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Epidemiological Studies on the Association between Cat and Dog Exposure and Atopic Outcomes in Young Children

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Chapter 1

Introduction

1.1 Allergic Sensitization and Allergic Diseases

The prevalence of allergic diseases has been rapidly increasing in the past 50 years [1]. Based on the largest-scaled international study on asthma and allergy in children (ISSAC), the prevalence of asthma symptoms in 13 to 14 year-olds reaches 31% in the U.K., the prevalence of allergic rhino-conjunctivitis reaches 45% in Paraguay, and the prevalence of eczema symptoms reaches 22% in Sweden [2]. High prevalence of childhood allergy and asthma is associated with significant costs in the health care system. In the United Kingdom, one of the countries with the highest prevalence of allergic disorders, the estimated annual cost on treatments for allergic diseases in year 2000 were 0.7 billion pounds, which accounts for 11% of the primary care prescribing cost budget [3]. Furthermore, these diseases result in low quality of life of millions of children and adults. Severe asthma and systemic allergic reactions are even potentially life-threatening conditions. On the other hand, the prevalence rate of allergic diseases varies significantly between geographical regions. The reported prevalence of diagnosed asthma in children and young adults was about 30% in Australia but 5% in Taiwan [1]. In Germany, between year 2002 and 2003, the reported prevalence rates of asthma, eczema and allergic rhinoconjunctivitis symptoms in school age children are 12.8%, 7.9%, and 6.9% respectively [2]. The prevalence of diagnosed

asthma has also reached 8% after year 2000 [1]. Despite considerable research effort has been carried on for many years to understand allergic diseases, the puzzles of the abrupt increase and the geographical variation of the prevalence of the diseases are not yet solved. Further understanding on the complex factors and the mechanisms that drive the development of these diseases are on demand.

It has been observed that the pattern of allergic sensitization and diseases changes with age. Sensitization to food allergens and eczema start in early infancy while sensitization to cat and dog dander and other inhalant allergens and hay fever are more frequent at preschool and school age. However, a recent study showed that sensitization to perennial inhalant allergens in early childhood is associated with a loss of lung function in school age [4]. Little is known about the natural course of the development of sensitization and within the individual. It seems that a sequence of events, which finally leads to the development of the diseases, begin as early as during fetal life. Exposure to different stimuli during the prenatal period and in the first year of life may be crucial. As infants' immune system are vulnerable and the development of the immune response are still underway. Therefore studies begin at the prenatal phase and during infancy on the newborns and young children's development of allergy are fundamental for further understanding the natural course of the diseases and the development of effective therapies.

Asthma, on the other hand, is a complex syndrome and no standard method can be used to identify asthma with certainty. The association between asthma and allergies has long been recognised. However, not all asthma is associated with allergy and the mechanisms of these associations are still puzzles. For example, children who suffered from recurrent wheezing can be due to decline of lung function which was caused by passive tobacco exposure in utero, viral airway infection, or smaller airways and lung size and only a minority will go on to have persistent asthma in later life [5]. However, for most of the asthmatic patients the disease initiates in early childhood. Various phenotypes of the disease and little understanding of the underlying pathobiology resulted in poor primary disease prevention strategies.

1.1.1 Epidemiology of Allergic Diseases

Studies in the past 50 years show significant increase in the prevalence of allergic diseases [1, 6, 7]. It is recognised that the reported prevalence rates were constantly higher in affluent western countries than in developing countries [7]. Until recently, the report from the International Study of Asthma and Allergy in Childhood (ISAAC) shows that the prevalence of allergic symptoms in children in some Latin America countries are similarly high as in some developed countries[2]. The influence of westernised lifestyle on the increasing prevalence of allergic disorders has been investigated in several studies by comparing the prevalence of allergic disorders in former Eastern and Western Germany after their reunification in 1989. Increasing prevalence of allergic sensitization and hay fever in former Eastern Germany were observed and the prevalence rates between former Eastern and Western German cities have been converging [8, 9]. A recent study in China also gives a good evidence of the influence of westernised lifestyle. It shows that children living in Beijing reported significantly more asthma symptoms than in Urumqi, while children living in Hong Kong reported the highest rates of asthma and other allergic symptoms[10].

Other studies observe that the prevalence of allergic diseases varies in different socioeconomic groups. Previous studies have shown that some allergic diseases such as hay fever and eczema are more prevalent in the population with high socioeconomic status (SES). A British national cohort study has concluded that social advantage is a consistent determinant of hay fever and eczema in children up to the age of 16 years old [11]. Studies in the U.S. and Germany have suggested similar results [12, 13, 14]. However, recent Scandinavian studies have shown that the association between SES and clinical outcomes has changed in the last decades [15, 16]. The occurrence, severity, and hospitalization of asthma on the other hand, seems to be negatively associated with parental education level and subjects' socioeconomic status [17, 18]. The prevalence of subjects having asthma as well as sensitization reactions to common allergens, however, seems to be similar between different socioeconomic classes [17].

The observed phenomena demonstrated that the development of allergy is a result

of the interaction of multiple factors. Numerous environmental factors including changes in environmental chemical and microbiological exposure, diet habit and lifestyle in general have been examined. However, up to now, no conclusive explanation of the increasing prevalence of allergic disorders has been found.

1.1.2 Immune Mechanism and Pathology of Allergy and Atopy

Allergens are proteins and are often proteases with high solubility. When an individual encounter with allergens, usually through mucosal surfaces, the allergens are taken by the dendritic cells. The dendritic cells present allergens to the immune system and the CD4 T cell will then differentiate to type 1 helper T cells (Th1) or type 2 helper T cells (Th2). Th1 cells produce cytokines interleukin-2 and interferon-γ, which inhibits Th2 mediated responses. Th2 cells, on the other hand, produce interleukin-4, 5, and 13. Interleukin-4 and 13 provide signals to B cells to switch to the production of IgE isotype. Once released by B cells, IgE antibodies will bind itself to high-affinity IgE receptors ($Fc\epsilon RI$) on the surface of mast cells and cause the activation of mast cells. The activated mast cells will then release histamine, lipid mediators, and cytokines that lead to both acute and chronic allergic reactions [19, 20, 21, 22](figure1.1.2).

Non-sensitized individuals response to allergen exposure with a low-grade immunologic response with a moderate proliferation of interferon-γ by type 1 helper T cells as well as production of allergen specific IgG1 and IgG4 antibodies without IgE. Sensitized individuals, on the other hand, response with production of cytokines by type 2 helper T cells and elevated allergen specific IgE antibodies which may eventually lead to allergic inflammatory reactions [19, 22]. However, the immune response for newborns is dominated by Th2 cells. It has been proposed that for non-sensitized individuals, a shift to Th1-mediated immune response happens during their early childhood when the development of the immune system is still underway. For sensitized individuals, on the other hand, the Th2 type of immune response is enhanced [23]. It has also been proposed that perinatal exposure to microbes and viral infections may be crucial for the matu-

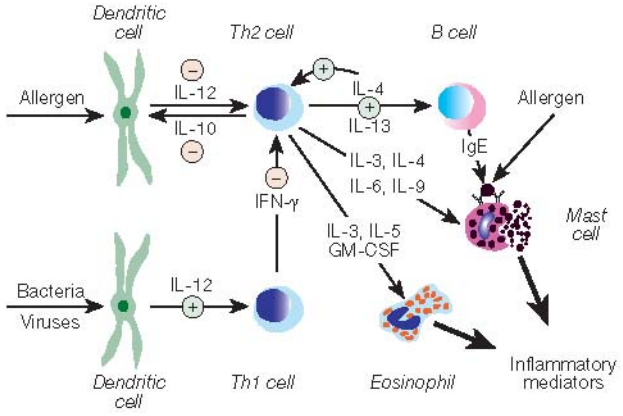


Figure 1.1: Proposed cellular and molecular mechanisms of allergy (Reprint from Holgate 2000 [19])

ration of young children's immune system and enhance the Th1 cells dominated immune response [24, 25, 26].

1.1.3 Allergen and Microbial Exposure - Environmental Risk / Protective Factors for Allergic Diseases

The development of allergic diseases depends on both genetic and enviromental factors. Environmental risk factors have been associated with the epidemic of allergic diseases particularly in the developed countries, because the genetic predisposition alone cannot explain the rapid increase of the prevalence of allergic diseases. Associations between tobacco smoking, air pollution, diet, and indoor environment and allergic diseases have been extensively studied. Allergen exposure is one of the known environmental risk factors associated with the symptoms and the severity of allergy, and prevention of allergen exposure is likely to have the strongest effect on short-term disease management [27, 28, 29, 30, 31, 32]. However, the effect of allergen exposure on the onset of allergy is not yet clear. On the other hand, lack of indoor environmental exposure to microbes has also been associated with allergy. Based on the hygiene hypothesis [33], lack of early life exposure to unhygienic microbial products or infectious components may also

have a causational link to the development of allergy. However, it is not yet clear which agents contribute to the proposed mechanism [24, 25, 26].

The relationship between allergen exposure and the development of allergic diseases is complex. Cross-sectional and cohort studies in the U.S. and Europe have observed dose-response relationships between mite allergen exposure and specific sensitization in children [34, 35, 36, 37, 38]. In Scandinavia, where the domestic mite allergen level is generally low [39], the prevalence of sensitization to house dust mite is also low [40]. Cat and dog allergen, on the other hand, are ubiquitous and constantly airborne. Exposure to cat and dog allergens cannot be actively avoided. The allergens can attach to the clothes of those people who have regular contact with cats and dogs, which facilitates it to be transferred to public places such as offices and schools [41, 42, 43, 44, 45]. There, through direct contact with cat owners or by catching cat allergen in the air, the clothes of those who do not have cats at home are contaminated, and the allergens are further spread. Non-cat owners living in a district of communities with high numbers of cat owners can therefore increase the amount of cat allergen exposure. With the difficulty to sufficiently control the amount of allergen exposure, the casual link between these common indoor allergens and the development of allergic diseases is yet not clear.

It is usually difficult to determine the causal role for most perennial indoor allergens. However, it is easier to observe the causal link between the seasonal or episodic allergen exposure and the allergic diseases. For example, a Finish study has shown that a higher percentage of children who were born between February and April, just before the birch pollen season in Scandinavia, are sensitized to the specific allergen [46]. Another study in Stockholm showed that children who were born between February and April 1993, when extremely high level of birch pollen was recorded, are at higher risk of developing birch pollen sensitization compared to children who were born during the same period but in the years of normal amount of birch pollen exposure [47].

Conflicting results reported by randomized controlled trials on mite allergen avoidance have also raised uncertainty to the observed causal link. Environmental manipulations, which were aimed to reduce the domestic mite allergen

exposure during pregnancy and early childhood of high-risk children, did not show a significant effect on the reduction of sensitization rate [48, 49, 50]. Although most of the trails have achieved to significantly reduce the mite allergen level on the children's sleeping area, it dose not seem to effectively protect children from developing allergy. The Isle of Wight study, on the other hand, has shown a significant effect of the combined food and mite allergen avoidance during infancy on preventing the development of mite sensitization and allergic diseases including asthma up to age of 8 years [51]. Children are exposed to mite allergens in all sorts of indoor environments. Interventional studies aimed to reduce allergen exposure from home cannot prevent children to be exposed to allergens from day-care centre or relatives' homes. However, it is generally accepted that there is a close association between allergen exposure and allergic sensitization. Individuals only develop an IgE mediated immune response if they were exposed to sufficient allergen. For example, several studies have observed positive links between the cockroach and mouse allergen exposure in the poorer inner city areas and allergen specific sensitization [52, 53, 54, 55]. On the other hand, there is insufficient evidence for the proposed mechanism that allergen exposure causes the development of sensitization and continuous exposure leads to inflammatory response and the development of allergic diseases.

It has also been proposed that insufficient microbial exposure during infancy and early childhood may be associated with the increasing prevalences of allergies. Studies showed that children growing up on farms have a lower prevalence of hay fever and atopic sensitization [56, 57, 58]. Regular contact with stable and farm animals showed a protective effect on the development of allergic diseases [59, 60]. Studies which have investigated the microbial exposure from the farm environment observed that the level of endotoxin, a constituent of the outer membrane of gram-negative bacteria, and fungal are significantly high [61, 62]. Studies have shown that exposure to higher level of indoor endotoxin is associated with a decreased risk of allergic sensitization and disorders in pre-school and school children [63, 64, 65, 66]. Laboratory experiments on mice have provided further evidence that endotoxin exposure induces cytokines which may shift the infants' developing immune system to a predominantly TH1 type responses that

protect children from developing allergy [67, 68]. Exposure to other microbial agents such as mold and fungi components, on the other hand, may also have immune stimulatory properties that may reduce the risk of allergic sensitization and allergic symptoms such as wheezing [69, 70, 71]. However, since the amount of these microbial agents measured from settled house dust samples are significantly correlated, no conclusion can be drawn to which specific components contribute to the observed negative associations. Furthermore, the studies agents might be markers for exposure to a wider range of microbes.

1.2 Pet Ownership and Pet Contacts

1.2.1 Cats and Cat Allergen

The influence of cat allergen exposure in early childhood on the subsequent development of sensitization and allergic symptoms and diseases is complex and controversial. Most of the prospective cohort studies have reported positive associations between cat allergen exposure in infancy and elevated specific immunoglobulin E to cat allergen during childhood. For example, the German Multi-Centre Allergy Study (MAS) has reported that domestic cat allergen exposure during the first 2 years of life is associated with the IgE response to cat up to age 7 [72, 73]. The Dutch Prevention and Incidence of Asthma and Mite Allergy Study (PIAMA) have also suggested that higher amount of domestic cat allergen measured from settled house dust sampled in infancy is associated with an increased risk of cat sensitization in children at age 4 with non-atopic mothers [34]. Similar results have also been reported by the Asthma Multicentre Infants Cohort Study (AMICS), which combines three ongoing European birth cohort studies conducted in Ashford, Kent, UK; Barcelona city, and Menorca Island, Spain [74]. Cross-sectional studies on children and adults have also found increased cat sensitization rates in current cat owners. However, with the retrospectively collected information on cat ownership during childhood these studies have also suggested that cat ownership during the first year of life is protective against the development of allergic sensitization [75, 76]. Negative associations between current cat or pet

ownership and the prevalence of the elevated IgE reaction to cat allergen have also been observed in cross-sectional studies [77, 78]. These results correspond to the inverse u-shaped association between indoor cat allergen exposure and cat allergen sensitization observed in a U.S. study on pupils and a British study in adults [38, 79]. These observations led to the speculation that exposure to extremely high level of cat allergen may induce immune tolerance which involves a modified Th2 response by increasing the expression of IgG4 isotype to cat specific allergen but not IgE [38]. The IgG4 antibody, like IgE, is regulated by IL-4 cytokine but does not trigger inflammatory reactions. It has also been observed in *vitro* that IgG4 may have a protective role in the Th2-mediated inflammation [80]. However, a high IgG4 level to cat allergen is not associated with a lower risk of allergic respiratory symptoms [81, 77]. It has also been proposed that pet keeping may increase the exposure to bacterial components such as endotoxin [63, 64, 82, 83] which may enhance young children's type 1 lymphocyte (T-helper 1) development as described in the previous section and therefore protect them from allergen sensitization [66]. This observed protective effect, however, may be partly due to selective cat avoidance by parents with allergic diseases [84, 85, 86] or cat removal in families with sensitized children [78, 87].

To date, the role of cat allergen exposure during childhood is still disputed. The discrepancies between studies may be due to different study designs, study populations, definitions of allergic sensitization, the amount of domestic cat allergen exposure, and cat allergen exposure outside the domestic area. It is known that the spectrum of allergic sensitization and symptoms changes with age. Sensitization to food allergen starts in early infancy while sensitization to cat dander and other inhalant allergens is more frequent at preschool and school age. However, sensitization to perennial inhalant allergens in early childhood was found to be associated with a loss of lung function in school age [4]. On the other hand, the fact that some older children have a higher chance to be exposed to unmeasured cat allergen outside their home could also play a role in the discrepant associations observed in different studies. Svanes et al. have observed from the ECRHS study that the effect of domestic cat allergen exposure during childhood on allergic diseases in adulthood may be modulated by the community preva-

lence of cats. Those who were cat owners during childhood and were living in a community with low cat prevalence are in higher risk of adulthood asthma and other respiratory symptoms [88]. Exposure simultaneously to different amount of other microbial components in the indoor environment and to cat allergen may also lead to different observed associations. Finally, expressions of the amount of cat allergen in the settled house dust samples vary between studies. Most of the studies expressed the level of indoor cat allergen exposure using the allergen concentration in settled dust, which is the amount of allergen per gram of sampled dust [73, 38, 74]. A few studies reported their results using surface allergen load, the amount of allergen per m^2 of sample surface [34, 89]. However, it has been argued in the previous study that microbial level expressed per square meter has a stronger effect on health outcome [90] and that surface load is a better indicator as it simultaneously adjusts for the total amount of dust present at the sampling sites [91].

1.2.2 Dog Ownership

Previous studies on the associations between pet ownership and pet contact on the development of allergic sensitization and diseases have mainly focused on cat allergen exposure and cat ownership. Only a few prospective studies have specifically discussed the effect of childhood dog contact. It has been reported in the cohort studies from the U.S. that early childhood dog ownership is associated with reduced risk of wheezing at age of 1, 5 to 9 years, and from birth up to 13 years of age [92, 93, 94]. The Stockholm Children Allergy and Environmental Prospective Birth Cohort Study (BAMSE) has reported that dog ownership in infancy seems to reduce the risk of asthma at age 4 [95]. The Dutch PIAMA cohort, on the other hand, has reported that dog allergen exposure during infancy has no effect on the development of wheezing nor asthma up to age 4 [34]. Some cross-sectional studies have collected retrospective information on dog ownership in early childhood. In the European Community Respiratory Health Survey, Svanes et al has reported that childhood dog ownership is associated with decreased risk of hay fever but promote non-allergic asthma [88]. Associations between childhood dog ownership and allergic sensitization have also been

reported by both longitudinal studies and cross-sectional studies in Europe and the U.S. [95, 76, 96, 97]. Most of the studies have observed a negative association between childhood dog ownership and sensitization to aeroallergens, particularly outdoor aeroallergens. The Tucson birth cohort study, however, reported that no such protective effect was found on both skin prick test to local aeroallergens and total serum IgE results in children up to 13 years old [93]. It has been reported by the Swedish BAMSE study that dogs are less common in families with than in families without parental atopic eczema/dermatitis syndrome and families with smoking mothers are more likely too keep dogs [84]. Therefore, the observed protective effect of dog ownership may be partly due to selective dog avoidance by atopic parents [98]. On the other hand, the simultaneous exposure to higher level of indoor endotoxin may be the biological mechanism behind the observed protective effect [99, 82].

A higher level of endotoxin exposure in early childhood has been inversely associated with the development of allergic sensitization and diseases as shown in the previous section. Dog ownership has been directly associated with higher endotoxin level measured in the settled house dust [92, 100, 101]. Therefore it has been speculated that the observed protective effect of dog ownership during early childhood in young children from developing allergic sensitization and diseases is partly due to the simultaneous exposure to a higher level of endotoxin in the dog owner's home. On the other hand, dogs require more outdoor activities than most of the other pets. Dog fur is likely to carry wide range of microbes other than endotoxin from outdoor environment such as soil. Close contact with dogs at very young age may increase the exposure to variety of microbes and stimulate the maturation of the immune system. Keeping dogs also means a different lifestyle that involves more outdoor activities. Dog keepers are also more likely to live in a less dense area or have access to the ground floor. These factors may also have an effect on the development of the immune system during childhood.

1.3 Objectives

Indoor environment has been associated with allergic diseases. The role of the exposure to cat and dog during early childhood in the development of allergy in young children is still debated. Further, it has been observed that the prevalence of allergic sensitization is different in different social groups. The aims of this thesis are first to assess the variance of the amount of domestic cat allergen levels in families with different socioeconomic status. Second, to investigate the associations between the observed domestic cat allergen level and the prevalence of cat sensitization and allergic symptoms and diseases in young children using both longitudinal and cross-sectional epidemiological studies in three European countries. Finally, to assess the associations between dog ownership during early childhood and the development of allergy in young children in two German cohort studies.

Chapter 2

Study Populations and Designs

The associations between cat and dog exposure during childhood and allergy and allergic diseases in young children were assessed using two prospective German birth cohort studies and one European cross-sectional study.

2.1 The LISA Study

The Influences of lifestyle-related factors on the immune system and the development of allergies in childhood study (LISA) is an ongoing population-based prospective birth cohort study. Parents of neonates admitted to maternity hospitals in Munich, Leipzig, Wesel, and Bad Honnef, Germany were contacted between December 1997 and January 1999. Overall, 3097 neonates fulfilled the inclusion criteria were recruited in the study. The inclusion criteria of the study are: both of the parents were born in Germany and having German nationality, materity $>$ 37 gestational weeks, birth weight $>$2,500 gram, the child did not have congenital malformation, or symptomatic neonatal infection, or antibiotic medication, the child was not hospitalized or admitted to intensive medical care during neonatal period, and the mother did not have any immune-related diseases such as autoimmune disorders, diabetes, hepatitis B, and long-term medication or abuse of drugs and alcohol. LISA is designed as a population based study and the participants were not pre-selected based on family history of allergic diseases.

The cohort was followed up at the age of six months, twelve months, eighteen months, two years, four years, and six years. When the children were two years and six years old, the blood samples were collected for allergen sensitization tests. In the Munich and the Leipzig subgroup, the house dust samples were collected when the children were 3 months old.The flow chart of the LISA cohort study is presented in figure 2.1. The study was approved by local ethics committees.

2.1.1 Questionnaire Data

Information on parental educational level, family history of allergic diseases, and family equivalent income were collected using self-administered questionnaires at birth and when the children were two years old. Information on children's allergic symptoms, doctor diagnosed asthma, eczema, hay fever, and allergic rhinitis, pet ownership, contact with pets outside home, moving home, and exposure to environmental tobacco smoking at home were collected at each follow up using self-administered questionnaires.

2.1.2 Collection and Analysis of Blood Samples

With parents' consent, blood samples were collected from 2176 (82%) and 1193 (50%) children at age two and six respectively. Specific IgE antibodies to allergens were measured using RAST FEIA CAP system (Pharmacia, Freiburg, Germany). At the first blood sampling, common food allergens (food mix fx5: egg white, milk, fish, wheat, peanut, soybean), inhalant allergens (house dust mix hx2: *D. pteronyssinus*, *D. farinae*, German cockroach, mould mix mx1: *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria alternata*, cat, grass pollen, weed, and tree pollen mix rx1: timothy grass, mugwort, ribwort, wall pellitory, birch were tested. At the second blood sampling, a screening test for sensitization was used to detect specific IgE antibodies against inhalant allergens (SX1: timothy grass, rye, birch, mugwort, house dust mite, cat, dog, and moulds) and food allergens (fx5: egg white, milk, fish, wheat, peanut, and soybean) in the serum. Those children who were positive to SX1 or fx5 were

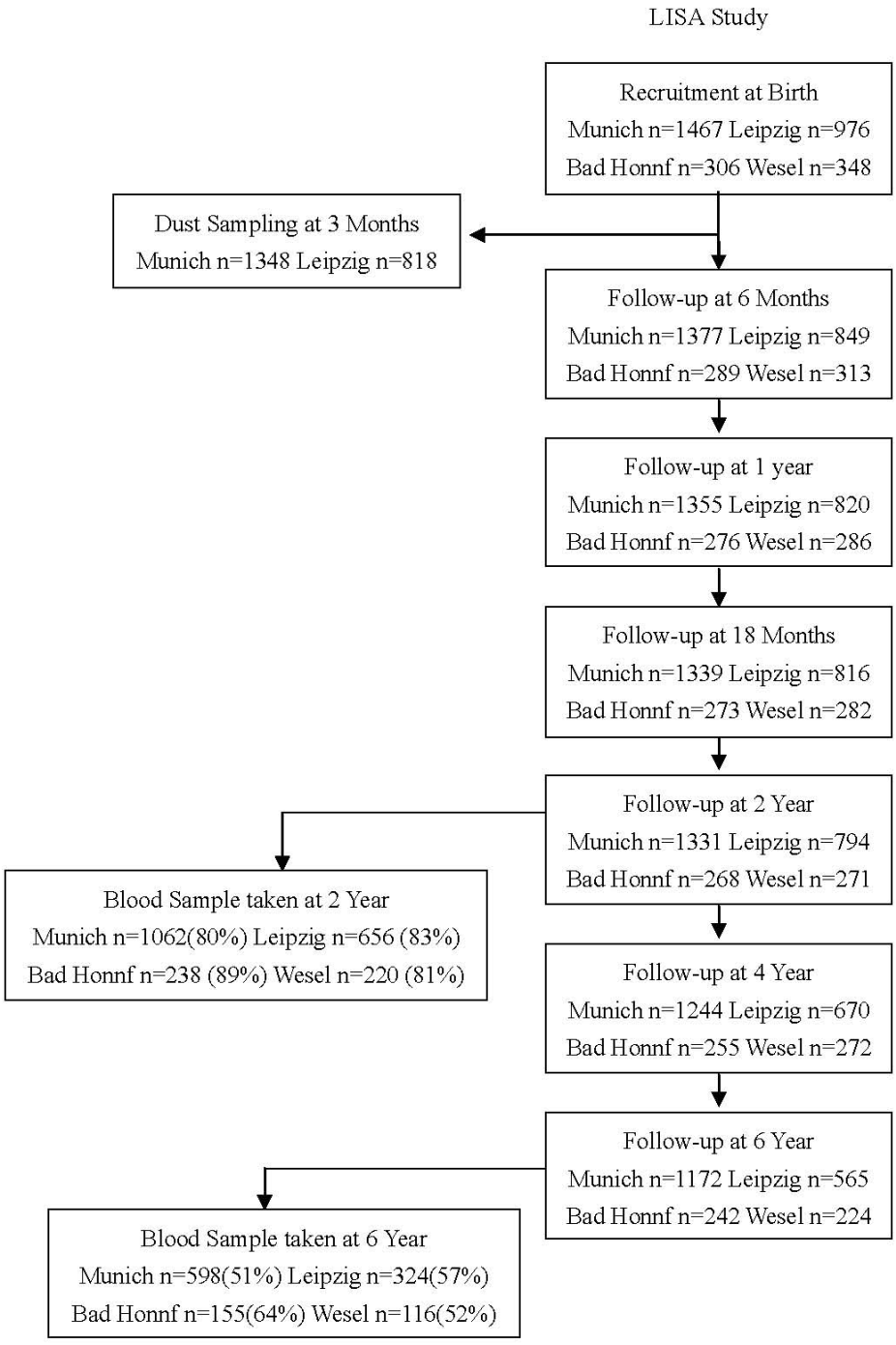


Figure 2.1: Flow Chart of the LISA study

tested for single specific allergens including cat allergen Fel d 1 and dog allergen Can f 1. Sensitization to mixed pollen allergens included timothy grass, rye, birch, and mugwort. Sensitization was defined as having a specific serum IgE titer >0.35 kU/L to specific allergen.

2.1.3 Collection and Analysis of Settled House Dust Samples

The house dust samples were collected from the Munich and the Leipzig subgroup when the children were 3 months old. 2166 (89%) families participated. Trained field workers carried out dust sampling based on a standardized operating procedure. Dust samples were taken from parents' and child's mattresses by vacuuming 1 m^2 of the mattress surface for two minutes using vacuum cleaners equipped with special nozzles (ALK-Abelló allergen mouthpiece, Hørsholm, Denmark). The samples were stored at -20°C until extraction to prevent bacteria growth. The dust samples plus filter paper were extracted with 0.125 mol/L NH_4HCO_3 plus 0.05% Tween-20 (vol/vol) for 2 hours at room temperature under constant shaking, with an extraction ratio of 1:10 to 1:100 (wt/vol), depending on the amount of sampled dust.

Major mite allergens Der p 1 and Der f 1 and cat allergen Fel d 1 were extracted using a two-site monoclonal enzyme-linked immunosorbent assay (ELISA). Endotoxin level was quantified using the kinetic Limulus Amebocyte Lysate assay. The detection limits were 10 ng/g dust for mite allergens, 15 ng/g dust for cat allergen and 50 EU/g dust for endotoxin. Samples under the detection limit were assigned 1/2 of the lower detection limit. The allergen and endotoxin levels were expressed as load, the amount of allergen or endotoxin per m^2 of sample surface and concentration, the amount of allergen or endotoxin in per gram of sampled dust. However, a previous study has shown that microbial level expressed per square meter has a stronger effect on health outcome [90]. In the LISA study, the measured allergens and endotoxin expressed per square meter and per gram dust were highly correlated (Spearman Correlation Coefficients =0.9, for all allergen and endotoxin measured from both parents' and children's mattresses). Further-

more, expressing the bio-contaminant level in per square meter of the sampling surface simultaneously adjusted the total amount of dust collected.

2.1.4 Information on household density in the living area

Information on household density were available in the Munich subgroups from the LISA cohort [102]. Demographic information including the number of households in every postcode area in Bavaria, Germany was collected from the company INFAS GEOdaten (Bonn, Germany). The data was updated in December 2003. The household density of a 2500 meter buffer was calculated for each residential address of the Munich subgroups from both cohorts. The proportions of the postcode area and the proportion of household counts were calculated for each buffer and the area weighted averages of the household numbers were used as household density.

2.2 The GINI Study

The German Infant Nutritional Intervention Program (GINI) recruited 5991 newborns from 16 maternity wards in Munich and Wesel, Germany, between September 1995 and June 1998. There are two subgroups in the GINI study, the interventional study group and non-interventional (observational) cohort group. However, the GINI study as a whole is a population based study. A total of 2252 infants with at least one parent or sibling having a history of allergic diseases agreed to participate in the randomized trial. The aim of the trial was to compare the effect of hydrolyzed formulas and conventional cow's milk formula on the prevention of the development of allergic diseases in high-risk children. The infants with no family history of allergic disease and those whose parents did not want to participate in the trial were allocated in the non-interventional cohort group. The exclusion criterias of the study are: severe acquired or congenital diseases, maternity less then 37 gestational weeks, birth weight less then 2,500 gram, the child was more than 14 days old, intake of any cow's milk-based formula before inclusion, or parents were unable to fill in the questionnaires. Children from both

groups were followed-up at the age of one year, two years, three years, four years, and six years. The flow chart of the GINI interventional study group and non-interventional cohort group are presented in figure 2.2. The study was approved by local ethics committees.

2.2.1 Questionnaire Data

Information on parental educational level, and family history of allergic diseases were collected using self-administered questionnaires before birth. Information on children's allergic symptoms, doctor diagnosed asthma, eczema, and allergic rhinitis, pet ownership, contact with pets outside home, and exposure to enviomental tobacco smoking at home were collected at each follow up using self-administered questionnaires.

2.2.2 Collection and Analysis of Blood Samples

With parents' consent, blood samples were collected from 2252 (54%) and 1962 (51%) children at age three and six respectively. Specific IgE antibodies to allergens were measured using RAST FEIA CAP system (Pharmacia, Freiburg, Germany). At the first blood sampling, egg white, cow's milk protein, soybean, house dust mites *D. pteronyssinus* and *D. farinae*, cat, timothy and birch pollen allergen were tested. At the second blood sampling, a screening test for sensitization was used to detect specific IgE antibodies against inhalant allergens (SX1: timothy grass, rye, birch, mugwort, house-dust mite, cat, dog, and moulds) and food allergens (fx5: egg white, milk, fish, wheat, peanut, and soybean) in the serum. Those children who were positive for SX1 or fx5 were tested for single specific allergens including cat allergen Fel d 1 and dog allergen Can f 1. Sensitization to mixed pollen allergens included timothy grass, rye, birch, and mugwort. Sensitization was defined as having a specific serum IgE titer >0.35 kU/L to specific allergen.

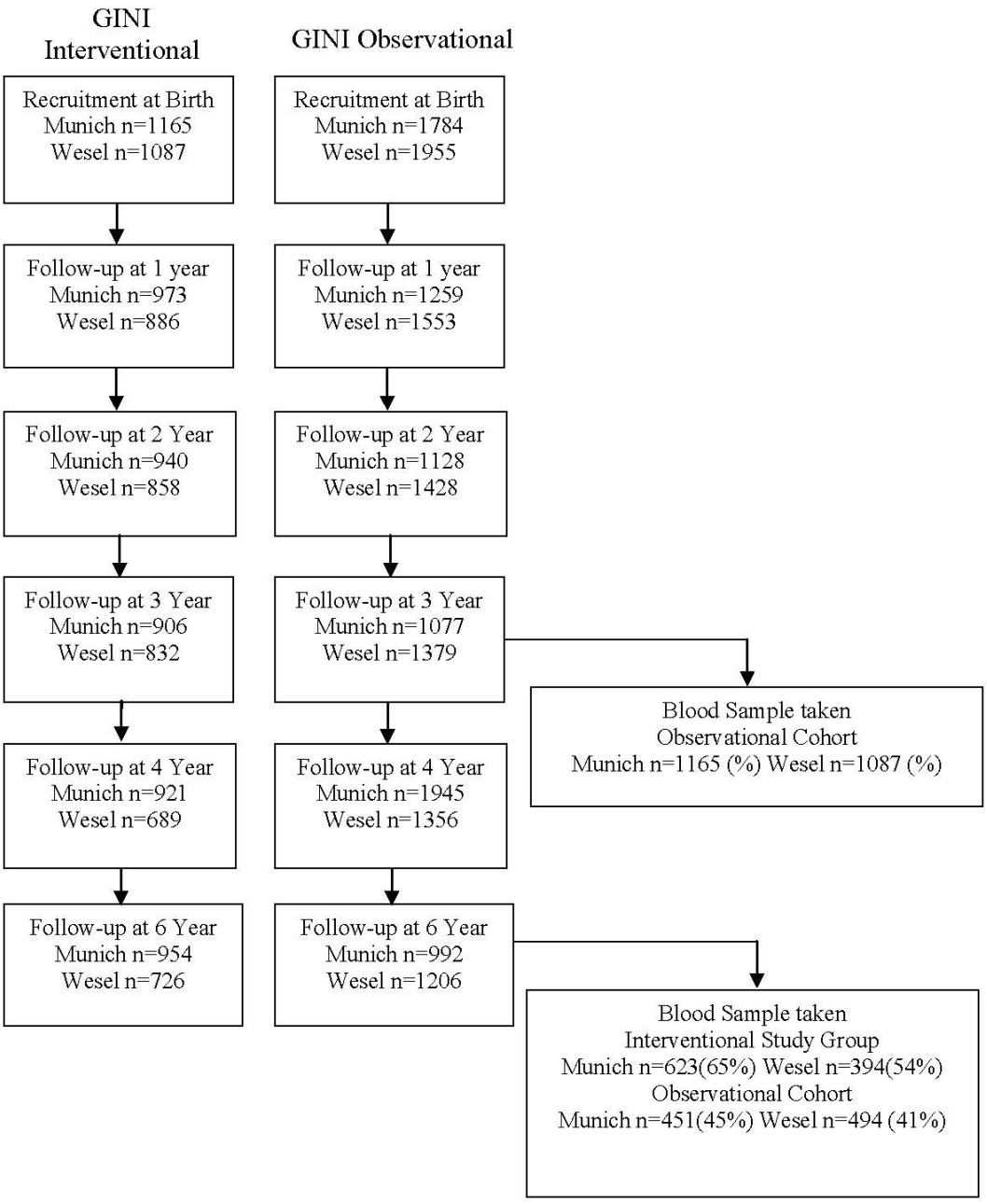


Figure 2.2: Flow Chart of the GINI study

2.2.3 Information on household density in the living area

Information on household density was available in the Munich subgroups from the GINI cohort [102]. Demographic information including the number of households in every postcode area in Bavaria, Germany was collected from the company INFAS GEOdaten (Bonn, Germany). The data was updated in December 2003. The household density of a 2500 meter buffer was calculated for each residential address of the Munich subgroups from both cohorts. The proportions of the postcode area and the proportion of household counts were calculated for each buffer and the area weighted averages of the household numbers were used as household density.

2.3 The AIRALLERG Study

The AIRALLERG study was a nested case-control study composed of four ongoing birth cohorts conducted in 3 centres. The GINI and LISA cohorts were based in Germany, the PIAMA cohort in the Netherlands, and the BAMSE cohort in Sweden. The LISA and BAMSE cohort are population based studies and the participants were not pre-selected based on family history of allergic diseases. GINI study design is described in detail in the previous section. The Dutch PIAMA cohort, similar to the GINI study, was subgrouped into the interventional study group, and the natural history group. 855 neonates with atopic mothers agreed to participate in the randomized trial which aimed to evaluate whether the application of mite impermeable mattress covers reduces the incidence of asthma and mite allergy in high-risk children. 3291 neonates participated in the natural history group. The children in the natural history group were followed-up without any intervention.

Before the recruitment of the AIRALLERG study, blood samples from the cohorts were taken when the children were 2 (LISA), 3 (GINI), and 4 years old (the Dutch and Swedish cohorts). Based on serum IgE examinations, all children who were sensitized to common inhalant allergens, and some who were sensitized to common food allergens were invited to participate in the AIRALLERG study.

Equal numbers of non-sensitized random controls from the four cohorts were also invited to participate in the study. Overall, 358, 347, and 364 subjects from Germany, the Netherlands, and Sweden respectively were recruited and 554 (52%) children were not sensitized to any common allergens at the time of the recruitment (controls). These non-sensitized control groups were defined as the reference group in the analysis. Within the 515 cases, 106 children were sensitized to cat allergen. Baseline information of the participants was extracted from the existing database of the four cohorts. The study was approved by local ethics committees.

2.3.1 Serum Assays

As described in the previous section, blood samples were taken when the children were 2 (LISA), 3 (GINI), and 4 years old (the Dutch and Swedish cohorts). In the Dutch centre, IgE antibodies to common allergens were measured using a radioallergosorbent test according to the standard operating procedure used at the Sanquin Institute[103]. In the German and Swedish centres, the RAST FEIA CAP (Pharmacia UniCAP) system was used (Pharmacia, Freiburg, Germany and Phadia AB, Uppsala, Sweden respectively). Common allergens tested in each original cohort were slightly different as each cohort had its own protocol adapted to locally most prominent allergens. The allergens tested in the GINI and LISA study are described in detail in the previous sections. In the PIAMA study, egg white, milk, soybean, house dust mites *D. pteronyssinus*, cat, dog, *Dactylus glomerata*, birch pollen, and *Alternaria alternata* were tested. In the BAMSE study, mixed food allergens food mix fx5(egg white, milk, fish, wheat, peanut, soybean), and Phadiatop airborne allergen mix (house dust mite *D. pteronyssinus,* cat, dog, horse, timothy, birch pollen, *Cladosporium herabarum*, and mugwort) were tested. Those children who were positive in aeroallergen mix test were tested for specific IgE to cat allergen. Sensitization was defined as having a serum IgE titer >0.35 kU/L to specific allergens.

2.3.2 Collection and Analysis of Settled House Dust Samples

Dust samples were collected during home visits when the German study children were on average 5 (LISA) and 6 (GINI) years old, the Dutch study population was 6 years old and the Swedish study population was 7 years old. Trained field workers conducted dust sampling using vacuum cleaners equipped with special nozzles (ALK-Abelló allergen mouthpiece, Hørsholm, Denmark). The three centres conducted dust sampling using a common standard operating procedure in the cool seasons of 2001/2002 and 2002/2003 respectively. Mattress dust samples were collected by vacuuming the entire mattress surface for two minutes. Floor dust samples were collected by vacuuming 1 m^2 of wall-to-wall carpets for 2 minutes, or 1 m^2 of large rugs for 2 minutes, or 2 m^2 of smooth floor for 4 minutes depending on the flooring type in the living room. Collected samples were stored frozen and transported on dry ice to the IRAS; Utrecht Laboratory for extraction and analysis. Field workers also collected site characteristics information by interviewing the occupants with a standard questionnaire developed for the AIRALLERG study during the home visit. Major allergens extraction including cat allergen Fel d 1 from children's mattress and living room floor dust was done by Sandwich Enzyme Immuno Assay (EIA). The detection limit of the assay in this study was 1.1 ng/ml. Samples with cat allergen level under the detection limit were assigned with 2/3 of the lowest measured value. Endotoxin, extracellular polysaccharides (EPS), and $\beta(1 \rightarrow 3)$ glucan were also sequentially extracted from the dust samples. The kinetic Limulus Amebocyte Lysate (LAL) assay was used to determine indoor endotoxin levels. EPS was analysed with a specific Sandwich Enzyme Immuno Assay (EIA) for EPS of Aspergillus and Penicillium spp. Glucan levels were measured with a $\beta(1 \rightarrow 3)$-specific inhibition EIA. The allergen, endotoxin, EPS, and $\beta(1 \rightarrow 3)$ glucan levels were expressed as bio-contaminant load, the amount of bio-contaminant per m^2 of sample surface and bio-contaminant concentration, the amount of bio-contaminant in per gram of sampled dust.

Chapter 3

Statistical Analysis

3.1 Descriptive Analysis

The distribution of the amount of the sampled dust and the bio-contaminants loads and concentrations were highly skewed, therefore, the amount of indoor dust, mite and cat allergens, and endotoxin were described using median and quartiles.

Spearman Correlation Coefficients were used to assess the correlations between the amounts of different bio-contaminants, and the amounts of the bio-contaminants collected from different sampling areas.

Spearman Correlation Coefficient is a nonparametric approach to assess the degree to which two variables are linearly related.

$$r_s = 1 - \frac{6\Sigma D^2}{N(N^2 - 1)}$$

N= the number of paired bio-contaminants
D= the difference between each paired bio-contaminants

It is a special case of the Pearson product-moment coefficient that the data are converted to ranks before calculating the coefficient.

Wilcoxon Rank-Sum Test and Kruskal-Wallis test were used to test if the amount of allergens, dust mass and endotoxin vary between families with different demographic characters such as socioeconomic status and different history of pet ownership.

Wilcoxon Rank-Sum Test is a nonparametric approach to compare two populations. It is used to test the null hypothesis that two populations have identical distribution functions against the alternative hypothesis that the two distribution functions differ with respect to location. The comparison is based on the order in which the observations from the two populations fall. The test begins by ranking the combined data set of the two groups of observations to be compared. The sum of the ranks of the first group and those in the second group should be close to the same value when the two populations have the same distribution.

$$T = \frac{R_1 - \frac{n_1 n_2}{2}}{\sqrt{\frac{n_1 n_2 (n_1+n_2+1)}{12}}} \sim N(0,1) \ , n_1 + n_2 \geq 20$$

n_1 and n_2 = the numbers of observations in each groups to be compared
R_1 = the sum of the ranks in group 1

Kruskal-Wallis test is an extension of the Wilcoxon Rank-Sum Test for comparing three or more groups.

$$T = \frac{12}{N(N+1)} \sum_{i=1}^{k} \frac{R_i^2}{n_i} - 3(N+1) \sim X_{k-1}$$

n_i = the numbers of observations in the ith groups to be compared
R_i = the sum of the ranks assigned to the ith group
k =number of groups to be compared
$N = \sum_{i=1}^{k} n_i$

Chi-square tests were used to describe the association between two categorical variables. For example, the demographic characteristics of the study populations between different study centres were compared using chi-square test.

Chi-square test measures the difference between the observed and the expected values. The expected values were calculated assuming that there is no association between the two categorical variables.

$$\chi^2 = \sum_i \sum_j \frac{(O_{ij} - E_{ij})^2}{E_{ij}} \sim X_{(r-1)(c-1)}$$

where O_{ij} is the observed value and E_{ij} is the expected value
r and c are the numbers of the categories in each categorical variable

The analyses were performed with SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

3.2 Analysis for Single Outcome Measurement

3.2.1 Logistic Regression Model

Logistic regression is a generalization of chi-square test to examine the association of a binary outcome with one or more explanatory variables. The logistic regression models the population probability $\pi(x)$ of the presence of certain characteristic given x. In the following analysis, $\pi(x)$ is the population probability of having positive allergic sensitization outcome.

Using the logistic distribution

$$\pi(x) = \frac{e^{\beta_0+\beta_1 x}}{1 + e^{\beta_0+\beta_1 x}}$$

and the logit transformation is applied to π so that

$$logit(\pi(x)) = \ln \frac{\pi(x)}{1 - \pi(x)}$$

and

$$logit(\pi(x)) = \beta_0 + \beta_1 X_1$$

The maximum likelihood technique [104] is used to obtain the estimates for the parameter β_i.

The model can be extended to multiple logistic regression which allows more than one explanatory variables

$$logit(\pi) = \beta_0 + \beta_1 X_1 + ... + \beta_p X_p$$

and the results are presented as odds ratios adjusted for the remaining explanatory variables X_k where $k \neq j$

$$OR_j = exp(\hat{\beta}_j)$$

with 95% confidence intervals

$$exp\left[\hat{\beta}_j \pm Z_{1-\alpha/2} \times \sqrt{\hat{\text{Var}}(\hat{\beta}_j)}\right]$$

When exposure data were in the form of continuous measures, such as allergen exposures, the results were presented as odds ratios for every inter-quartile range increase in exposure variable.

The analyses were performed with proc logistic procedure, SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

3.2.2 Generalized Additive Models

Generalized additive models using local regression smoothing operation were fitted to assess the linear dependence of the associations between continuous indoor bio-contaminants exposures and the categorical health outcomes.

The generalized additive model extends the generalized linear model [105] by fitting nonparametric functions between the transformed outcome and the explanatory variables to estimate their relationships [106].

The standard linear logistic regression model assumes that the logit of the probability π of the binary outcome is linearly associated with the exposure variables X_1, ... ,X_p. The additive model replaces the linear function $\beta_j X_j$ used in the linear logistic regression by a non-linear function.

$$logit(\pi) = \beta_0 + f_1(X_1) + ... + f_p(X_p)$$

where $f_j(X), j = 1, ..., p$ are smooth functions which are estimated in a nonparametric fashion.

The smooth functions were fitted using locally weighted regression (LOESS) methods which robust locally linear fits to protect against outliers. The default span of 0.6 was used.

The analyses were performed with the gam function, s-plus version 6.2 (Insightful ,USA).

3.2.3 Ordinal Logistic Regression Model

To estimate the association between indoor dust and bio-contaminants levels and family SES, the observed dust and bio-contaminant levels were categorized into tertiles and ordinal logistic regression models adjusting for confounders were fitted.

The ordinal logistic regression model (or proportional-odds model) takes into account the rank ordering nature of a multiple category outcome. The model uses maximum likelihood estimation to compare the effect of a given explanatory variable on the likelihood of being above certain category in an ordinal outcome variable to the likelihood of being in the category and the categories below. The reference group in a proportional-odds model is all respondents below the cut point and it assumes that the odds ratio is constant for all outcome categories.

$$\begin{aligned} logit(\pi_1) &= \ln \tfrac{\pi_1}{1-\pi_1} = \alpha_0 + \mathrm{x}'\,\beta \\ logit(\pi_1 + \pi_2) &= \ln \tfrac{\pi_1+\pi_2}{1-\pi-\pi_2} = \alpha_0 + \mathrm{x}'\,\beta \\ &\vdots \\ logit(\pi_1 + \pi_2 + ... + \pi_{k+1}) &= \ln \tfrac{\pi_1+\pi_2+...+\pi_k}{1-\pi-\pi_2-...-\pi_k} = \alpha_k + \mathrm{x}'\,\beta \\ & \qquad \text{and } \pi_1 + \pi_2 + ... + \pi_{k+1} = 1 \end{aligned} \tag{3.1}$$

The results were presented as odds ratios with 95% confidence intervals.

The analyses were performed with proc logistic procedure, SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

3.3 Analysis for Repeated Outcome Measurements in Cohort Study Design

The association between pet ownership and pet contact during childhood and cat allergen exposure in infancy and the development of specific sensitization and allergic symptoms and diseases at multiple follow-ups were assessed using longitudinal analysis techniques. In the GINI and LISA birth cohort study, health outcomes such as cat allergen specific IgE measurements and allergic symptoms and diseases were collected repeatedly from the same individual through time to allow analyses of the changes over time. The longitudinal analysis approach takes the dependence of repeated outcome measures within each subject into account.

Two types of models were used to handle the longitudinal data. Marginal models are also known as "population-average models", estimate the mean response at each occasion and do not incorporate the dependence of responses within each individual. In contrast to the marginal model is the random effect model, which is the subject-specific model. The random effect model allows us to capture the changes within each individual as well as the between individual variation. Marginal model was used when only a small number of data were available that caused insolvable convergence problems with the random effects model.

3.3.1 Longitudinal analysis with marginal logistic regression model (GEE)

Marginal logistic regression models for longitudinal data focus on the population mean response at each repeated measurement that depends only on the covariates of interests. The correlations across the repeated measurements within each individual is assumed to exist but are treated as nuisance [107].

The probability of having allergic sensitization can be related to the exposure variables by a logit link

$$g(\pi_{ij}) = \ln \frac{\pi_{ij}}{1-\pi_{ij}} = \beta_0 + \beta_1 X_{ij} + ... + \beta_k X_{ij}$$

where i is each repeated measurement within the jth individual

The variance of each response Y_{ij}, given the effect of the covariates is

$$Var(Y_{ij}) = \pi_{ij}(1 - \pi_{ij})$$

The within subject association among the vector of repeated response is assumed to have an unstructured pair-wise log odds ratio pattern

$$\ln(OR(Y_{ij}, Y_{ik})) = \alpha_{jk}$$

where α is defined as the working correlation matrix, which handles the inter-correlated feature in the data of repeated measurements. The structure of α is not usually known but can be specified. However, wrongly specified working correlation matrix has no effect on the consistency of the regression parameter estimation.

The maximum likelihood technique is used to obtain the estimates for the parameter β_i.

The results were presented as odds ratios with 95% confidence intervals. In case of allergen exposure in the form of continuous measures, the results were presented as odds ratios for every inter-quartile range increase in allergen exposure.

The analyses were performed with proc genmod procedure, SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

3.3.2 Longitudinal analysis with random effects logistic regression models

Random effect models for longitudinal data are regression models in which the regression coefficients are allowed to vary across the subjects. These models include within-individual component, which describes an individual's change over time with a population-level intercept and slope, and between-individual component, which captures the variation in individual intercepts and slopes. For longitudinal studies, random effects models allows us to describe the trend over time while

taking account of the correlation that exists between repeated measurements, and the variation in the baseline measurement and in the rate of change over time between individuals. Here, only random intercept model were used, as our data varied significantly between the baseline measurement but not in the rate of change over time[108].

The random effect can be introduced to a one level logistic regression model using the following model:

$$g(\pi_{ij}) = \ln \frac{\pi_{ij}}{1-\pi_{ij}} = \beta_0 + \beta_1 X_{1ij} + ... + \beta_k X_{kij} + u_{0j} + e_{ij}$$

where i is each repeated measurement within the jth individual
$u_{0j} \sim N(0, \sigma_u^2)$ is the random intercept, assuming the probability of developing allergic sensitization varies randomly between each child.

and $e_{ij} \sim$ logistic distribution with mean= 0 and variance= $\sigma_e^2 = \pi^2/3$

We can then calculate the intra-cluster correlation (ICC) which describes the proportion of the total conditional outcome variation that lies between subjects

$$\text{ICC}(\rho) = \frac{\sigma_u^2}{\sigma_u^2 + \sigma_e^2}$$

The results were presented as odds ratios with 95% confidence intervals. In case of allergen exposure in the form of continuous measures, the results were presented as odds ratios for every inter-quartile range increase in allergen exposure.

The analyses were performed with proc glimmix procedure, SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

Chapter 4

Study Results

The association between socioeconomic status and the amount of indoor cat allergen and endotoxin in the families in the LISA cohort, Munich and Leipzig subgroups was first assessed in this chapter. This was followed by the main analysis focusing on the effect of cat allergen exposure and pet ownership and pet contact during childhood. The association between the amount of the observed domestic cat allergen and the prevalence rate of cat sensitization and allergic diseases and symptoms in children at different age was investigated using both the longitudinal LISA study and the cross-sectional AIRALLERG study. The distribution of domestic cat allergen loads and concentrations across 3 European countries was also described using the AIRALLERG study. Finally, the data from the German GINI and LISA cohort were analysed to describe the association between dog ownership and dog contact during childhood and the development of specific sensitization and allergic diseases and symptoms up to school age.

4.1 Inequality of Domestic Cat Allergen and Endotoxin Distribution between Families with Different Socioeconomic Status

The results presented here have been published in Indoor Air. early online publication Aug 2007 Chen et al. Social Factors, Allergen , Endotoxin, and Dust Mass in Mattress.

The dust samples were available from 2118 (98%) parents' mattress and 2098 (97%) children's mattress from 2166 (89%) families in the LISA study, Munich and the Leipzig subgroup. The samples were collected when the children were 3 months old. For those infants who slept occasionally on their parents' bed or on their own bed, only the mattress the infants had very frequent contact with was sampled.

The amounts of dust collected from the infants' mattresses were very low. The median load of the infants' mattresses dust samples was a quarter of the median dust load collected from the parents' mattresses (table 4.1). In general, there were no significant differences in the amounts of cat allergens in infants' mattress between the two study centres and the amount of endotoxin was slightly higher in the homes in Leipzig. For parents' mattress, there were higher amount of cat allergens in both concentration and surface load in Leipzig ($p < 0.01$).

Sampling site		Fel d 1 (nanogram)	Endotoxin (endotoxin unit)	Total dust mass (mg/m^2)
Parents' mattress	Unit /gram dust	180.2(68.6,820.5)	3008(1045.5,7913)	738.5(453.0,1166.0)
	Unit / m^2 dust	130.9(50.9,696)	2071(594.5,6916)	
	N (Detectable %)	88.5%	100%	N=2118
Infants' mattress	Unit /gram dust	326.0(84.7,1322.5)	5866(2336,14669)	188.0(120.0,289.0)
	Unit / m^2 dust	65.6(16.6,333.1)	1015(330,3022)	
	N (Detectable %)	77.5%	100%	N=2098

Table 4.1: The amount of domestic cat allergen and endotoxin measured from parents' and infants' mattress in the LISA study

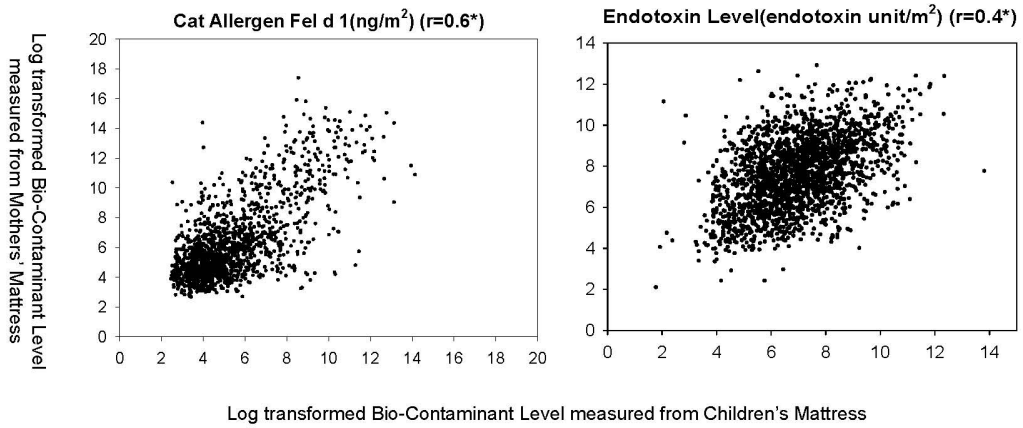


Figure 4.1: Correlation between the log-transformed domestic bio-contaminants collected from parents' and infants' mattress (LISA study)

The correlations between cat allergen and endotoxin loads measured from parents' and infants' mattresses were not strong (Figure 4.1). Similar correlation were observed in the concentrations(r=0.6 for Fel d 1 and r=0.4 for endotoxin). There were also no strong correlations between cat allergen and endotoxin loads and generally very weak correlations between concentrations (table 4.2).

The distribution of socioeconomic groups of the study population are listed in table 4.3. Parental educational level and family equivalent income were used independently as socioeconomic indicators. The parental educational level was categorised based on the German educational system, which takes both the highest completed grade in school and vocational training into account [109] . In the analysis, parental education level was defined as the higher education level from either parent. Family equivalent income was calculated by dividing the net household income per month, which was reported on an eleven-point scale ranging from less than €500 to more than €3000, by the equivalence scale that considers family size. The equivalence scale gives a weight of 1.0 to the first adult in the family, 0.5 to any other family members above 15 years old, and 0.3 to

	Fe l d 1 (ng/m^2)	Endotoxin (mg/m^2)	Total dust mass (g/m^2)		Fe l d 1 (ng/g)	Endotoxin (EU/g)
Fe l d1 (ng/m^2)	-	*0.36*	*0.48*	Fe l d1 (ng/g)	-	*0.14*
Endotoxin (EU/m^2)	**0.35**	-	*0.58*	Endotoxin (EU/g)	**0.16**	-
Total dust mass (mg/m^2)	**0.41**	**0.54**	-	-	-	-

[1]*Numbers in italic format are correlation between the amounts of bio-contaminants collected from infants' mattress*

[2]**Numbers in bold format are correlation between the amounts of bio-contaminants collected from parents' mattress**

Table 4.2: Correlation between the amount of bio-contaminants collected from both parents' and infants'mattress (LISA study)

family members below 14 years old. The calculation was based on the new Organisation of Economic Cooperation and Development (OECD) guidelines [110]. The families were categorised into tertiles based on their family equivalent income. Comparing the two study centres, families in Munich generally had higher percentages of highly educated parents and higher income. However, the cost of living in Munich is also higher.

Table 4.4 shows the housing characteristics and family history of allergic diseases

		Munich n(%)	Leipzig n(%)	Total n(%)
Parental Education level	Very High	903(67.0%)	356(44.5%)	1259(58.1%)
	High	217(16.1%)	168(21.5%)	385(18.8%)
	Median	162(12.0%)	237(29.0%)	399(18.4%)
	Low	53(4.9%)	43(5.3%)	96(4.4%)
Family equivalent income	High	502(37.2%)	86(11.5%)	588(27.1%)
	Median	402(30.8%)	173(21.1%)	575(27.5%)
	Low	226(17.8%)	383(47.8%)	609(28.1%)

Table 4.3: Socioeconomic level of the study population (LISA study)

	n/N (%)		
	Munich N=1348(62.2%)	Leipzig N=818(37.8%)	Total N=2166
Number of people sharing children's room[1]			
1	144/1348(11%)	70/818(9%)	214/2096(10.2%)
≥2	812/1348(60%)	591/818(72%)	1403/2096(66.9%)
Parents or siblings have a history of atopic disease[1]	856/1348(64%)	378/818(46%)	1234/1560(79.1%)
House type[1]			
Alone-standing house	280/1348(21%)	66/818(8%)	346/2151(16.1%)
Not alone-standing one family house	210/1348(16%)	39/818(5%)	249/2151(11.6%)
Flat	749/1348(56%)	666/818(81%)	1415/2151(65.8%)
Multi-storage house	106/1348(8%)	35/818(4%)	141/2151(6.5%)
Story of dwelling[1]			
ground floor	585/1348(43%)	173/818(21%)	758/2135(35.5%)
1st floor	321/1348(24%)	170/818(21%)	491/2135(23.0%)
2nd floor and higher	425/1348(32%)	461/818(56%)	886/2135(41.5%)
Having a dog currently in the house[1]	73/1348(5%)	71/818(9%)	144/2145(6.71%)
Having a cat currently in the house	124/1348(9%)	83/818(10%)	207/2145(9.65%)
Used to have a cat in the house[1]	139/1348(10%)	55/818(7%)	194/2087(9.3%)
Current signs of dampness	82/1348(6%)	50/818(6%)	132/2151(6.1%)
Current signs of mold[1]	408/1348(30%)	189/818(23%)	597/2151(27.8%)
Water damage (past 12 months)	121/1348(9%)	60/818(7%)	181/2151(8.4%)
Heating system[1]			
Heating in the flat	169/1348(13%)	118/818(14%)	287/2151(13.3%)
Central heating	1042/1348(77%)	649/818(79%)	1691/2151(78.6%)
Both	135/1348(10%)	38/818(5%)	173/2151(8.0%)

[1]The characteristic is statistically significantly different between the two study centres (α=0.05)

Table 4.4: Housing characteristics and family history of allergic disease of the study population (LISA study)

of the study population. We stratified the data by study centre and chi-square test (α=0.05) showed that in Leipzig, higher percentages of the recruited families lived in flats, and the families were more likely to have a dog. In Munich, higher percentages of the recruited families were using anti-allergen mattress cover, used to have a cat in their house, and reported signs of mold. We also recruited fewer parents with a history of allergic diseases in Leipzig than in Munich centre.

Comparisons between total amount of dust, cat allergen, and endotoxin loads and concentrations measured from families in different socioeconomic groups are listed in table 4.5. It showed that there was a steady decrease of the amount of cat allergen Fel d 1 on the mattress from families with lower SES to families with

		Parental Educational Level				
Allergen	Sampling site	Low	Median	High	Very high	P-value
Fel d 1	Parents(ng/g)	589(144,7586)	280(95,2203)	232(77,1522)	148(59,463)	< .01
	Parents(ng/m^2)	424(86,11280)	193(63,1663)	171(60,1048)	107(46,465)	< .01
	Children(ng/g)	918(239,5363)	452(116,2883)	350(52,1640)	271(64,909)	< .01
	Children(ng/m^2)	174(51,1998)	89(25,573)	65(15,396)	56(15,221)	< .01
Endotoxin	Parents(EU/g)	2841(929,6253)	2838(1031,7708)	2478(926,7531)	3151(1110,8591)	0.23
	Parents(EU/m^2)	2096(792,7402)	1818(623,6393)	1715(514,6306)	2324(594,7368)	0.22
	Children(EU/g)	5846(2153,15515)	6100(2663,13473)	5552(2369,14413)	5850(2035,15094)	0.83
	Children(EU/m^2)	1272(345,2959)	1010(409,3434)	1038(318,2821)	1023(325,3394)	0.85
Dust Mass	Parents(mg/m^2)	813(503,1185)	701(450,1108)	706(427,1171)	759(460,1172)	0.31
	Children(mg/m^2)	191(109,296)	190(121,290)	178(115,266)	190(120,301)	0.36
		Family Equivalent Income				
Fel d 1	Parents(ng/g)	238(80,1571)	174(72,705)	131(51,441)	-	< .01
	Parents(ng/m^2)	193(593,1467)	127(49,652)	101(43,445)	-	< .01
	Children(ng/g)	447(92,1639.9)	273(58,1134)	270(91,959)	-	0.01
	Children(ng/m^2)	79(20,410)	52(13,276)	60(19,239)	-	0.02
Endotoxin	Parents(EU/g)	3090(1032,8332)	2970(1041,7293)	2797(1020,7563)	-	0.72
	Parents(EU/m^2)	2016(629,7103)	1896(585,6522)	2070(568,6978)	-	0.52
	Children(EU/g)	6063(2588,14532)	5021(1878,12990)	5746(1983,15143)	-	0.03
	Children(EU/m^2)	1201(357,3573)	861(320,2713)	1043(307,3581)	-	0.1
Dust Mass	Parents(mg/m^2)	732(464,1192)	720(428,1094)	792(471,1166)	-	0.18
	Children(mg/m^2)	181(122,289)	187(120,285)	197(118,303)	-	0.43

Table 4.5: The amount of cat allergen and endooxin measured from parents' and infants' mattresses by family's socioeconomics levels (LISA study)

higher SES. After adjusting for confounders, the amounts of cat allergen in both parents' and infants' mattress dust were significantly negatively associated with the two independent socioeconomic indicators. For example, in homes of parents with very high education, the odds of having a high cat allergen load (over 66th percentile of the overall cat allergen load from the study population) on their mattresses was 0.44 times smaller than having a middle and low cat allergen load (less than 66th percentile of the overall cat allergen loads from the study population). Likewise, in homes of parents with very high education, the odds of having a median and high cat allergen load (over 33rd percentile of the overall cat allergen load from the study population) on their mattresses versus low cat allergen load (less than 33rd percentile of the overall cat allergen loads from the study population) was 0.44 times smaller. However, the amount of endotoxin and dust sampled in both parents' and infant's mattress dust was not associated with the 2 social indicators (table 4.6).

			Parental Educational Level(OR(C.I.)[1])			
Allergen	Sampling site	Unit	Low	Median	High	Very high
Fel d 1	Parents' mattress	ng/g	1.0	0.56(0.3,1.03)	0.51(0.27,0.92)	0.38(0.21,0.67)
		ng/m^2	1.0	0.61(0.33,1.13)	0.58(0.31,1.05)	0.44(0.25,0.78)
	Infants' mattress	ng/g	1.0	0.66(0.37,1.17)	0.5(0.28,0.88)	0.44(0.26,0.75)
		ng/m^2	1.0	0.65(0.36,1.16)	0.51(0.28,0.9)	0.47(0.27,0.81)
Endotoxin[2]	Parents' mattress	EU/g	1.0	1.24(0.81,1.9)	1.09(0.71,1.67)	1.27(0.85,1.91)
		EU/m^2	1.0	0.86(0.56,1.31)	0.82(0.54,1.26)	0.98(0.66,1.45)
	Infants' mattress	EU/g	1.0	0.97(0.64,1.49)	0.98(0.64,1.51)	1.00(0.67,1.50)
		EU/m^2	1.0	0.9(0.6,1.4)	0.8(0.6,1.3)	0.9(0.6,1.3)
Dust Mass[3]	Parents' mattress	mg/g	1.0	0.8(0.52,1.22)	0.8(0.52,1.22)	0.92(0.62,1.36)
	Infants'mattress	mg/m^2	1.0	1.17(0.77,1.76)	0.97(0.64,1.46)	1.13(0.77,1.66)
			Family Equivalent Income(OR(C.I.)[1])			
Fel d 1	Parents' mattress	ng/g	1.0	0.87(0.66,1.15)	0.6(0.45,0.81)	-
		ng/m^2	1.0	0.77(0.58,1.01)	0.63(0.47,0.84)	-
	Infants' mattress	ng/g	1.0	0.83(0.63,1.09)	0.81(0.61,1.08)	-
		ng/m^2	1.0	0.83(0.63,1.09)	0.88(0.66,1.17)	-
Endotoxin[2]	Parents' mattress	EU/g	1.0	1.01(0.81,1.27)	0.94(0.74,1.19)	-
		EU/m^2	1.0	0.9(0.72,1.13)	0.94(0.74,1.19)	-
	Infants' mattress	EU/g	1.0	0.84(0.67,1.05)	0.97(0.76,1.23)	-
		EU/m^2	1.0	0.91(0.73,1.13)	1.04(0.82,1.31)	-
Dust Mass[3]	Parents' mattress	mg/g	1.0	0.95(0.76,1.18)	1.19(0.94,1.5)	-
	Infants' mattress	mg/m^2	1.0	0.96(0.77,1.21)	1.07(0.85,1.36)	-

[1]Confounders adjusted for the cat allergen (Fel d 1) model: season, study centre, current cat owners, family history of allergic diseases, and previous cat in the house

[2]Confounders adjusted for the endotoxin model: season, study centre, current signs of mold, and previous cat in the house

[3]Confounders adjusted for the dust model: season and study centre

Table 4.6: The associations between the amount of domestic cat allergen and endotoxin measured from parents' and infants' mattress and family socioeconomic levels (LISA study)

Due to the unequal distributions of socioeconomic groups of the recruited families in Munich and Leipzig and the differences of the amount of endotoxin between the two centres, stratified analysis was performed. In each stratified group, there were no association between family SES and dust mass and endotoxin load and concentration in mattresses. The negative associations between the amount of cat allergen and family SES was detected in both centres. However, only 38% of the recruited families were from Leipzig. The power of detecting the differences of the amount of cat allergen between socioeconomic groups is lower in this subgroup, although the magnitudes of the effect estimates were similar (table 4.7).

In our study population, families of lower parental education were more likely to keep cats (20% in low parental educational families vs. 6% in high parental educational families). Further sensitivity analysis showed that the observed negative associations between family SES and domestic cat allergen loads and concentrations were restricted to the families who are not cat owners at the time of dust sampling. For the families who were cat owners at the time of dust sampling and those who used to have a cat in their house, we re-categorised the cat allergen loads and concentrations based on the amount of cat allergen in each sub-group. In homes of current cat owners, the associations between the amount of cat allergen and families SES were not consistent (table 4.8).

The associations between socioeconomic status and the amount of indoor cat allergn and endotoxin in the families in the LISA study were assessed. The results showed a negative association between the amount of indoor cat allergen and family SES, particularly in non-cat- owner families . The total amount of dust, and endotoxin loads and concentrations in mattresses, however, do not vary between families of different social classes.

Munich								
				Parental Educational Level (OR(C.I.)[1])			Family Equivalent Income (OR(C.I.)[1])	
Allergen	Sampling site	Unit	Low	Median	High	Very high	Median	High
Fel d 1	Parents' mattress	ng/g	1.0	0.7(0.4,1.4)	0.6(0.3,1.1)	0.4(0.2,0.8)	0.7(0.5,1)	0.5(0.4,0.7)
		ng/m^2	1.0	0.8(0.4,1.6)	0.7(0.4,1.3)	0.6(0.3,1)	0.6(0.5,0.9)	0.6(0.4,0.8)
	Infants' mattress	ng/g	1.0	0.9(0.4,1.6)	0.5(0.3,1.0)	0.5(0.3,0.8)	0.6(0.5,0.9)	0.6(0.5,0.9)
		ng/m^2	1.0	0.9(0.4,1.6)	0.5(0.3,0.9)	0.5(0.3,0.9)	0.6(0.5,0.9)	0.7(0.5,0.9))
Endotoxin[2]	Parents' mattress	EU/g	1.0	1.1(0.6,2)	1(0.6,1.7)	1.2(0.7,2.1)	1.2(0.9,1.6)	1.1(0.8,1.5)
		EU/m^2	1.0	0.7(0.4,1.3)	0.7(0.4,1.2)	0.9(0.5,1.5)	1(0.7,1.3)	1.1(0.8,1.4)
	Infants' mattress	EU/g	1.0	1.0(0.6,1.9)	1.0(0.5,1.7)	1.0(0.6,1.7)	0.9(0.7,1.3)	1.1(0.8,1.5)
		EU/m^2	1.0	1(0.6,1.7)	0.8(0.4,1.3)	0.9(0.6,1.5)	1(0.8,1.4)	1.2(0.9,1.6)
Dust Mass[3]	Parents' mattress	mg/g	1.0	0.7(0.4,1.2)	0.7(0.4,1.2)	0.8(0.5,1.3)	0.9(0.7,1.2)	1.1(0.8,1.5)
	Infants'mattress	mg/m^2	1.0	1.2(0.7,2.2)	1(0.6,1.8)	1.4(0.9,2.3)	1(0.7,1.4)	1.2(0.9,1.6)
Leipzig								
Fel d 1	Parents' mattress	ng/g	1.0	0.5(0.2,1)	0.5(0.2,1)	0.4(0.2,0.8)	1(0.7,1.4)	0.6(0.4,1)
		ng/m^2	1.0	0.6(0.3,1.3)	0.6(0.3,1.3)	0.5(0.2,1)	0.9(0.6,1.2)	0.6(0.4,0.9)
	Infants' mattress	ng/g	1.0	0.5(0.2,0.9)	0.5(0.2,0.9)	0.5(0.3,0.9)	0.9(0.6,1.2)	1.2(0.8,1.8)
		ng/m^2	1.0	0.6(0.3,1.1)	0.5(0.3,1)	0.6(0.3,1.1)	0.9(0.6,1.2)	1.1(0.7,1.7)
Endotoxin[2]	Parents' mattress	EU/g	1.0	1.4(0.7,2.6)	1.2(0.6,2.3)	1.2(0.6,2.3)	0.8(0.6,1.2)	0.6(0.4,1)
		EU/m^2	1.0	1.1(0.5,2)	1.1(0.6,2.1)	1.1(0.6,2)	0.9(0.6,1.2)	0.6(0.4,1)
	Infants' mattress	EU/g	1.0	0.9(0.5,1.7)	1.0(0.5,1.9)	0.9(0.5,1.7)	0.7(0.5,1.0)	0.7(0.5,1.1)
		EU/m^2	1.0	0.9(0.5,1.6)	0.9(0.5,1.7)	0.8(0.5,1.6)	0.8(0.6,1.1)	0.8(0.5,1.3)
Dust Mass[3]	Parents' mattress	mg/g	1.0	1(0.5,1.9)	1(0.5,2)	1.2(0.6,2.2)	1(0.7,1.3)	1.5(1,2.3)
	Infants'mattress	mg/m^2	1.0	1(0.5,1.8)	0.9(0.5,1.6)	0.8(0.4,1.4)	1(0.7,1.4)	0.8(0.5,1.3)

[1]Confounders adjusted for the cat allergen (Fel d 1) model: season, study centre, current cat owners, family history of allergic diseases, and previous cat in the house

[2]Confounders adjusted for the endotoxin model: season, study centre, current signs of mold, and previous cat in the house

[3]Confounders adjusted for the dust model: season and study centre

Table 4.7: The associations between the amount of domestic cat allergen and endotoxin measured from parents' and infants' mattress and family socioeconomic levels by study centre (LISA study)

Cat Ownership	Sampling site	unit	Low	Median	High	Very high
Never[1]				Parental Educational Level(OR(C.I.)[1])		
	Parents' mattress	ng/g	1.0	0.56(0.29,1.07)	0.53(0.28,1)	0.4(0.22,0.73)
		ng/m^2	1.0	0.64(0.33,1.22)	0.61(0.32,1.15)	0.49(0.26,0.89)
	Infants' mattress	ng/g	1.0	0.74(0.4,1.37)	0.54(0.3,1)	0.49(0.28,0.87)
		ng/m^2	1.0	0.78(0.41,1.45)	0.56(0.3,1.05)	0.54(0.3,0.97)
				Family Equivalent Income(OR(C.I.)[1])		
	Parents' mattress	ng/g	1.0	0.98(0.74,1.31)	0.65(0.48,0.88)	-
		ng/m^2	1.0	0.85(0.64,1.13)	0.68(0.5,0.91)	-
	Infants' mattress	ng/g	1.0	0.9(0.68,1.19)	0.86(0.64,1.17)	-
		ng/m^2	1.0	0.88(0.66,1.17)	0.9(0.67,1.21)	-
Current[3]				Parental Educational Level(OR(C.I.)[1])		
	Parents' mattress	ng/g	1.0	0.62(0.17,2.16)	0.75(0.2,2.74)	0.53(0.16,1.74)
		ng/m^2	1.0	0.37(0.1,1.35)	0.53(0.13,2.01)	0.51(0.15,1.73)
	Infants' mattress	ng/g	1.0	0.74(0.19,2.85)	1.46(0.38,5.67)	1.2(0.34,4.31)
		ng/m^2	1.0	0.68(0.17,2.68)	1.1(0.27,4.5)	0.85(0.22,3.16)
				Family Equivalent Income(OR(C.I.)[1])		
	Parents' mattress	ng/g	1.0	1.42(0.59,3.46)	0.5(0.2,1.26)	-
		ng/m^2	1.0	2.21(0.91,5.48)	1.25(0.5,3.15)	-
	Infants' mattress	ng/g	1.0	1.43(0.56,3.66)	0.64(0.24,1.64)	-
		ng/m^2	1.0	2.07(0.8,5.46)	0.51(0.2,1.29)	-
Used to have a cat in the house (not necessarily cat owners)[3]				Parental Educational Level(OR(C.I.)[1])		
	Parents' mattress	ng/g	1.0	0.18(0.01,1.7)	0.19(0.01,1.9)	0.09(0,0.72)
		ng/m^2	1.0	0.26(0.01,2.45)	0.2(0.01,2.04)	0.1(0.01,0.85)
	Infants' mattress	ng/g	1.0	0.16(0.01,1.57)	0.08(0,0.78)	0.07(0,0.57)
		ng/m^2	1.0	0.32(0.03,2.41)	0.26(0.03,2.03)	0.23(0.03,1.4)
				Family Equivalent Income(OR(C.I.)[1])		
	Parents' mattress	ng/g	1.0	0.21(0.07,0.66)	0.22(0.06,0.72)	-
		ng/m^2	1.0	0.13(0.04,0.42)	0.22(0.06,0.73)	-
	Infants' mattress	ng/g	1.0	0.16(0.05,0.49)	0.25(0.07,0.81)	-
		ng/m^2	1.0	0.29(0.09,0.84)	0.45(0.14,1.4)	-

[1]Families had no cat at the time of dust sampling and had no cat previously in their house

[2]Confounders adjusted: season, study centre, and family history of allergic diseases

[3]The cat allergen loads and concentrations re-categorised into the tertiles based on the amount of allergen in each sub-group.

Table 4.8: Associations between the amount of domestic cat allergen measured in parents' and infants' mattress dust and family socioeconomic levels stratified by family cat ownership (LISA study)

4.2 Associations between Cat Allergen Exposure and the Development of Allergy in Young Children-LISA Study

The results presented here have been published in J.Allergy Clin.Immunol.2007;119:1148-1155 Chen et al. Longitudinal study on cat allergen exposure and the development of allergy in young children.

To assess the effects of infancy cat allergen exposure and cat ownership / cat contact during childhood on the development of allergy in children, we used the data from the LISA study, Munich and Leipzig centres. In the following analysis, the allergen and endotoxin levels were expressed as allergen load, the amount of allergen per m^2 of sample surface only. In the LISA study, Munich and Leipzig centres, the measured cat allergen level expressed per square meter and per gram dust were highly correlated (Spearman Correlation Coefficients =0.9, for both parents' and children's mattresses). Furthermore, in the following section, the study results of the AIRALLERG study will show that the estimated associations between sensitization outcomes and domestic cat allergen levels do not differ significantly when allergen levels were expressed in concentrations in dust or surface allergen loads.

The amount of dust and cat allergen load collected from mattresses is listed in the previous section (table 4.1). The estimated correlation between cat allergen loads from parents' and children's mattress was 0.6 (Figure 4.1). At age 2 and 6, 21 (1.3%) and 43 (5.0%) children respectively were sensitized to cat allergen (see table 4.9 for baseline information). In our study, there was no significant difference of the prevalence of cat ownership between the two study centres. The prevalence of cat ownership between birth and age 2 were 11% in Munich and 12% in Leipzig and the prevalence between age 2 and 6 were 12% in Munich and 15% in Leipzig.

The effect of cat allergen exposure in infancy on the development of cat sensitization changed during follow-up. Higher cat allergen exposures from both parents' and children's mattresses significantly increased the risk of the development of

		n/N(%)
Sensitization to cat at age 2		21/1591(1.3%)
Sensitization to cat at age 6		43/857(5.0%)
Doctor diagnosed allergic diseases		
Eczema	at age 6 months	128/2047(6.3%)
	at 12 months	173/1998(8.7%)
	at 18 months	187/1985(9.4%)
	at age 2	194/1955(9.9%)
	at age 3	199/1713(11.6%)
	at age 4	178/1510(11.8%)
	at age 5	169/1606(10.5%)
	at age 6	150/1602(9.4%)
Hay Fever	at age 4	33/1655(2.0%)
	at age 5	44/1604(2.7%)
	at age 6	66/1599(4.1%)
Asthma	at age 4	16/1660(1.0%)
	at age 5	35/1603(2.2%)
	at age 6	33/1603(2.1%)
Study Centre	Munich	1348 /2166(62.2%)
	Leipzig	818 /2166(37.8%)
Parents had asthma, eczema, or hay fever		1170/2095(55.8%)
Parental Education level[1]	Very high	1259/2139(58.9%)
	High	385/2139(18.0%)
	Median	399/2139(18.7%)
	Low	96/2139(4.5%)
Sex	Male	1115/2166(51.5%)
	Female	1051/2166(48.5%)

[1]The parental educational level was categorised based on the German educational system, which takes both the highest completed grade in school and vocational training into account [109]. The higher education level from either parent was taken

Table 4.9: Baseline information of the study population (LISA study)

	Adjusted mixed model(OR (C.I.))[2]	
	Parents' mattress	Children's mattress
Cat Sensitization at age two[1]	6.2 (2.6,14.8)	3.7 (1.5,9.1)
Cat Sensitization at age six[1]	1.3 (0.2,9.4)	1.3 (0.2,9.7)
Intra-cluster correlation coefficient (ICC)	32%	32%
Reference range of the probability of cat sensitization at baseline (i.e. at age 2)	1%	2%

[1]Specific IgE titer >0.35kU/L to cat allergen

[2]Adjusted for sex, study centre, parental educational level, move to another accommodation, and parental allergy (For every inter-quartile range increase in indoor cat allergen load (table 4.1))

Table 4.10: Cat allergen exposure from mattress dust during infancy and cat sensitization rate at age 2 and 6 (LISA study)

cat sensitization when the children were 2 years old, but showed no effect when the children were 6 years old (table 4.10). The effect of the allergen exposure from parents' mattress dust was stronger compared to the effect of the allergen exposure from children's mattress dust. The wide inter-quartile range of cat allergen loads in parents' mattress dust compared to children's mattress dust also added to the difference between the effect estimates. The intra-class correlation (ICC) was 32% for each model. In other words, 32% of the total conditional outcome variation (i.e. the variation of the probability of developing cat sensitization) lies between subjects. Additionally adjusting for endotoxin loads from parents' and children's mattresses made no change to the associations between cat allergen exposure and cat sensitization development (table 4.11).

Figure 4.2 describes the estimated association between early life cat allergen exposure from mattress dust and the probability of cat sensitization development at age 2 and 6 based on the models used in table 4.10. The figure shows that age and parental history of allergy were significant risk factors for the development of cat sensitization during childhood. Very high level of cat allergen exposure in

	Adjusted mixed model(OR (C.I.))[2]	
	Parents' mattress	Children's mattress
Cat Sensitization at age two[1]	6.3(2.7,15.0)	4.1(1.6,10.4)
Cat Sensitization at age six[1]	1.4(0.2,9.5)	1.5(0.2,10.9)

[1]Specific IgE titer >0.35kU/L to cat allergen

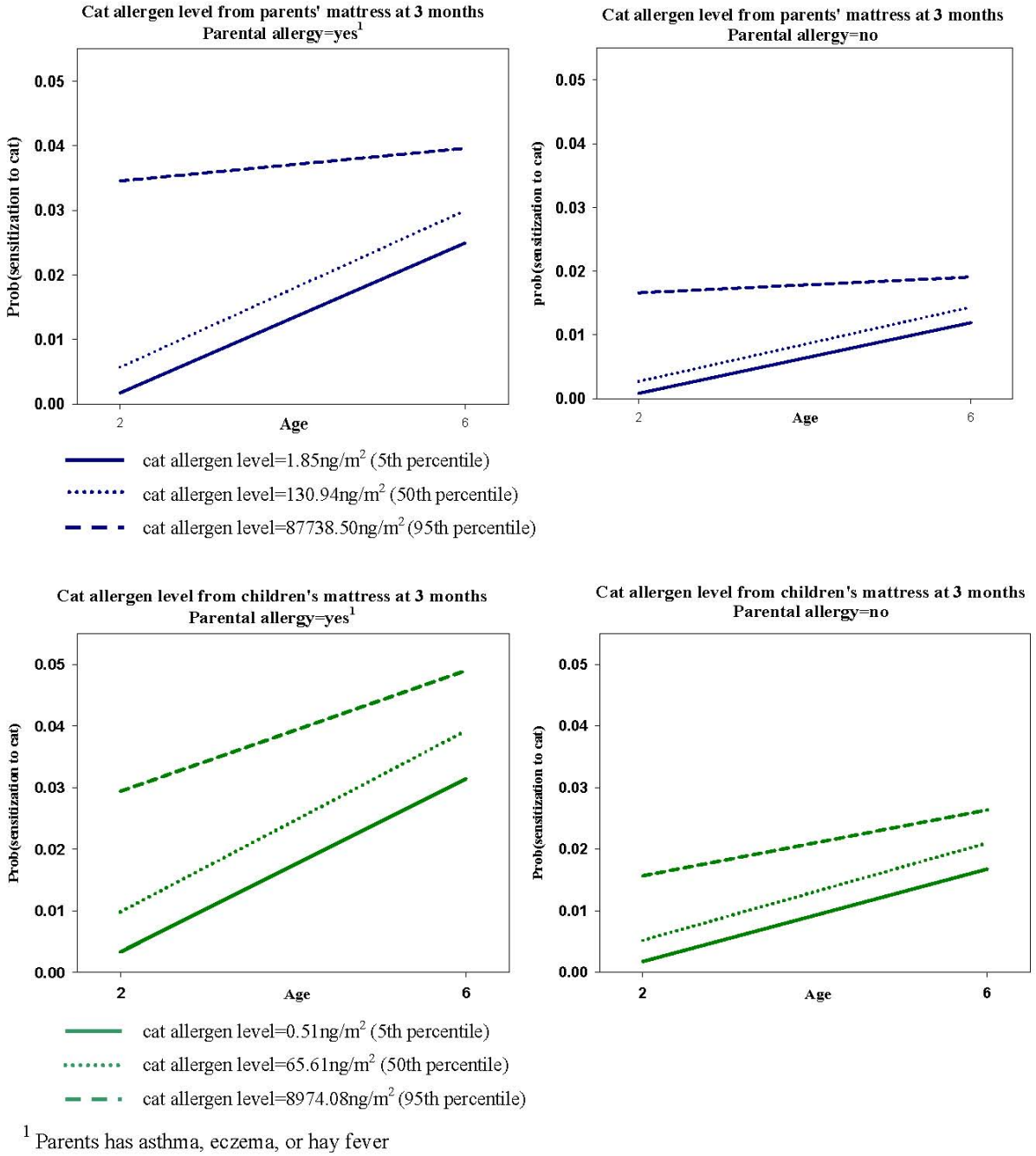
[2]Adjusted for sex, study centre, parental educational level, move to another accommodation, parental allergy, and the exposure to the endotoxin load measured from the mattress dust samples at age of 3 months (For every inter-quartile range increase in indoor cat allergen load (table 4.1))

Table 4.11: Cat allergen exposure from mattress dust during infancy and cat sensitization rate at age 2 and 6 with additional adjustment of the endotoxin exposure from mattress (LISA study)

infancy significantly increased the risk of cat sensitization at age 2 but the effect weakened as children grew older.

Early life cat allergen exposure from mattress dust had no effect on the development of allergic symptoms and diseases during childhood (table 4.12). Furthermore, the associations between cat allergen exposure in infancy and the development of allergic symptoms and diseases were constant over the follow-up period. Our data showed that cat allergen exposure in infancy induced the development of cat sensitization in early childhood, but it did not directly increase the risk of the development of allergic symptoms and diseases up to age 6.

Using family cat ownership during the first 6 years and regular cat contact during the first 4 years of life as surrogates of continuous cat allergen exposure during childhood, we analyzed its effect on the development of cat sensitization. Both cat ownership and regular cat contact without ownership increased the risk of sensitization to cat during childhood (at both age 2 and 6) (table 4.13). Unlike the effect of early life cat allergen exposure from mattress dust, the effect of cat ownership and regular cat contact during childhood on the development of cat sensitization was consistent over the follow-up period. However, previous cohort study in Sweden has shown a strong negative association between maternal pet



[1] Parents has asthma, eczema, or hay fever

Figure 4.2: The estimated association between early life cat allergen exposure from parents' and children's mattress dust and the cat sensitization rate at age 2 and 6 (LISA study)

		Adjusted mixed model (OR (C.I.))[1]	
		Parents' mattress	Children's mattress
Doctor diagnosed allergic diseases	Eczema(6, 12, 18 months, 2, 3, 4, 5,and 6 years)	1.0(0.8,1.3)	1.0(0.8,1.2)
	Hay Fever(4, 5, and 6 years)	0.9(0.5,1.5)	0.7(0.4,1.1)
	Asthma(4, 5, and 6 years)	1.2(0.7,2.0)	1.0(0.7,1.6)
Symptoms of allergic diseases	Eczema[2](6, 12, 18 months, 2, 3, 4, and 6 years)	1.0(0.9,1.2)	1.0(0.9,1.1)
	Hay Fever[3](4 and 6 years)	1.2(0.9,1.6)	1.1(0.9,1.4)
	Wheeze[4](4 and 6 years)	1.2(0.9,1.6)	1.1(0.8,1.4)

[1]Adjusted for sex, study centre, parental educational level, move to another accommodation, and parental allergy (For every inter-quartile range increase in indoor cat allergen load)

[2]Defined as itchy rash that effected skin crease, face, neck, extremities, hands, or feet

[3]Defined as sneezing, running nose, or nasal congestion without cold

[4]Defined as wheezing sound in the chest

Table 4.12: Cat allergen exposure from mattress dust during infancy and development of allergic symptoms and diseases (LISA study)

allergy and pet ownership [84]. To overcome possible bias from selective avoidance of cats by children with parental history of allergy, a stratified analysis was performed. We stratified the cohort by the family history of allergic diseases and re-analyzed the association between cat sensitization rate and cat ownership and regular cat contact. The results showed that regular cat contact was positively associated with higher risk of cat sensitization during childhood (at both age 2 and 6) only for those children whose parents ever had asthma, eczema, or hay fever. For children whose parents never had allergic diseases, cat contact only during childhood was not a risk factor for the development of sensitization. Cat ownership, however, was associated with the development of cat sensitization for children from families with or without history of allergic disease at a 10% significance level (table 4.13). The high intra-class correlation (ICC) suggested that there are still unknown factors during childhood that determine the progressive development of sensitization in each individual. These individual differences in the probability of sensitization development in early childhood were more pronounced between the children whose parents ever had asthma, eczema, or hay fever.

In the LISA study, not all children who have been followed-up agreed to participate in the blood examination. At year 6, only 50% of the followed-up children from the Munich and Leipzig subgroups had their blood sampled (figure 2.1). We therefore compare the demographic characteristics between children with and without IgE measurements (Table 4.14). We found that parents with higher educational level are more willing to let their children to participate in the IgE measurement. Lower proportion of the children who have moved between birth and age 6 had their blood sampled then the non-movers. Parental allergic disease, however, was not associated with the participation of the IgE measurement. We also assessed whether doctor diagnosed allergic diseases or allergic symptoms were associated with the willingness of participation. In the Munich subgroup, slightly more children who have been diagnosed with asthma at age 6 participated in the IgE measurement at age 6 (55% vs. 51%). Differences between participation rates of children whose parents reported and did not report allergic symptoms in the questionnaire were between 0% and 9%. Non of the differences between the

	Adjusted mixed model(OR (C.I.)) [2]			
	Cat Ownership		Cat Contact only[3]	
Cat Sensitization[1]	2.4(1.2,4.7)		2.4(1.3,4.5)	
Intra-cluster correlation coefficient (ICC)	33%		32%	
Reference range of the probability of cat sensitization at baseline(i.e. at age 2)	6%		9%	
	Stratified Analysis by Parental Allergy			
	Parental Allergy =Yes (n=925)		Parental Allergy =No (n=1170)	
	Cat Ownership (n=156)[4]	Cat Contact[3] (n=422)[5]	Cat Ownership (n=167)[4]	Cat Contact[3] (n=317)[5]
Cat Sensitization[1]	2.2(0.9,5.5)	2.8(1.3,6.0)	2.4(0.9,6.7)	1.51,(0.4,5.2)
Intra-cluster correlation coefficient (ICC)	37%	35%	5%	9%
Reference range of the probability of cat sensitization at baseline(i.e. at age 2)	8%	8%	3%	2%

[1]Specific IgE titer>0.35kU/L to cat allergen

[2]Adjusted for sex, study centre, and parental educational level

[3]Cat owners were excluded

[4]Ever had a cat as pet between birth and age 6

[5]Ever had regular contact with cat between birth and age 4

Table 4.13: Cat ownership and regular cat contact during childhood and the cat sensitization rate at age 2 and 6 (LISA study)

	Munich n/N(%)		Leipzig n/N(%)	
IgE Measurement available	at age 2	at age 6	at age 2	at age 6
Parents had asthma, eczema, or hay fever				
Yes	627/820(76%)	357/820(44%)	265/350(76%)	131/350(37%)
No	359/499(72%)	199/499(40%)	302/426(71%)	153/426(36%)
Parental Education level[1]				
Very high	681/903(75%)[3]	402/903(45%)[3]	274/356(77%)[3]	142/356(40%)[3]
High	173/217(80%)	88/217(41%)	129/168(77%)	64/168(38%)
Median and Low	143/215(67%)	71/215(33%)	183/280(65%)	84/280(30%)
Move in the first 2/6 years[2]				
Yes	276/358(77%)	258/570(45%)[3]	213/277(77%)[3]	144/379(38%)[3]
No	720/898(80%)	302/581(52%)	358/431(83%)	143/226(63%)

[1]The parental educational level was categorised based on the German educational system, which takes both the highest completed grade in school and vocational training into account[109]. The higher education level from either parent was taken

[2]The association between IgE measurement at age 2 and move in the first 2 years of life and the association between IgE measurement at age 6 and move in the first 6 years of life were assessed

[3]Chi-square test, p-value<0.05

Table 4.14: Demographic characteristics between children with and without IgE measurements by study centre (LISA study)

participation rates were statistically significant except in the Munich subgroup, those who reported hay fever symptoms at age 6 were more likely to participate in the IgE measurement at age 6 (59% vs. 50%, chi-square test, p=0.03).

The analysis results using the LISA study Munich and Leipzig subgroups showed a positive association between cat allergen exposure during infancy and the sensitization rate up to age 2 but not at age 6. There were no associations between cat allergen exposure in infancy and allergic symptoms and diseases up to age 6. Cumulative allergen exposure due to cat ownership and regular cat contact outside the domestic area during childhood were found to increase the risk of cat sensitization development at school age. The results also confirmed that age and family history of allergic disease are risk factors for the development of sensitization.

4.3 Domestic Cat Allergen Level and Allergic sensitization in Young Children-AIRALLERG Study

The results presented here have been presented in Chen et al. Domestic Cat Allergen Level and Allergic Sensitization in Young Children. Preliminary Accepted by ***Int J Hyg Environ Health****.*

Overall 15, 8, 34 and 49 children from the GINI, LISA, PIAMA, and BAMSE cohort respectively were sensitised to cat and these children were compared to the non-sensitised controls (table 4.15).

The cat allergen loads and concentrations measured from children's mattress and living room floor dust samples are summarized in table 4.16. The estimated correlations between surface allergen loads on mattress and living room floor were

	GINI(N=141) Germany(%)	LISA(N=75) Germany(%)	PIAMA(N=219) The Netherlands(%)	BAMSE(N=225) Sweden(%)
Sensitised to cat allergen[1]	n=15	n=8	n=34	n=49
Having cat as pet at home at dust sampling	10(7.1%)	7(9.3%)	50(22.8%)	21(9.3%)
Had a cat at age of blood sampling[2]	16(11.3%)	10(13.3%)	60(27.4%)	20(8.9%)
Had a cat but not anymore at age of blood sampling[2]	0(0.0%)	0(0.0%)	14(6.4%)	10(4.4%)
Never had a cat up to the age of blood sampling[2]	99(70.2%)	63(84.0%)	145(66.2%)	192(85.3%)
Age of blood sample collection (mean(95% C.I.))	3.0(3.0, 3.0)	2.0(2.0, 2.0)	4.0(4.0,4.1)	4.0(4.0, 4.0)

[1]Defined as having specific IgE titer >0.35 kU/L to cat allergen

[2]Information on cat ownership were available from 188, 219 and 222 children from German, Dutch and Swedish study population respectively

Table 4.15: Descriptions of the study populations of the four cohorts defined as children who had specific IgE titer > 0.35 kU/L to cat allergen and children who were not sensitized to any common allergens tested (controls)

between 0.4 (GINI and LISA) and 0.6 (BAMSE) and the correlations between allergen concentrations in dust from mattresses and living room floors were between 0.5 (GINI and LISA) and 0.7 (BAMSE).

The association between domestic cat allergen level and the prevalence of cat sensitization varied between the study populations. In the German study population (GINI and LISA cohort), higher level of domestic cat allergen was associated with higher prevalence of cat sensitization. In the Dutch study population (PIAMA cohort), no association was observed. In the Swedish study population (BAMSE cohort), cat allergen level was negatively associated with sensitization rate to cat dander (table 4.17). For the Dutch and Swedish study populations the estimated associations between sensitization and domestic cat allergen level did not differ when the amount of allergen was expressed in concentration in dust or surface allergen load. However, for the German study population, allergen levels expressed as concentration in dust showed a stronger association (OR (C.I.) =7.6 (2.1, 29.5)).

The amount of bacteria and mould components including bacterial endotoxin, ß(1→3) glucans, and fungal extracellular polysaccharides (EPS) in the settled house dust samples were quantified. Further adjusting for the bacteria and mould components individually in the model made no substantial change to the estimated associations between cat sensitization rate and domestic cat allergen level (table 4.18).

Sampling Site	Cat Allergen Level1	Germany GINI and LISA	The Netherlands PIAMA	Sweden BAMSE
Children's	ng/m^2	120(50 - 437)	305(88 - 1,888)	90(37 - 292)
Mattress	ng/g	475(174 - 1,708)	1,006(450 - 8,573)	626(274 - 1,848)
Living room	ng/m^2	101(13 - 424)	189(14 - 961)	141(42 - 438)
Floor	ng/g	408(155 - 1,369)	832(295 - 5,846)	392(179 - 1,180)

[1]Samples with cat allergen level under the detection limit were assigned with 2/3 of the lowest measured value

Table 4.16: Cat allergens levels in the three study centres (median, 25th, and 75th percentile)

Sampling Site	Germany OR(95%C.I)[1]	The Netherlands OR(95%C.I)[1]	Sweden OR(95%C.I)[1]
Children's Mattress	3.01(1.16, 7.99)	0.83(0.22, 2.93)	0.55 (0.22, 1.27)
Living room floor	1.64(0.62, 4.42)	1.61(0.67, 3.84)	0.41 (0.16, 0.98)

[1]Adjusted for gender, parental history of allergies, parental education, breast feeding, maternal smoking during pregnancy, mattress cover (Germany and the Netherlands) and cohort (Germany only). Odds Ratios expressed per inter-quartile range increase in level (table 4.16)

Table 4.17: The association between cat sensitization and log transformed cat allergen level (ng/m^2) measured from children's mattress and living room floor of the three study populations

Centre	Additional Adjustment	Sensitization to cat allergen	
		Mattress Cat Allergen(ng/m^2) OR(95%C.I)[1]	Floor Cat Allergen(ng/m^2) OR(95%C.I)[1]
Germany	Endotoxin	5.57(1.85,16.79)	2.31(0.74,7.26)
	$\beta(1 \rightarrow 3)$glucan	6.22(2.03,19.04)	2.82(0.89,8.89)
	EPS	4.77(1.62,14.06)	3.25(1.01,10.44)
The Netherlands	Endotoxin	0.81(0.21,3.06)	0.97(0.31,3.01)
	$\beta(1 \rightarrow 3)$glucan	0.86(0.22,3.43)	1.12(0.36,3.45)
	Eps	0.69(0.17,2.78)	0.98(0.31,3.05)
Sweden	Endotoxin	0.58(0.23,1.46)	0.54(0.19,1.54)
	$\beta(1 \rightarrow 3)$glucan	0.62(0.23,1.67)	0.61(0.22,1.67)
	EPS	0.54(0.21,1.35)	0.56(0.2,1.59)

[1]Adjusted for gender, parental history of allergies, parental education, breast feeding, maternal smoking during pregnancy, mattress cover (Germany and the Netherlands) and cohort (Germany only). Odds Ratios expressed per inter-quartile range increase in level (table 4.16)

Table 4.18: The association between cat sensitization and domestic cat allergen level (ng/m^2) of the three study populations with additional adjustment of the amount of bacteria and mould components in the dust

Sampling Site	Germany OR(95%C.I)[1]	The Netherlands OR(95%C.I)[2]	Sweden OR(95%C.I)[2]
Children's Mattress	2.83(0.62,13)	0.19(0.03,1.35)	0.19(0.03,1.01)
Living room floor	1.21(0.35,4.17)	0.78(0.23,2.71)	0.03(0,0.37)

[1]Crude OR. Odds Ratios expressed per inter-quartile range increase in level (table 4.16)

[2]Gender, parental history of allergies, parental education, breast feeding, maternal smoking during pregnancy, and mattress cover (the Netherlands) were adjusted. Odds Ratios expressed per inter-quartile range increase in level (table 4.16)

Table 4.19: The association between cat sensitization and domestic cat allergen level (ng/m^2) restricted to the children who have never moved since birth

In the German, Dutch and Swedish study populations 60%, 32%, and 51% of the children respectively have moved from their accommodation at birth. A sensitivity analysis was performed by restricting the study populations to those children who have never moved from their original accommodation until dust sampling. The negative associations observed between the amount of domestic cat allergen and cat sensitization rate in the Swedish study population was more pronounced. In the German study population, only 9 of the children who has never moved were sensitised to cat. Therefore, the crude odds ratios were calculated. In this small subgroup, there was no association between cat sensitization and domestic cat allergen level (table 4.19).

Genetically pre-disposed children are more likely to develop allergic disorders. Their parents, on the other hand, are more likely to perform allergen avoidance due to their own allergic symptoms. To overcome possible bias from selective avoidance of cat due to parental history of allergic diseases, a stratified analysis was performed. The definition of parental history of allergy in the German study population was if one of the parent ever had asthma, hay fever, or eczema. In the Dutch study population, the defination was if one of the parent ever had asthma, allergy to house dust (mite) or pets, or hay fever, and the defination in the Swedish study population was if one of the parent ever had asthma. Due to the small number in each stratum, simple logistic regression analyses were performed . The results showed that there were no association between domestic

cat allergen level and cat sensitization rate in the Dutch study population. In the German study population, the high-risk children who were living in an eviroment with higher surface load of cat allergen on the mattress were more likely to have cat sensitization (OR (C.I)=2.8 (1.0, 7.9)). In the Swedish study population, no association between cat sensitization rate in high-risk children and domestic cat allergen level was observed. However, for children whose parents did not have asthma, cat allergen load on the living room floor was negatively associated with cat sensitization rate (OR (C.I)=0.3(0.1, 0.9))

The associations between cat sensitization and cat ownership between children's birth up to the age when the children had their blood sampled were evaluated. The prevalence of cat keeping varied between countries, 12%, 34% and 13% of the families from the German, Dutch and Swedish study population respectively were cat owners. Fewer families with allergic parents kept cats. The differences were about 10% in the Dutch and Swedish study population and 5% in the German study population. Cat allergen levels increased significantly (Kruskal Wallis test $p<0.01$) from families who never had a cat, used to have a cat but not anymore at the age of blood sampling, and had a cat at the age of blood sampling. In the Dutch and the Swedish study population, the lowest prevalence of cat sensitization was found in children who had a cat at the age of blood sampling and the highest prevalence was found in those who used to have a cat but not anymore at the age of blood sampling (13% compared to 43% in the Dutch study population, 5% compared to 30% in the Swedish study population). In the German study population, children who had a cat at the age of blood sampling had higher prevalence of cat sensitization than those children whose families never had a cat (table 4.20). The Dutch study population was the only study population with information addressing reasons for removing the cat from home. Six (21.4%) of 28 families who removed their cat indicated that it was due to allergy in the family. Finally, we stratified each study population by cat keeping history (never had a cat, had a cat but not anymore at the age of blood sampling, and had a cat at the age of blood sampling). The stratified analysis showed that there was no association between domestic cat allergen level and cat sensitization in each stratum (table 4.21).

Cat ownership up to the age of blood sampling	Children who have developed cat sensitization			Children who have not developed sensitization		
	Germany N=23	The Netherlands N=34	Sweden N=49	Germany N=193	The Netherlands N=185	Sweden N=176
Never have had a cat[1]	13	20	45	149	125	147
Had cat in the past but not anymore at age of blood sampling[1]	0	6	3	0	8	7
Have a cat at the age of blood sampling[1]	8	8	1	18	52	19

[1]Information on cat ownership was available from 188, 219 and 222 children from German, Dutch and Swedish study population respectively

Table 4.20: Past cat ownership up to the age of blood sampling stratified by the sensitization outcome (sensitized to cat vs. control group)

	Sampling Site	Germany OR(95%C.I)[1]	The Netherlands OR(95%C.I)[1]	Sweden OR(95%C.I)[1]
Never have had a cat[2]	Children's Mattress	2.31(0.56,9.55)	0.42(0.04,4.17)	0.57(0.22,1.47)
	Living room floor	0.86(0.15,4.82)	1.08(0.34,3.48)	0.55(0.22,1.43)
Had cat in the past but not anymore at age of blood sampling[2]	Children's Mattress	-	0.12(0,6.82)	0.02(0,36.25)
	Living room floor	-	0.75(0.07,7.51)	0.26(0.01,6.45)
Have a cat at the age of blood sampling[2]	Children's Mattress	1.12(0.29,4.39)	0.52(0.06,4.83)	1.42(0.02,99.6)
	Living room floor	0.63(0.16,2.42)	2.9(0.58,14.59)	0.02(0,18.38)

[1]Crude OR, due to the small number of subjects in each stratum

[2]Information on cat ownership was available from 188, 219 and 222 children from German, Dutch and Swedish study population respectively

Table 4.21: The association between cat sensitization and domestic cat allergen level (ng/m^2) stratified by the history of pet ownership

This cross-sectional study showd a mixed picture of the association between the observed domestic cat allergen level and sensitization rate in children. The association varies between the three study centres. In the German study population we found a positive association between the observed amount of domestic cat allergen and cat sensitization rate. In the Swedish study population, a negative association was observed. No association was observed in the Dutch study

population. Looking into the family history of cat keeping, we found the lowest prevalence of cat sensitization in children who were cat owners at the age of blood sampling and the highest prevalence was found in those who have had a cat but not anymore at the age of blood sampling.

4.4 Associations between Dog Ownership and Dog Contact and the Development of Allergy in Young Children-LISA and GINI Study

The results presented here have been presented in Chen et al. Dog Ownership and Contact with Dogs during Childhood and Later Development of Allergy; Results of Combined German Birth Cohort Studies. Submitted.

Information of children's health outcomes and demographic characteristics is listed in (Table 4.22). Due to the study design, there were only 26% of the parents with a history of allergic diseases in the GINI non-interventional cohort but 90% in the GINI intervention study group. The rest 10% of the children in the GINI intervention study group have at least one sibling with a history of allergic diseases. The high genetic risk in the children from the GINI intervention study group explains the higher rate of sensitization to dog, mixed pollen, and inhalant allergens and of diagnosed asthma, atopic dermatitis, and allergic rhinitis. The prevalence of dog ownership between birth and age 1 were 10% in the LISA cohort, 10% in the GINI non-interventional cohort and 8.5% in the GINI interventional study group. However, at age 6, the prevalence of dog ownership was significantly different between the three study groups (p=0.02). Ten percent of the families in the LISA study had a dog, while 12% of the families in the GINI non-interventional groups owned a dog and 11% of the families in the GINI interventional study group are dog owners.

We investigated the associations between dog ownership and contact with dogs outside the domestic area and the allergic sensitization rate at age 6. Dog ownership during infancy, during early childhood, and at age 6 was not associated with dog sensitization (Table 4.23). However, dog ownership was associated with a significant lower rate of mixed pollen and inhalant allergen sensitization, particularly if the child's family had a dog in the child's first year of life. On the other hand, contact with dogs outside the domestic area without ownership was not associated with any of the sensitization outcomes. Further investigation showed that dog ownership and contact with dogs outside the domestic area during child-

hood had no effect on the development of doctor diagnosed allergic diseases or reported allergic symptoms up to age 6 (Table 4.24).

	LISA Study	GINI Non-Intervention	GINI Intervention	Total
	n/N(%)	n/N(%)	n/N(%)	n/N(%)
Sensitization to dog allergen at age 6	49/1193(4.1%)	43/945(4.6%)	64/1017(6.3%)	156/3155(4.9%)
Sensitization to mixed pollen at age 6	221/1193(18.5%)	182/945(19.3%)	238/1017(23.4%)	641/3155(20.3%)
Sensitization to inhalant allergens at age 6	318/1193(26.7%)	257/945(27.2%)	335/1017(32.9%)	910/3155(28.8%)
Doctor diagnosed allergic diseases				
Eczema at age 4	229/2362(9.7%)	181/2379(7.6%)	193/1598(12.1%)	603/6339(9.5%)
at age 5	212/2179(9.7%)	177/2175(8.1%)	196/1663(11.8%)	585/6017(9.7%)
at age 6	187/2171(8.6%)	156/2083(7.5%)	171/1632(10.5%)	514/5886(8.7%)
Allergic Rhinitis at age 4	56/2353(2.4%)	50/2378(2.1%)	66/1601(4.1%)	172/6332(2.7%)
at age 5	90/2174(4.1%)	66/2179(3%)	120/1659(7.2%)	276/6012(4.6%)
at age 6	126/2165(5.8%)	77/2085(3.7%)	137/1632(8.4%)	340/5882(5.8%)
Asthma at age 4	23/2349(1.0%)	20/2383(0.8%)	32/1597(2%)	75/6329(1.2%)
at age 5	48/2173(2.2%)	34/2176(1.6%)	49/1664(2.9%)	131/6013(2.2%)
at age 6	45/2169(2.1%)	34/2088(1.6%)	53/1634(3.2%)	132/5891(2.2%)
Study Centre				
Munich	1467/3097(47.4%)	1784/3739(47.7%)	1165/2252(51.7%)	4416/9088(48.6%)
Leipzig	976/3097(31.5%)	n.a.	n.a.	976/9088(10.7%)
Wesel	348/3097(11.2%)	1955/3739(52.3%)	1087/2252(48.3%)	3390/9088(37.3%)
Bad Honnef	306/3097(9.9%)	n.a.	n.a.	306/9088(3.4%)
Parents had asthma, eczema, or hay fever	1585/2857(55.5%)	940/3656(25.7%)	2006/2241(89.5%)	4531/8754(51.8%)
Parental Education level[1] High	1263/3060(41.3%)	1098/3706(29.6%)	859/2241(38.3%)	3220/9007(35.6%)
Medium	1191/3060(38.9%)	1193/3706(32.2%)	737/2241(32.9%)	3121/9007(34.7%)
Low	606/3060(19.8%)	1415/3706(38.2%)	645/2241(28.8%)	2666/9007(29.6%)
Environmental Tobacco Smoke at home up to age 6	1049/2427(43.2%)	1265/2425(52.2%)	845/1657(51%)	3070/6498(47.3%)
Sex Male	1586/3097(51.2%)	1441/2814(51.2%)	1173/2252(52.1%)	4200/8163(51.5%)
Female	1511/3097(48.8%)	1373/2814(48.8%)	1079/2252(47.9%)	3963/8163(48.6%)

[1]The parental educational level was categorised based on the German educational system, less, equal, and more than grade 10. The higher education level from either parent was taken

Table 4.22: Children's health outcomes and demographic characteristics

		Adjusted model(OR (C.I.))[1] Sensitization outcome at age 6[2]		
	Sensitization outcome at age 6[2]	dog	mixed pollen	inhalant allergen
LISA	Dog ownership at age 1	0.9(0.4,2.6)	0.6(0.1,1.2)	0.6(0.3,1.0)
	Dog ownership at age 6	1.0(0.2,2.8)	0.7(0.3,1.3)	0.8(0.4,1.3)
	Dog ownership up to age 6	0.5(0.2,1.4)	0.6(0.4, 1.0)	0.6(0.3,0.9)
GINI Non-I	Dog ownership at age 1	1.7(0,4.2)	0.5(0.8,1.1)	0.5(0.2,0.9)
	Dog ownership at age 6	1.9(0.6,4.1)	1.1(0.2,1.8)	0.8(0.5,1.3)
	Dog ownership up to age 6	1.9(0.8,3.9)	0.8(0.7,1.3)	0.8(0.5,1.2)
GINI IN	Dog ownership at age 1	0.7(0,1.9)	0.5(0.4, 1.0)	0.5(0.3,0.9)
	Dog ownership at age 6	1.2(0.2,2.7)	0.6(0.2,1.0)	0.6(0.3,0.9)
	Dog ownership up to age 6	1.0 (0.4,2.1)	0.5(0.3,0.9)	0.5(0.3,0.8)
Total[4]	Dog ownership at age 1	1.1(0.5,1.9)	0.6(0.4,0.8)	0.5(0.4,0.7)
	Dog ownership at age 6	1.4(0.8,2.3)	0.8(0.6,1.1)	0.7(0.5, 1.0)
	Dog ownership up to age 6	1.1(0.7,1.8)	0.7(0.5,0.9)	0.6(0.5,0.8)
LISA	Dog Contact only at age 1[3]	1.4(0.4,2.9)	1.0(0.4,1.5)	0.9(0.6,1.2)
	Dog Contact only up to age 6[3]	0.8(0.7,1.7)	0.9(0.7,1.3)	0.8(0.6,1.1)
GINI Non-I	Dog Contact only at age 1[3]	0.9(0.4,1.9)	0.8(0.3,1.3)	1.0 (0.7,1.4)
	Dog Contact only up to age 6[3]	0.7(0.4,1.8)	0.8(0.55,1.3)	1.2(0.8,1.8)
GINI IN	Dog Contact only at age 1[3]	0.9(0.4,1.7)	1.0 (0.6,1.5)	0.9(0.6,1.3)
	Dog Contact only up to age 6[3]	1.2(0.4,2.2)	0.9(0.7,1.3)	0.9(0.6,1.3)
Total[4]	Dog Contact only at age 1[3]	1.0(0.7,1.6)	1.0 (0.76,1.2)	0.9(0.7,1.1)
	Dog Contact only up to age 6[3]	0.9(0.6,1.4)	0.9(0.7,1.1)	0.9(0.8,1.1)

[1]Adjusted for sex, study centre, parental educational level, and parental allergy

[2]Specific IgE titer>0.35kU/L to tested allergen

[3]Dog owners were excluded

[4]Additionally adjusted for studies

Table 4.23: Dog ownership and dog contact during childhood and sensitization to dog, mixed pollen, and inhalant allergens at age 6

Dog ownership and dog contact in the first year of life							
		Adjusted mixed model (OR (C.I.))[1]					
		LISA Study		GINI Non-Intervention		GINI Intervention	
		Dog Ownership	Dog Contact only[5]	Dog Ownership	Dog Contact only[5]	Dog Ownership	Dog Contact only[5]
Doctor diagnosed	Asthma (4, 5, and 6 years)	1.5(0.6,3.9)	1(0.5,2.2)	2.1(0.9,5.0)	2.1(1.0,4.3)	0.9(0.4,2.2)	1.1(0.5,2.1)
allergic diseases	Allergic Rhinitis (4, 5, and 6 years)	0.5(0.2,1.2)	1.4(0.9,2.3)	0.9(0.4,2.0)	0.5(0.3,0.9)	0.8(0.4,1.6)	1.3(0.8,1.9)
	Eczema(4, 5,and 6 years)	0.6(0.4,1.1)	1.1(0.8,1.6)	1.2(0.8,1.9)	0.8(0.6,1.2)	1.0(0.6,1.6)	1.0(0.7,1.4)
Symptoms of	Asthma (4, 5, and 6 years)[2]	0.9(0.6,1.3)	1.3(1.0,1.8)	1.0(0.6,1.5)	0.9(0.7,1.3)	0.8(0.5,1.4)	1.0(0.7,1.5)
allergic diseases	Allergic Rhinitis (4, 5, and 6 years)[3]	1.1(0.8,1.6)	1.0(0.8,1.4)	1.4(0.9,2.0)	0.8(0.6,1.1)	0.7(0.4,1.1)	1.3(1.0,1.9)
	Eczema (4, 5,and 6 years)[4]	0.7(0.5,1.0)	1.1(0.8,1.4)	1.4(1.0,2)	0.8(0.6,1.1)	1.1(0.7,1.6)	1.0(0.7,1.3)
Dog ownership and dog contact in the first 4 / 6 year of life							
		Adjusted mixed model (OR (C.I.))[1]					
		LISA Study		GINI Non-Intervention		GINI Intervention	
		Dog Ownership	Dog Contact only[5]	Dog Ownership	Dog Contact only[5]	Dog Ownership	Dog Contact only[5]
Doctor diagnosed	Asthma (4, 5, and 6 years)	1.2(0.5,3-0)	0.8(0.4,1.5)	1.5(0.7,3.4)	1.3(0.5,3.2)	0.8(0.3,1.8)	1(0.5,2)
allergic diseases	Allergic Rhinitis (4, 5, and 6 years)	0.5(0.2,1.0)	1.3(0.9,2.1)	0.8(0.4,1.6)	1.2(0.7,2.0)	0.8(0.4,1.5)	1.1(0.7,1.7)
	Eczema (4, 5,and 6 years)	0.7(0.4,1.0)	1.0(0.8,1.4)	1.2(0.8,1.8)	1(0.7,1.5)	0.7(0.5,1.2)	1.0(0.7,1.4)
Symptoms of	Asthma (4, 5, and 6 years)[2]	1.0(0.7,1.4)	1.0(0.8,1.4)	1.1(0.8,1.5)	1.1(0.8,1.6)	1.2(0.8,1.8)	1.2(0.8,1.8)
allergic diseases	Allergic Rhinitis (4, 5, and 6 years)[3]	0.9(0.6,1.3)	1.1(0.8,1.4)	1.1(0.8,1.6)	1.1(0.8,1.49)	0.8(0.6,1.2)	1.2(0.8,1.6)
	Eczema (4, 5,and 6 years)[4]	0.7(0.5,1.0)	1.0(0.8,1.2)	1.4(1.0,1.9)	1.0(0.8,1.4)	0.9(0.6,1.3)	0.9(0.7,1.2)

[1]Adjusted for sex, study centre, parental educational level, and parental allergy

[2]Defined as wheezing sound in the chest

[3]Defined as sneezing, running nose, or nasal congestion without cold

[4]Defined as itchy rash that effected skin crease, face, neck, extremities, hands, or feet

[5]Dog owners were excluded

Table 4.24: Dog ownership and dog contact during childhood and development of allergic diseases and symptoms between age 4 and 6

The amount of the endotoxin were available from 2108 parents' mattress dust samples and 2093 children's mattress dust samples from 2166 families. All of the analysed dust samples contained endotoxin above the detection limit. The median (25th, 75th percentile) of the load and concentration of endotoxin measured from parents' and children's mattress dust were listed in table (4.1). The correlation between the amount of endotoxin in parents' and children's mattress dust samples were 0.4 (Figure 4.1). The amount of endotoxin in parents' and children's mattress dust samples were significantly different between families with and withouta dog in the household within the first year of child's life ($p<0.01$). However, further analysis showed that there were no associations between early childhood endotoxin exposure from mattress and sensitization to dog (OR (95% C.I.)= 1.01 (0.80, 1.26)), mixed pollen (OR (95% C.I.)= 1.07 (0.95, 1.19)), and inhalant allergens (OR (95% C.I.)= 1.08 (0.97, 1.19)) (when using the endotoxin loads sampled from children's mattresses as exposure). The associations between endotoxin exposure and sensitization outcome in homes with and without dog ownership in their child's first year of life were also assessed by including an interaction term of dog ownership in the first year and mattress endotoxin level in the model. The results showed that in homes with and without dog ownership, early childhood endotoxin exposure had no effect on the sensitization outcomes at age 6 (Table 4.25).

In a further subgroup analysis, we investigated whether the household density where the dog owners live confounds the observed protective effect of dog ownership. In the Munich subgroups of both GINI and LISA cohorts, information on household density of their home addresses were available. We observed that dog owners are more likely to live in areas with lower household density ($p<0.01$). Further adjusting for household density in the model, however, did not modify the negative effect of dog ownership on sensitization outcomes (OR (95% C.I.)= 0.53(0.24, 1.10) for pollen sensitization and OR (95% C.I.)= 0.51(0.26,0.92) for inhalant sensitization).

To overcome possible bias from selective avoidance of dog due to parental history of allergic diseases, a stratified analysis was performed. We stratified the cohort by whether the parents ever had asthma, eczema, or hay fever and re-analyzed

	Sensitization to inhalant allergens (OR(C.I.))[1]		Sensitization to mixed pollen (OR(C.I.))[1]	
Dog Ownership in the child's first year of life	No	Yes	No	Yes
Endotoxin Level measured from Parents' Mattress	0.8(0.1,4.6)	0.8(0.0,24.2)	0.3(0.0,2.2)	0.1(0,7.0)
Endotoxin Level measured from Children's Mattress	3.0(0.5,16.9)	4.7(0.1,154.3)	1.5(0.2,11.0)	1.1(0.0,63.2)

[1]Adjusted for sex, study centre, parental educational level, and parental allergy. Odds Ratios expressed per inter-quartile range increase in level (table 4.2)

Table 4.25: The association between inhalant sensitisation and log transformed endotoxin level (EU/m^2) measured from parents and children's mattresses stratified by dog ownership in the child's first year of life (LISA study)

the association between dog ownership and contact and the sensitization rate at age 6. The results confirmed that the negative association between dog ownership during childhood and sensitization to mixed pollen and inhalant allergens at age 6. The negative associations were slightly stronger in families with a history of allergic diseases (Table 4.26).

To overcome possible confounding effect from exposure to environmental tobacco smoking, we performed sensitivity analysis restricted to those children who have not been exposed to tobacco smoke at home up to age 6 (n=1752). The negative association between dog ownership during childhood and sensitization to mixed pollen (OR (95%C.I.)= 0.7(0.5, 1.0)) and inhalant allergens (OR (95%C.I) = 0.7(0.5, 1.0) at age 6 remained.

Furthermore, in both GINI and LISA study, not all children participated in the blood examination. We therefore cohmpared the demographic characteristics between children with and without IgE measurements (Table 4.27). We found that parents with higher educational level were more likely to agreed to participate in the IgE measurement, while parental history of allergic diseases was negatively associated with the participation of the IgE measurement. However, all children in the GINI interventional study group are with at least one parent or sibling

	Adjusted model(OR (C.I.))[1]					
	Sensitization to dog at age 6[2]		Sensitization to mixed pollen at age 6[2]		Sensitization to inhalant allergen at age 6[2]	
	Parental allergy		Parental allergy		Parental allergy	
	Yes	No	Yes	No	Yes	No
Dog ownership at age 1	0.7(0.2,1.5)	1.7(0.6,4.0)	0.5(0.3,0.8)	0.6(0.3,1.1)	0.4(0.3,0.7)	0.6(0.3,1.0)
Dog ownership at age 6	1.3(0.6,2.4)	1.7(0.7,3.8)	0.6(0.4,1)	1.0(0.6,1.6)	0.6(0.4,0.9)	0.8(0.5,1.2)
Dog ownership up to age 6	0.9(0.4,1.6)	1.6(0.7,3.5)	0.5(0.3,0.8)	0.8(0.5,1.2)	0.5(0.4,0.8)	0.7(0.5,1.0)
Dog Contact only at age 1[3]	1.1(0.6,1.7)	0.9(0.4,2.3)	1.0(0.7,1.3)	0.9(0.6,1.3)	1.0(0.7,1.3)	0.8(0.6,1.2)
Dog Contact only up to age 6[3]	1.0(0.6,1.6)	0.7(0.3,1.7)	0.9(0.7,1.2)	0.9(0.6,1.4)	0.9(0.7,1.2)	0.9(0.7,1.4)

[1]Adjusted for sex, study centre, parental educational level, and studies

[2]Specific IgE titer >0.35kU/L to tested allergen

[3]Dog owners were excluded

Table 4.26: Dog ownership and dog contact childhood and sensitization to dog, mixed pollen, and inhalant allergens at age 6 by parental history of allergic diseases (GINI and LISA study)

		Children who had IgE measurement at age 6	
		LISA n/N(%)	GINI n/N(%)
Dog ownership at age 6	Yes	105/211(50%)	216/446(48%)
	No	1083/1977(55%)	1739/3411(51%)
Parents had asthma, eczema,	Yes	460/1272(36%)[2]	766/2951(26%)[2]
or hay fever	No	655/1585(41%)	1179/2946(40%)
Parental Education level[1]	High	554/1263(44%)[2]	757/1957(39%)[2]
	Medium	446/1191(37%)	686/1930(36%)
	Low	1827606(30%)	517/2060(35%)
ETS at home up to age 6	Yes	396/1049(38%)[2]	780/2021(39%)[2]
	No	762/1378(55%)	1080/2050(53%)

[1]Chi-square test, p-value<0.05

Table 4.27: Demographic characteristics between children with and without IgE measurements at age 6 by study

having a history of allergic diseases.

The analysis results using the two German cohorts showed that dog ownership in early childhood was associated with a significant lower rate of mixed pollen and inhalant sensitization but not dog specific allergic sensitization. The negative associations remained when the data are stratified by parental history of allergic disease or restricted the analysis to those children who have never been exposed to ETS at home. Dog contact outside domestic area solely had no effect on the development of allergic sensitization. No association between dog ownership and dog contact and the development allergic symptoms and diseases were found. In homes with and without dog ownership, early childhood endotoxin exposure had no effect on the sensitization outcomes at age 6.

Chapter 5

Discussion

5.1 Cat Exposure and Allergy in Young Children

The influence of cat allergen exposure in early childhood on the subsequent development of sensitization and allergic symptoms and diseases is complex and controversial. Many conflicting and intriguing finding have been published in the last couple of years. The three epidemiological studies included in this thesis provided further evidence on the association between cat exposure during early childhood and the development of cat specific sensitization. However, due to the young age of the selected cohorts, we cannot determine whether exposure to cat during childhood is associated with the development of allergic disorders.

5.1.1 Domestic Cat Allergen and Endotoxin Distribution between Families with Different Socioeconomic Status

In order to assess the effect of cat allergen exposure during early childhood on the development of allergy, we first described the amount of indoor cat allergen and endotoxin exposure in families with different socioeconomic status. We observed

a negative association between the amount of indoor cat allergen and family SES, particularly in families who are not cat owners. We also found that the endotoxin loads and concentrations and the total amount of dust in infants and parents' mattresses do not vary between families of different social classes.

A few studies have explored the associations between family socioeconomic conditions and the amount of domestic bio-contaminants. Elevated domestic cat allergen has been associated with white families in the U.S. studies [111, 112], and low SES families in the German MAS cohort study and the Swedish cohort study in Stockholm and Uppsala [84, 113]. Finally, it has been observed that endotoxin concentrations in settled house dust in homes of public health professionals and their neighbours are lower than those samples collected from homes of low-income families in Denver; Colorado, U.S [114].

Our observation confirmed that the amount of domestic cat allergen is lower in the socially advantaged families, which is in line with the observations described in other European studies [84, 113]. In our study population, families of lower parental education were more likely to keep cats. However, the stratified analysis showed that families who are not cat owners mainly contributed to the negative association between the amount of indoor cat allergen and family SES. As described in the section 1.1.3, cat allergen is ubiquitous and constantly airborne. Exposure to cat allergen is passive, through direct or indirect contact with cat owners, cat allergen is widely spread in homes without cats. Therefore, living in a district of communities with high numbers of cat owners can increase the amount of cat allergen exposure at home.

However, the observed results in this study may be under representative to the true difference of the amount of domestic cat allergen and endotoxin exposure across families of different SES, since a high percentage of families with highly educated parents were recruited in the LISA study, Munich and Leipzig subgroups. Furthermore, it is common for epidemiological studies that only a few families of extremely high or low social classes agree to participate. The recruitment process and using self-administered questionnaires for collecting baseline information may have also caused higher refusal rate from the families of extreme socioeconomic conditions. In addition, the amount of cat allergen and endotoxin

were measured only from parents' and infants' mattresses, which cannot represent the overall domestic allergen exposure. As cat allergen and endotoxin are consistently airborne and ubiquitously distributed throughout accommodations [115], there may be wider differences between the amounts of bio-contaminant in families with different SES levels.

Our results showed that the correlations between bio-contaminant from the two sampling sites were poor. Infants' mattresses appeared to contain less amount of dust and the amounts of bio-contaminant in the dust were very low. This implied that when measuring childhood indoor bio-contaminants exposure, it is necessary to collect settled house dust samples from both parents' and child's mattress.

Finally, we have used parental educational level and family equivalent income as two independent indicators for the family SES. The impacts of these two socioeconomic factors are likely to vary across the populations with time. In our study population, the minimum age of the fathers was 19 years old, 26% of the families of both parents under 30 years old. It should be taken into account that these younger parents in our study population may still in the process of education or professional training, which indicates high educational but low or median income level. Our study investigated the association between SES conditions and the amount of domestic bio-contaminants and dust only at one point in time. It cannot be considered as the reflection of life long impact of SES on the environmental influence on health.

5.1.2 Associations between Cat Allergen Exposure and the Development of Allergy in Young Children-German Longitudinal Study

The associations between cat allergen exposure during early childhood and the development of allergy in young children were assessed using the LISA cohort, Munich and Leipzig subgroups. The results showed a positive association between cat allergen exposure during infancy and the sensitization rate up to age 2 but not at age 6. There were no associations between cat allergen exposure in infancy

and allergic symptoms and diseases up to age 6. Cumulative allergen exposure due to cat ownership and regular cat contact outside the domestic area during childhood were found to increase the risk of cat sensitization development at school age. The results also confirmed that age and family history of allergic disease are risk factors for the development of sensitization.

In the LISA study, Munich and Leipzig subgroups, high cat allergen exposure during infancy significantly increased the risk of cat sensitization at age 2 but the effect weakened when the time lag between exposure and health outcome became bigger. The German MAS Study has reported that cat allergen exposure during the first 2 years of life correlates with cat sensitization at age of 5 and 7 but not 10 years [73]. For older children domestic cat allergen is no longer the major source of exposure to cat allergen and exposure from community, school, and regular contact with cat from their friends and relatives may be more important for the later cat sensitization development [43, 116]. We also found that children who had regular cat contact but were not cat owners, particularly in the subgroup of children whose parents ever had asthma, eczema, or hay fever, had an increased risk of developing cat sensitization. Cumulative passive cat allergen exposure in later childhood may also partly explain the higher sensitization rate at preschool and school age. It should be acknowledged that sensitization is not a clinical symptom and our study cohort was still too young to assess the effect of cat allergen exposure on the development of allergic diseases. However, it has been reported by the large scaled cohort study that sensitization to perennial inhalant allergens in early childhood is highly associated with loss of lung function at school age [4]. Therefore, our study results could be interpreted as the initiation of allergic disease development, as allergic sensitized subjects are at higher risk of developing allergic disease.

Contradictory results in the association between cat allergen exposure and cat sensitization rate have been reported. Several cross-sectional studies have suggested that high level of cat allergen exposure is negatively associated with sensitization development [77, 96]. This protective effect may be due to the modified T helper cell type 2 (Th2) response that correspond to the production of IgG4 antibodies but not IgE when in contact with cat allergen [38]. There was, however, no

direct evidence showing that IgG4 antibodies directly mediate a protective effect [73, 77]. The observed phenomena in cross-sectional studies can also be explained by the selective avoidance effect [84, 78]. In the recruited families, the level of cat allergen concentration in the house dust samples was much lower than the sampled allergen concentration in the studies by Platts-Mills et al and Custovic et al [38, 79]. Therefore the dose of exposure might not be sufficient for the induction of the immune tolerance suggested by Platts-Mills et al [38]. However, in our study population, cat ownership was associated with the development of cat sensitization for children from families with or without history of allergic disease. This was a strong indication that even very high amount of cat allergen exposure is a risk factor for the development of cat sensitization and no evidence of the induction of immune tolerance were seen in our study. It has also been suggested that pet keeping may increase the indoor bacterial components such as endotoxin [82] which may down regulate infants' type 2 immune response [66]. However, our study showed that additional adjustment for endotoxin in domestic dust did not modify the associations between cat allergen exposure and cat sensitization.

The major difficulty in the interpretation of the results is that cat allergen is ubiquitous and none of the pet allergen exposure studies can precisely measure the overall allergen exposure of each individual. Particularly if the child spends time in indoor environment other than the family home such as day care centre or relatives' home. In our study, we used the fel d 1 levels in mattress at age 3 months as a surrogate of subjects' cat allergen exposure in infancy. Although infants tend to spend most of their time in bed, they may also be exposed to different levels of cat allergen on other furniture, carpet, in the air or even outdoor. Furthermore, the allergen level may change dramatically once the family gets a cat or moves to another accommodation where a cat used to live. Similarly, cat ownership and cat contact are also not the precise index of cat allergen exposure during childhood. In the section 4.1, we have demonstrated that the amount of domestic cat allergen exposure significantly varies between non-cat-owners' homes. Finally, as every longitudinal cohort study, we have lost participants during the follow-up and some participated in the IgE test at age 2 did not participated in the IgE test at age 6. We do not think that selective participation can explain the study

results since the sensitivity analysis showed that predisposed children and children with allergic disease or symptoms did not have a much higher participation rate. However, the results must be interpreted with caution.

Due to practical and economic reasons, we were not able to monitor allergen levels in our cohort's household over 6 years and had to use cat ownership and regular cat contact to approximate the allergen exposure during childhood although pet ownership has been considered as a poor surrogate for the specific allergen exposure [41, 117, 118]. Sensitivity analysis showed that cat ownership and regular cat contact outside the residence at age of three months and in the first 2 years of age statistically significantly increased the risk of cat sensitization at age 2. This was in line with the positive association between cat allergen exposure in infancy and cat sensitization rate in early childhood. On the other hand, the Dutch PIAMA study has shown that domestic cat allergen loads can be considered very stable over more than 4 years, as the ratio of within- to between-home variances is always below unity [119]. Therefore, the domestic cat allergen load collected when the children were 3 months old may be considered as an appropriate measurement for the exposure during early childhood.

5.1.3 Domestic Cat Allergen Level and Allergic sensitization in Young Children-European Cross-sectional Study

We further investigated the association between the observed domestic cat allergen level and allergic sensitization in young children using an European multicentre cross-sectional study. The results show a mixed picture of the relationship between cat allergen levels and cat sensitization, positive in Germany, absent in the Netherlands, and negative in Sweden.

The AIRALLERG study is composed of four distinct study populations in three European countries, which may contribute to the mixed results. The ages of children at blood sampling were different between the study populations. German children were relatively young compared to the Dutch and Swedish children,

which may have contributed to the positive association shown in the German study populations. The spectrum of allergic sensitization and symptoms changes with age. Sensitization to cat dander and other inhalant allergens becomes more frequent at preschool and school age. Inverse associations between cat allergen exposure and cat sensitization was primarily reported in studies on schoolchildren [120, 38] and adults [79] while positive associations were reported in studies of young children [95, 34, 88, 72]. The fact that older children have higher chance to be exposed to unmeasured cat allergen outside their home could also be associated with the mixed observations. As the results showed in the section 4.2, domestic cat allergen exposure is an important risk factor for the development of cat sensitization in early childhood. For children at school age, however, exposure to cat allergen outside the domestic area plays an important role [121]. The observed mixed associations between domestic cat allergen level and cat sensitization from this nested case-control study with health outcomes evaluated at different age provided further evidence that the effect of exposure to cat allergen from home plays different roles to children at different age.

The relationship between allergen exposure and subsequent sensitization development is complex, especially for cat allergens. As described in the previous section, a few studies have suggested that extremely high cat allergen exposure is associated with increased IgG antibody production, which may leads to immune tolerance [38, 79, 77]. The elevated IgE antibody level, on the other hand, has been observed in theses studies, only in subjects who were exposed to at intermediate cat allergen levels. Yet again, there was no direct evidence showing that IgG4 antibodies directly mediate a protective effect [77, 73]. We also investigated the suggested effect that pet keeping may increase the exposure to bacterial components such as endotoxin [82] which may down regulate the immune response [66]. However, our study results again showed that additional adjustment for bacteria and mould components in domestic dust did not modify the associations between cat sensitization and domestic cat allergen levels.

Cat avoidance may be a plausible explanation to the low prevalence of cat sensitization in subjects with the highest exposure. In the BAMSE birth cohort [84], a strong negative association between maternal pet allergy and pet ownership was

reported. Cat allergen levels in homes without cats also differed between families with and without maternal pet allergy. In the Globe Allergy and Asthma European Network (GA^2LEN), in which 12 European birth cohorts have been investigated using a cross-sectional study design, showed a significant lower frequency of cat ownership in families which at least one parents or siblings ever had a history of allergy, especially pet-related allergy (Eller et al, paper in progress). In the Dutch and Swedish AIRALLERG study populations, significantly fewer families with allergic parents ever had a cat at home. There was no difference in cat allergen levels between non-cat keeping families with or without allergic parents, and one would expect children from allergic parents are more likely to develop cat allergen sensitization when expose to equal amount of cat allergen compared to those non-predisposed children. Therefore, pet avoidance may contribute to the negative association between cat allergen levels and cat sensitization in the Swedish subjects.

In our study a significantly higher percentage of children whose family had kept a cat but not anymore at the age of blood sampling were sensitised to cat compared to those who never had a cat. These children had an intermediate sensitization rate, and those who had a cat at the time of blood sampling had the lowest sensitization rate. Similar patterns were observed in a Dutch questionnaire study in 1992 for respiratory and allergic symptoms [87]. It was also reported in the BAMSE birth cohort that cat ownership decreased from birth to two years of age even in families without parental history of allergic disease [84]. The high prevalence of cat sensitization among previous cat keepers may indicate a generally positive association between cat allergen exposure and sensitization and cats were obscured after the development of sensitization in children.

The community prevalence of cat also was different between the three study populations. A recent publication investigating the association between pet-keeping in childhood and adulthood allergic disease using the European Community Respiratory Health Survey (ECRHS) data suggested that the effect of cat keeping in childhood varies between communities with different prevalence of cat ownership [88]. The association between childhood cat keeping and asthma in sensitised adults was stronger in those who grew up in communities with low cat ownership

prevalence. Based on the data from the ECRHS, the prevalence of cat keeping in adults aged 20 to 44 years was 15% in Germany, 23% in the Netherlands and 19% in Sweden. In the AIRALLERG study, the prevalence of cat keeping in the German study population was 8%, 34% in the Netherlands, and 13% in the BAMSE study population. The significant difference in the community prevalence of cat ownership may have also contributed to the observed mixed results.

One last issue that needs to be considered is that in the AIRALLERG study, exposure was measured 2 to 3 years after sensitization. Study children were selected to have lived in the same home from at least 6 months before the measurement of sensitization. As described in the previous section that in the Dutch PIAMA study, it has been demonstrated that cat allergen levels in the home are very stable over long periods of time, as the ratio of within- to between-home variances was always below unity [119]. We therefore argue that although cat allergen was measured after sensitization; the allergen measurements provide a reasonable reflection of the levels that were present in the period immediately preceding the sensitization measurements.

The strengths of the AIRALLERG study are that the house dust sampling were highly standardised across the 3 study populations and the quantifications of the house dust components were conducted centrally in one laboratory. Furthermore, the additionally measured endotoxin, $\beta(1 \rightarrow 3)$ glucan, and extracellular polysaccharides (EPS) level provided us the chance to investigate the complex associations between multiple exposures to cat allergen and mold and bacteria components and the sensitization outcomes.

5.2 Dog ownership and Dog Contact and Allergy in Young Children

The association between dog ownership and dog contact and allergy in young children were examined using two German cohort studies. The results showed that dog ownership in early childhood was associated with a lower rate of mixed pollen and inhalant sensitization but not dog specific allergic sensitization. The

negative associations remained when the data are stratified by parental history of allergic disease or restricted the analysis to those children who have never been exposed to ETS at home. Dog contact outside domestic area solely had no effect on the development of allergic sensitization. No association between dog ownership and dog contact and the development allergic symptoms and diseases were found. In homes with and without dog ownership, early childhood endotoxin exposure had no effect on the sensitization outcomes at age 6.

Protective effects of dog ownership in childhood on the development of inhalant allergen sensitization in both low- and high-risk children have been reported [95, 76, 122, 88]. Cumulative evidence suggests that dog ownership in early childhood seems to prevent the development of allergy. Simultaneous exposure to endotoxin has been speculated as the explanation behind the observed negative association. Particularly due to a large number of studies which reported that children raised on farms where high level of endotoxin have been measured have a lower prevalence of hay fever and allergic sensitization [63, 62, 123, 98]. Laboratory experiments on mice have provided further evidence that endotoxin exposure induces cytokines which may shift the infants' developing immune system to a predominantly Th1 type responses that protect children from developing allergy [67, 68]. Gern et al have also observed the association between dog ownership and higher IL-10 and IL-13 cytokine secretion in 1 year old children [124]. However, the results from our study and the cohort study in Boston [94] showed that the negative association between dog ownership and allergic sensitization and symptoms are independent of the effect of endotoxin exposure.

It has also been speculated that the observed protective effect of dog ownership may be partly due to selective dog avoidance by atopic parents. It has been reported by the Swedish BAMSE study that dogs are less common in families with than in families without parental atopic eczema / dermatitis syndrome. However, the study has also reported that less dog avoidance behaviour were observed comparing to cat avoidance [84]. The recent publication from the European Community Respiratory Health Survey reported that selective avoidance subsequent to asthma or allergy was not observed for childhood dog keeping and adult dog acquisition [125]. In our study, the negative association between

dog ownership during childhood and sensitization to mixed pollen and inhalant allergens at age 6 was found in families with and without parental history of allergic disease. However, a stronger protective effect was observed in subjects with parental allergy. Since not all parents with a history of allergy also have pet especially dog allergy, we cannot rule out the possibility that dog avoidance may partly contributed to the observed protective effect.

In the 2 cohort studies, no associations between dog ownership and dog contact during childhood and the prevalence of allergic diseases and symptoms between age 4 and 6 were found. In the previous publication of the GINI study, however, a negative association between keeping a dog in the 1st year of life and the development of eczema in the 1st and the 2nd years of life was observed [126]. When children grow older, they are more likely to have frequent contact with multiple triggers for allergic disease and symptoms, the observed protective effect in infancy may therefore disappear when the children are in school age. In addition, one might conclude that dog ownership during the first year of life is associated with a lower risk of early onset eczema, but this association disappeared beyond age of 2 years.

Based on the current information, we were not able to establish how dog ownership leads to a lower atopy, especially the observation that dog ownership was associated with a lower prevalence of mix pollen and inhalant sensitization but not with sensitization to specific dog allergens. As we did not quantify the dog allergen Can f 1 level in the collected house dust samples, we could only use the information on dog ownership as a surrogate of dog allergen exposure. However, the observed protective effect is more likely due to other unknown factors associated with dog ownership. Dogs require more outdoor activities than most of the other pets. Dog fur is likely to carry wide range of microbes other than endotoxin from outdoor environment such as soil. Close contact with dogs at very young age may increase the exposure to variety of microbes and stimulate the maturation of the immune system. Keeping dogs also means a different life style that involves more outdoor activities, which may explain why such protective effect was not observed in children who only had regular contact with dogs outside the domestic area but were not dog owners. Furthermore, some families may have had their

dog before the birth of the child. It has been observed in laboratory experiment on mice that prenatal plus postnatal exposure to endotoxin is associated with a robust shift toward predominantly Th1 immune response [127]. This result corresponds to the finding that exposure to farm environment during pregnancy and the first year of life leads to a lower prevalence of allergy [59]. It has also been observed in the LISA study that exposure to high levels of endotoxin is negatively associated with cord blood IgE level [83]. Finally, Ownby et al have reported that exposure to only one dog is not sufficient to stimulate children's immune system. Exposure to 2 or more dogs or cats in the first year of life is associated with a lower risk of inhalant sensitization at age 7 [97]. Unfortunately, we do not have information on the number of pets in each household of our cohorts. Therefore, we are unable to replicate the analysis. The fact that more indoor pets is associated with higher level of endotoxin may indicate that the amount of endotoxin exposure in most of the homes with only one dog is not sufficient to cause a modification in the development of children's immune system. The household density where the dog owners live, on the other hand, had no effect on the negative associations between dog ownership and sensitization outcomes.

One difficulty in the interpretation of our results is that as every longitudinal cohort study, we have lost participants during the follow-up and some did not participate in the IgE test at age 6. Although parental history of allergic disease was negatively associated with the participation of the IgE measurement, the GINI non-interventional study arm, LISA study population, and GINI interventional study arm represented low, normal and high-risk children respectively. Furthermore, there was no difference between the dog ownership of the participants and non-participants. However, the results should be interpreted with caution.

5.3 Conclusion

The results of the four epidemiological studies included in this thesis show that:
1. Domestic cat allergen levels were higher in the in the homes of lower socioeconomic groups, particularly for those families who are not cat owners. The amount of endotoxin in the settled house dust and total amount of dust, on the other hand, were similarly distributed between families of different social classes.

2. Cat allergen exposure in infancy increases the risk of sensitization development in early childhood but not in school age. Cumulative allergen exposure from cat ownership and regular cat contact during childhood contribute to sensitization development up to school age.

3. When examining the association between the observed domestic cat allergen level and sensitization rate in children using a multi-centre cross-sectional study, we found a mixed picture. The associations were generally positive in Germany, negative in Sweden and absent in the Netherlands. However, for all three centres, we found that the highest prevalence of cat sensitization is amount children who have had a cat but not anymore at the age of blood sampling. Therefore, this mixed results may be explained by differences in age of the study populations and avoidance patterns.

4. The study results suggest that dog ownership in early childhood might be protective against the development of inhalant sensitization but not allergic symptoms and diseases up to age 6. This protective effect could not be attributed to the simultaneous exposure to endotoxin.

It is clear from our studies that cat and dog exposure during early childhood do not influence the development of childhood allergy in the same manner. Therefore, when discussing effective methods for allergy prevention, it is necessary to look at these two most popular pets separately. Cat allergen is ubiquitous and

constantly airborne. Our studies demonstrated that community is a major source of cat allergen exposure for non-cat-owner families, especially in communities of low SES. Further investigations on the effect of unequally distributed cat allergen between different socioeconomic groups on the prevalence of allergic sensitization are in need. The longitudinal LISA study further supports that cat allergen avoidance at home alone might be not effective to prevent the development of allergic sensitization in young children. As it shows that frequently expose to cat allergen outside the domestic area during early childhood is a significant risk factor for the development of allergic sensitization in school age children. Finally, by comparing study outcomes of a cross-sectional and a longitudinal study, we show that it is important to consider the study design when interpreting the conflicting study results on the influence of cat exposure in early life on the subsequent development of allergy. Differences in age and different avoidance behaviour of the study populations may have contributed to the mixed picture.

Exposure to dog, on the other hand, seems to have a protective effect, which was unlikely due to the exposure to dog allergen. The observed associations are less clear compared to the possitive associations observed between cat allergen exposure and cat sensitization. Unfortunately, we do not have data on the level of dog allergen during infancy, therefore, we cannot conclude from our study the effect of dog allergen exposure in infancy on the development of allergy. However, it is unlikely that exposure to high level of dog allergen in infancy protects children from developing allergic sensitization to pollen allergens. Unknown factors associated with dog ownership in early childhood protect children against the development of inhalant sensitization and this protective effect cannot be attributed to the simultaneous exposure to endotoxin. Therefore, further studies on the effect of wider range of perinatal microbial exposure through dog ownership are in need.

Chapter 6

Summary

Indoor environment has been associated with allergic diseases. The role of the exposure to cat and dog during early childhood in the development of allergy in young children is still debated. Exposure to cat allergen in early childhood has been observed as both risk and protective factor for development of sensitization and allergic symptoms and diseases in different epidemiological studies. The effect of dog ownership during early childhood, on the other hand, has only been studied in a few prospective cohorts.

The aim of this thesis is to assess the associations between cat/dog exposure during early childhood and the development of allergy in young children using epidemiological studies. We investigated the association between the observed domestic cat allergen level and the prevalence of cat sensitization and allergic symptoms and diseases in young children in both longitudinal and cross-sectional epidemiological studies. We also examined the associations between early childhood dog ownership and frequent contact with dogs and the development of allergy up to age 6.

The results of the four epidemiological studies included in this thesis show that domestic cat allergen levels were negatively associated with the families' socioeconomic status in non-cat-owners' home. Based on the data from a cohort study, we have also demonstrated that cat allergen exposure in infancy increases the risk of sensitization development in early childhood but not in school age. On the

other hand, cumulative allergen exposure from cat ownership and regular cat contact during childhood contribute to sensitization development up to school age. Interestingly, when examining the association between the observed domestic cat allergen level and sensitization rate in children using a multi-centre cross-sectional study, we found mixed associations. However, looking into the family history of cat keeping, we observed the lowest prevalence of cat sensitization in children who were cat owners at the age of blood sampling and the highest prevalence was found in those who have had a cat but not anymore at the age of blood sampling for all study centres. We also observed that dog ownership in early childhood might be protective against the development of inhalant sensitization but not allergic symptoms and diseases up to age 6. This protective effect could not be attributed to the simultaneous exposure to endotoxin.

Based on our findings, we can conclude that

1. When discussing effective methods for allergy prevention, it is necessary to look at these two most popular pets separately.
2. Cat allergen avoidance at home alone might be not effective to prevent the development of allergic sensitization in young children.
3. When comparing study results on the influence of cat exposure on the development of allergy, it is necessary to consider the study design. The mixed picture may be explained by differences in study design (cross-sectional vs. longitudinal cohort study), age, and avoidance patterns in different study population.
4. Further studies on the effect of wider range of perinatal microbial exposure through dog ownership are in need.

Chapter 7

Zusammenfassung

Innenraum-Faktoren sind bereits seit langem als wichtige Einflussfaktoren allergischer Erkrankungen bekannt. Die Rolle von Katzen- und Hundekontakt im frühen Kindesalter für die Entwicklung von Allergien wird immer noch kontrovers diskutiert. Der Einfluss einer Exposition zu Katzen-Allergenen im frühen Kindesalter wurde in zahlreichen epidemiologischen Studien untersucht. Katzen-Allergene konnten dabei sowohl als Risikofaktor als auch als protektiver Faktor für eine allergische Sensibilisierung oder die Entwicklung allergischer Symptome und Erkrankungen identifiziert werden. Der Einfluss der Hundehaltung bzw. des Kontaktes zu Hunden im frühen Kindesalter dagegen wurde bislang nur in wenigen prospektiven Studien untersucht.

Hauptthema dieser Doktorarbeit ist die Assoziation zwischen Katzen-/Hunde-Kontakt sowie der Katzenallergen-Konzentration im frühesten Kindesalter und der Entwicklung von Allergien. Dazu haben wir potentielle Assoziationen zwischen dem beobachteten Katzen-Allergen-Level und der Prävalenz von Katzen-Allergen-Sensibilisierung und allergischen Symptomen bzw. Erkrankungen sowohl in longitudinalen Follow-up-Studien als auch in Querschnittsstudien untersucht. Außerdem haben wir Assoziationen zwischen Hundehaltung bzw. regelmäßigem Kontakt mit Hunden und der Allergieentwicklung bis zum Alter von 6 Jahren untersucht.

Die Ergebnisse der vier epidemiologischen Studien, die in diese Doktorarbeit mit

einbezogen wurden zeigten, dass die häuslichen Katzen-Allergen-Konzentration im Hausstaub bei Familien ohne eigene Katzen negativ mit dem Sozialstatus assoziiert sind. Basierend auf den Daten einer Kohortenstudie konnten wir außerdem zeigen, dass eine Katzen-Allergen-Exposition im Kleinstkindalter die Sensibilisierungsrate im Kleinkindalter erhöht, jedoch nicht im Schulalter. Andererseits zeigt der kumulierte Effekt von Katzen-Allergen-Kontakt durch Katzenhaltung und regelmäßigen Kontakt zu Katzen im Kindesalter einen deutlichen Zusammenhang mit der Sensibilisierungsrate sogar bis ins Schulalter. Bei Untersuchung der Assoziationen zwischen Katzen-Allergen-Exposition und der Sensibilisierungsrate der Kinder in einer europäischen Multi-Center-Querschnitt-Studie fanden wir keine konsistenten Assoziationen. Wenn man jedoch die Katzenkontaktanamnese betrachtet, sieht man in allen Studienzentren die geringste Sensibilisierungsrate unter den Kindern, die zum Zeitpunkt der Blutentnahme selber Katzen besaßen und die höchste unter denen, die zwar vorher eine Katze besaßen, jedoch nicht mehr zum Zeitpunkt der Blutproben. Diese Assoziation wurde als Folge von Vermeidungsverhalten zur Katzenhaltung interpretiert. Hundebesitz im frühesten Kindesalter schützt möglicherweise vor der Entwicklung einer inhalativen Sensibilisierung, aber nicht vor dem Auftreten allergischer Symptome oder Erkrankungen bis zum Alter von 6 Jahren. Dieser potenzielle protektive Effekt kann nicht einer gleichzeitigen Endotoxin-Exposition zugeordnet werden.

Basierend auf unseren Ergebnissen lässt sich zusammenfassend folgern:

1. Für die Diskussion effektiver Maßnahmen für eine Allergieprävention müssen die zwei beliebtesten Haustiere (Katze und Hund) getrennt voneinander betrachtet werden.
2. Eine Meidung von Katzen-Allergenen im eigenen Haushalt ist nicht ausreichend, um eine allergische Sensibilisierung im Kleinkindalter zu vermeiden.
3. Wenn man die Ergebnisse verschiedener Studien zum Zusammenhang zwischen Katzenkontakt und der Entwicklung von Allergien vergleichen will, muss man das Studiendesign kritisch bedenken. Unklare Ergebnisse können beeinflusst sein vom Studiendesign selber (longitudinal, Querschnitt), aber auch durch Altersunterschiede und verschiedene Meidungsstrategien der unterschiedlichen Stu-

dienpopulationen.

4. Es sind noch weitere Studien nötig, um genauer perinatale mikrobiologische Expositionen durch Hundebesitz und deren Einfluss erörtern zu können.

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Hiermit erkläre ich, Chih-Mei Chen, dass ich die vorliegende Dissertation selbständig angefertigt habe. Ich habe mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind als solche kenntlich gemacht und nach ihrer Herkuft unter Bezeichnung der Fundstelle einzeln nachgewissen. Ich habe bisher noch keinen Promotionsversuch unternommen, und die vorliegende Dissertation wurde nicht in gleicher oder ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht

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