* ☐ ☐

If **"NO",** go directly to question 85

83) At what age were the problems first seen? **…….** years of age

84) At what age did the mother last have the problems? **……** years of age

85) Has the mother reacted with allergy or intolerance to **any foodstuff?** ☐ ☐

86) Has the mother reacted with allergy or intolerance to **any foodstuff last year**? ☐ ☐

If **"NO",** go directly to question 88

87) If yes, to which foodstuffs?

☐ Milk ☐ Egg ☐ Fish ☐ Wheat (and other types of flour)
☐ Soy ☐ Apple ☐ Peanuts ☐ Other nuts
☐ Other foodstuffs, state
what/which……

……

Questions on family and environment

88) In which gestational week was the child born?.....week

	Yes	No
89) Was the child born in Sweden?	☐	☐

If ”No”, in which country …….

90) Was the father born in Sweden?	☐	☐

If ”No”, in which country …….

91) Which year was the father born? 19……

92) What is the father’s profession?.....

93) What is the father’s education? (mark the appropriate box with a cross ”x”)

Father’s education		
		Primary school
		Secondary school 2-4 years or corresponding
		College or university, max 120 university points
		College or university, more than 120 university points

94) Was the mother born in Sweden?	☐	☐

If ”No”, in which country …….

95) Which year was the mother born? 19……

96) What is the mother’s profession?.....

97) What is the mother’s education? (mark the appropriate box with a cross ”x”)

Mother’s education		
		Primary school
		Secondary school 2-4 years or corresponding
		College or university, max 120 university points
		College or university, more than 120 university points

	Yes	No
98) Does the child have siblings?	☐	☐

If "Yes", fill in the table below

Give the siblings':			**Do the siblings have:**			
First name	**Gender**	**Birth year**	**Asthma**	**Hay fever**	**Eczema**	**Food allergy**

99) Did the mother smoke during the pregnancy? ☐ ☐

100) Has the father or somebody else in the household smoked regularly at home when the mother was pregnant with the child? ☐ ☐

101) Has anybody smoked at home during the child's first year? ☐ ☐

102) Have you any time during the pregnancy had dogs, cats or other animals who lived or was mainly kept indoors? ☐ ☐

If "Yes", state:
Number of dogs……..
Number of cats …….
Other animals……..

103) Have you any time during the child's first year of life had dogs, cats or other animals who lived or was mainly kept indoors? ☐ ☐

If "Yes", state:
Number of dogs……..
Number of cats …….

Yes **No**

Other animals……..

If "No", tell why:
☐ We/I did not want to have animals
☐ Wanted, but could not due to allergy problems in a family member
☐ We/I wanted to avoid the child becoming allergic
☐ Other reason…….

104) Have you had a dog, cat or another animal during the last year? ☐ ☐

105) Are y of the animals regularly inside at home? ☐ ☐

If "Yes", state what/which:…….

106) Approximately, how many airway infections (colds) has the child had?

During the first year of life?

☐ 0-5 ☐ 6-10 ☐ more than 10 ☐ do not remember

during the last year ?

☐ 0-5 ☐ 6-10 ☐ more than 10

107) How many times did the child need to take antibiotics?

During the first year of life …….. ……

In total until now? approximately……..

108) Has the child been at a day care centre/at a family day nursery? ☐ ☐

If "**Yes**":
At what age did the child start at the day-care centre?.....years of age

109) Does the child live together with

Yes **No**

☐ both parents
☐ one parent and a step parent
☐ single mother
☐ single father

110) Does the family live in:

☐ detached house, villa
☐ apartment
☐ semi-detached house, linked house
☐ farm

Living space……m^2
Number of inhabitants……persons

111) Do you have damp or damage by mould in the house/apartment? ☐ ☐

The child's food/diet

112) Was the child breastfed? ☐ ☐

If "**Yes**" for how many months?.....

113) At what age did the child start to get formula or gruel? …months of age

114) At what age did the child start to get purée and other food?……. months of age.

115) Did you use to cook yourself for the child during the first year of life?

☐ never or almost never
☐ sometimes
☐ approximately half of the time
☐ usually
☐ always

116) How often does the child eat fruit?
☐ never or almost never

☐ 1-2 fruits per week
☐ 3-6 fruits per week
☐ At least 1 fruit per day
☐ Several fruits per day

117) How often does the child eat vegetables?
☐ never or almost never
☐ 1-2 times per week
☐ 3-6 times per week
☐ At least once a day
☐ Several times per day

118) How often does the child eat yoghurt?
☐ Never
☐ 1-3 times per week
☐ more than 4 times per week

119) How often does the child eat fish?
☐ Never or almost never
☐ 1-2 times per month
☐ Once a week
☐ Several times per week

120) Does the child eat food that contains fermented (soured) vegetables (for example sauerkraut, sour cucumber – not pickled cucumber) or other food that is fermented?
☐ Never or almost never
☐ 1-2 times per month
☐ Once a week
☐ Several times per week

121) How often do you use semi-manufactured food when you cook for the child(for example fish fingers, meat balls, sausages)?
☐ Never or almost never
☐ 1-3 times per month
☐ 1-3 times per week
☐ Every day or almost every day

122) How often does the child usually eat ”fast food” (for example hamburgers, pizza)?
☐ Never or almost never
☐ 1-3 times per month
☐ 1-2 times per week

Yes **No**

☐ more than 3 times per week

123) How often does the child usually eat peanuts?
☐ Never
☐ 1-2 times per month
☐ 1-2 times per week
☐ more than 3 times per week

124) How often does the child usually eat other nuts (for example almonds, hazel nuts etc)?
☐ Never
☐ 1-2 times per month
☐ 1-2 times per week
☐ more than 3 times per week

125) How often does the child usually drink soft drinks?
☐ Never
☐ 1-2 times per month
☐ 1-2 times per week
☐ Every day or almost every day

126) How much milk does the child drink?
☐ Never drinks milk
☐ 1-2 glassfuls per week
☐ 1-2 glassfuls daily
☐ more than 3 glassfuls daily

127) How often does the child eat game-meat, elk, reindeer, venison, roe deer or wild boar?
☐ Never or almost never
☐ 1-5 times a year
☐ 1-2 times a month
☐ once per week or more often

128) Do you sometimes buy egg, meat or unpasteurised milk directly from the farm? ☐ ☐

129) How do you usually wash the dishes?
☐ usually hand dish
☐ usually with dishwasher

Thank you for your participation!

The questionnaire should be given to the school. Please use the attached envelope.

Appendix III Diagnostic criteria birth cohort

	ARC	Asthma	Eczema	Food allergy
18 m	symptoms from eyes and/or nose on exposure to pollen or animals **with** positive IgE/SPT to respective allergen	≥ 3 episodes of wheeze last year **and any of**: -symptoms outside infections or -other manifestations of allergy OR persistent wheeze ≥ 4 weeks	itching spots on typical locations which has come and gone for at least 6 months or based on William's criteria	An immediate or late onset reaction after ingestion of the specific food, followed by a clear and prompt clinical improvement when eliminating the suspected food allergen, **and any of**: -other signs of allergic disease or more than one organ system involved or supported by positive allergy tests, biopsies, or challenge tests
3 yr	Same as 18 m, with symptoms during last 12 m	≥ 3 episodes of wheeze with the last episode after 2 years of age, **and any of**:	Same as 18 m, with symptoms during last 12 m	Same as 18 m, with symptoms during last 12 m

		-symptoms outside infections or -other allergic manifestations OR wheeze with onset after 2 years of age, **and any of**: -triggered by colds in children with other manifestations of allergy or -triggered by exercise or -response to anti-inflammatory maintenance therapy OR persistent wheeze ≥ 4 weeks last year		
8 yr	Same as 18 m, with symptoms during last 12 m	wheeze/heavy breathing **and any of**: -response to anti-inflammatory maintenance therapy or -BHR with PD20 <0.6 mg methacholine or -FEV1rev >12%	Same as 18 m, with symptoms during last 12 m	symptoms of food allergy, **supported by** open food challenge, planned or accidental