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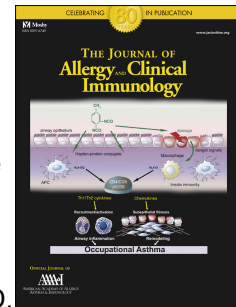
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Relevance of Specific IgE Antibody Titer to the Prevalence, Severity and Persistence of Asthma among 19-Year-Olds in Northern Sweden

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Abstract

Background: Although sensitization to indoor allergens is strongly associated with asthma, there are questions as to how this relates to asthma symptoms.

Objective: To study the relevance of IgE antibodies to cat and dog allergens in an area where i) the climate discourages cockroach, fungal and mite growth; and ii) dander allergens are known to be present in schools and houses without animals.

Methods: IgE to eight allergens was tested in 963 sera from a population based study on 19-year-olds, and associations with asthma symptoms, diagnosis, and treatment were examined. In positive sera, IgE to specific cat and dog allergens was also assayed.

Results: IgE specific for animal dander had the highest prevalence and the strongest relationship to asthma diagnosis. Furthermore, asthma severity, as judged by the frequency of symptoms and use of treatment, was directly associated with the titer of IgE antibodies to animal dander. Among the 103 subjects who had current asthma at age 19, 50 had asthma prior to age 12 years. Among those 50, the odds ratios for asthma related to any IgE antibodies to animal dander or high titer IgE antibodies (≥ 17.5 IU/mL) were 9.2 [4.9-17] and 13 [6.9-25], respectively. In multivariable analysis, IgE antibodies to Fel d 1 and Can f 5 were each associated with current asthma.

Conclusion: High titer IgE antibodies to cat and dog allergens were strongly associated with the diagnosis, severity and persistence of asthma; however, a large proportion of the subjects with current asthma did not live in a house with a cat or a dog.

Key Words: IgE titer, IgE antibodies, mammalian allergen, asthma severity, cat ownership

List of Abbreviations:

Ab Antibody/antibodies

Alpha-gal Galactose-alpha-1,3-galactose

ISAAC International Study on Asthma and Allergies in Childhood

OLIN Obstructive Lung Disease in Northern Sweden

Key Messages:

- In a community where, because of the climate, cat and dog derived allergens are the dominant perennial allergens, high titer IgE antibodies to dander allergens are strongly associated with the prevalence, severity and persistence of asthma.
- Asthma diagnosis after age 12 was, in many cases, no longer causing symptoms at age 19. In addition, many of these late onset cases were not sensitized and had low total IgE.
- A large majority of the cat sensitized individuals with current asthma did not live in a home with a cat and 48% had no cat or dog in the house. This observation is in keeping with the fact that animal dander allergens are present throughout the community, including schools and homes without a cat.

Capsule Summary:

Among students (n=963) living in a dry and cold climate, the presence of high titer IgE antibodies to cat and dog allergens, including Fel d 1, Can f 1, and Can f 5, had the strongest association with asthma prevalence and severity.

Introduction

Asthma in children over age 3 years and in young adults is strongly associated with sensitization to common inhalant allergens. This sensitization can be detected by skin tests or measurement of serum IgE antibodies (Ab). Generally in epidemiology and clinical studies, results from these tests have been analyzed as positive or negative rather than as a quantitative measure of risk (1-4). The allergens that are most strongly associated with asthma are perennial allergens, such as dust mite, cat, dog, cockroach, and *Alternaria* (5-8). Evaluating the relationship between sensitization and asthma is made complex by the fact that many patients are sensitized to more than one source of allergens (9-11). This can also make it difficult to evaluate either the effects of cat ownership or the modulating effects of specific IgG Ab on symptoms of asthma.

The northernmost province of Sweden, Norrbotten, reaches above the Arctic Circle, where the prolonged cold winters lead to dry indoor environments. As a result, houses do not become contaminated with dust mites (12-14). In addition, the climate is inhospitable to cockroaches, ticks, and fungi such as *Alternaria*. In keeping with the low levels of exposure, children raised in Norrbotten do not become allergic to many of the allergens that are strongly correlated with asthma in other climates (13-16). Following a large population of children through their primary and secondary school years, we have reported that sensitization identified either by a positive skin test or by the presence of measureable IgE Ab to cat and dog allergens were major risk factors for a physician diagnosis of asthma at age 12 years (13, 14). In addition, in keeping with other studies, we demonstrated that the presence of an animal in the home was not associated with increased prevalence or titer of IgE Ab to animal allergens (14).

The subjects in this cohort (n=3,430) were followed to age 19 years. Serum was collected from a representative sample of the cohort (n=963). We report here the results of serum IgE antibody assays to a range of inhalant allergens. In addition, we carried out studies on IgE Ab to components of cat and dog allergen extract, as well as assays of IgG and IgG4 antibodies to the major cat allergen, Fel d 1. The present study was designed to investigate the hypothesis that the titers of IgE to cat- and dog-derived allergens were significantly associated with the prevalence, persistence, and severity of asthma at age 19 years. Additionally, the analysis addressed the effects of cat ownership in an environment where most other perennial allergens are not relevant to asthma (14, 17-21).

Methods

Patient Population

Subjects were enrolled through the research program Obstructive Lung Disease in Northern Sweden (OLIN) in a prospective, population-based cohort, which has been ongoing since 1996 and includes data from 3,430 children (97% response rate) (12, 22). In 1996, all children in the first-grade and second-grade classes (age 7 and 8 years) in three municipalities in northern Sweden were invited to participate in a prospective study (23). The questionnaire used was based on the ISAAC design, with additional questions about asthma, rhinitis and eczema, and a broad screening for potential risk factors (12,15). The cohort was followed with repeated questionnaires until graduation from high school at age 19 years in 2006 or 2007. At age 19 years, 2,861 responded (83% of those enrolled in 1996). Both in 2006 and 2007, the follow up included a questionnaire, lung function test and skin prick test. In addition, in 2007 all participants in Kiruna and Lulea were invited to donate blood, and 963 participated (89% of those invited) (See Table E1 online for details of the population). The study was approved by the regional ethics committee of Umea University and the Human Investigation Committee at the University of Virginia.

ImmunoCAP IgE Assays

Total and specific IgE Ab were measured by using either commercially available ImmunoCAP (Phadia Thermo-Fisher, Portage, MI) or a modification of the assay with streptavidin on the solid phase (13, 24). The assays were performed with the ImmunoCAP 250 instrument, and the results were expressed as international units per milliliter (IU/mL), with the international unit both for specific and total IgE being approximately 2.4 ng. For specific assays, the standard cut-point for

a positive reaction was 0.35 IU/ml. The streptavidin CAP technique was used to measure IgE Ab to alpha-gal and the cat allergen Fel d 1, with approximately 1 µg of biotinylated antigen added to each CAP before adding 40 µl of serum (24). Sera were tested for IgE Ab to dust mite (*Dermatophagoides pteronyssinus*); cat dander, horse dander; both dog epithelium and dog dander; timothy grass; common silver birch; and *Alternaria*, as well as with ImmunoCAPs provided by Thermo Fisher for specific allergen components of cat and dog, including: the lipocalins Fel d 4, Can f 1, and Can f 2; the albumins Fel d 2 and Can f 3; and dog prostatic kallikrein Can f 5 (Table E2) (25-28).

Radioimmunoassays for IgG and IgG4 Ab to Fel d 1

Precipitation assays using radiolabeled Fel d 1 were carried out using established techniques to precipitate with either goat anti-IgG specific for all IgG subclasses or monoclonal anti-IgG4 followed by precipitation of the monoclonal antibody using goat anti-mouse IgG. The details of these assays have been published previously and are given in more detail in the online supplement (14, 18, 29, 30).

Statistical Analyses

Differences in the prevalence of specific IgE (≥ 0.35 IU/ml and ≥ 17.5 IU/ml) and pet ownership markers between groups were evaluated with univariable analysis using χ^2 or Fisher's exact test where appropriate and also presented as odds ratios with 95% confidence intervals. The outcome variables used were physician-diagnosed asthma, wheeze, and use of asthma medication. A multivariable binary logistic regression was used to analyze the relationship of multiple specific IgE sensitivities to physician diagnosis of asthma. Statistical analyses were performed using

115 Stata/IC 11 (StataCorp, College Station, TX) and GraphPad Prism 6 (GraphPad Software, La
116 Jolla, CA) and visualized using R version 3.0 and GraphPad Prism 6.

Results

Relevance of IgE antibody titer to prevalence, severity, and persistence of asthma

The prevalence of positive (≥ 0.35 IU/mL) or high titer IgE antibodies (≥ 17.5 IU/mL) to nine allergens was analyzed in relation to physician diagnosis of asthma at age 19 (Table 1). Using univariable (i.e., unadjusted) odds ratios, high titers of IgE to mammalian allergens and also to birch pollen were strongly associated with asthma ($p < 0.001$). The results for cat, dog, and horse showed extensive collinearity, and because of this we carried out logistic regression analysis for cat and dog allergens separately or for any mammal, in relation to the pollens (Table 2) (See also Table E7 online). The results are highly significant for dog or cat dander, or mammalian allergens, with consistently higher odds ratios for high titer IgE antibodies. For the three allergens with the strongest associations with asthma we also analyzed the effect of IgE Ab as a continuous variable (Fig 1). Those results show a strong relationship for either cat or dog dander and a weaker but still highly significant relationship for birch pollen, as well as a negative result for dust mite. In keeping with the results at age 11, only a few sera were positive for IgE to dust mite or *Alternaria* (14, 15) (Table 1 and Fig 1). The relationship between the titer of IgE Ab to animal dander and the severity of disease was also analyzed in relation to either frequency of symptoms (Fig 2A) or the use of treatment (Fig 2B). The results show a strong association between the titer of IgE Ab and either symptoms or treatment requirement at age 19. Similar results were seen with sensitization to cat or dog alone (Fig E1A-D).

Overall 79 (i.e., 54%) of the individuals who reported a physician diagnosis of asthma at age 19 were positive to one or more of the mammalian allergens tested. However, a surprisingly large number ($n=45$) were negative to all the allergens tested. We have previously reported on the

whole cohort that some of the students who received a diagnosis of asthma between age 11 and 19 had transient episodes only (12). To investigate this further we divided the subjects into persistent asthma, late onset asthma, and never asthma (See Table E1B) (Fig 3A). In addition, the results were analyzed for current asthma (i.e., individuals with a diagnosis of asthma who reported either use of treatment for, or symptoms of, asthma) in their 19th year (Fig 3B). For the 50 individuals with current asthma who had the diagnosis before age 11, 84% were sensitized to at least one allergen, 70% had IgE antibodies to a mammalian allergen, and 40% had high titer IgE antibodies to a mammalian allergen; odds ratios [95% confidence intervals] compared to subjects who never had an asthma diagnosis were 9.7 [4.5-21], 9.2 [4.9-17], and 13 [6.9-25], respectively (See Table E3 for details of groups).

The sera were also tested for total serum IgE, and the results show the expected relationship between total serum IgE and allergic asthma (Table E4). The geometric mean total IgE for IgE antibody negative subjects was low (18.1 IU/ml), and this value was not significantly different among IgE antibody negative subjects with a diagnosis of asthma. Among the subjects who had negative serum specific IgE ab assays, there were a few (n=17/576; 2.9%) who had total serum IgE ≥ 200 IU/ml. These sera were assayed for a wider range of allergens, but no significant positives were identified.

Relevance of Cat and/or Dog in the Home

In this cohort 279 subjects reported having a cat at home, 506 had a dog, 176 reported both, and 354 homes had neither animal. Cat ownership had a modest negative effect on the prevalence of IgE Ab to cat ($p < 0.05$) for IgE ≥ 0.35 IU/mL (Table E5). By contrast, reported dog ownership had no significant effect on IgE Ab to dog. Among the subjects with current asthma who were

sensitized to cat or dog (n=51), 50% were living in a home with no animal. Of the 46 cat sensitized subjects, 39 (85%) were living in a home without a cat and 22 (48%) reported no animals at home.

Using established techniques, IgG Ab to Fel d 1 were assayed in all 963 sera. The positive results (≥ 150 Units/mL) were analyzed as high titer (≥ 500 Units/ml) and low titer (150-500 Units/ml) (18). Cat ownership was associated with a high prevalence of both high and low titer IgG Ab ($p < 0.001$), as well as the modest decrease in IgE Ab (Fig 4). Surprisingly, the presence of IgG Ab showed no significant effect on symptoms of asthma when analyzed relative to different titers of IgE Ab (Fig E2A). When the titers of IgG Ab were analyzed relative to subjects requiring often or daily treatment, there was a lower prevalence in those with high titer IgG Ab, but the effect was not significant ($p = 0.12$) (Fig E2B). The sera that were positive for IgG to Fel d 1 were also assayed for IgG4 Ab (Fig E3). Those results correlated with IgG to Fel d 1 ($r = 0.40$, $p < 0.001$), and in keeping with that showed a strong association with cat ownership. The IgG4 Ab were also not a significant predictor of symptoms, and were equally present in sera with or without IgE Ab to cat (Fig E3).

Component Analysis for IgE Specific for Cat and Dog Allergens

Over the last few years, several allergens derived from cat or dog have become available to assay specific IgE Ab (See Table E2 and www.allergen.org). Where sufficient serum was available, the cat and dog positives were assayed for relevant components. The results were first evaluated on a matrix of intercorrelations for their correlation with IgE to other components and dander extracts (Fig 5). These results show correlations as high as 0.90 for Fel d 1 and cat, or 0.87 for Can f 1 and dog; or as low as 0.03 for Can f 5 and cat. The results for IgE specific components

183 were also evaluated for their relationship to asthma. Strong associations were found for Can f 1,
184 Can f 2 and Can f 5, as well as for Fel d 1 and Fel d 4 (Table 4A). The three components with
185 the strongest relationship to asthma were Can f 1, Can f 5, and Fel d 1. Strikingly, Fel d 1 and
186 Can f 5 showed little to no correlation with each other (Fig 5). Using multivariable analysis, the
187 odds ratios for these two allergens indicated that they had a strong independent association with
188 asthma: 4.8 (2.4-9.4) for Fel d 1 and 7.7 (2.2-27) for Can f 5 (Table 4B).

Discussion

It is now clear that there are populations of children and young adults living in westernized or post-hygiene societies, where the allergen sensitization associated with asthma is dominated by dust mite, cockroach, *Alternaria*, or, as in the present cohort, animal dander (2, 4, 6-8, 18). In each of these populations, the specificity of the sensitization associated with asthma correlates with the known presence of the relevant allergen in the community. However, the simple view that exposure in the child's home can predict sensitization is no longer tenable (31). In order to further understand the relationship between sensitization and asthma we studied IgE ab in sera from 963 19-year-old subjects who were part of a long-term population-based cohort living in an area where mite, cockroach, and *Alternaria* are not relevant. We report here that:

- i) The titer of IgE ab to cat and dog allergens was an important risk factor for both prevalence and severity of asthma;
- ii) Current cat owners have both lower prevalence of IgE ab and higher titers and prevalence of IgG ab to Fel d 1;
- iii) Sensitization to the specific proteins Fel d 1, Fel d 4, Can f 1, Can f 2, and Can f 5 was strongly associated with asthma, and in multivariable analysis Fel d 1 and Can f 5 showed strong independent relationships to asthma;
- iv) Sensitization to mammalian dander allergens showed the strongest association to asthma that had been diagnosed before age 12 and for individuals still symptomatic at age 19;
- v) A large proportion of the cases diagnosed for the first time between age 12 and 19 were not sensitized to any allergen.

Following the original report from Hesselmar and Bjorksten in 1998 that children raised in a house with a cat were less likely to be allergic to cats, there have been extensive publications related to this phenomenon (17, 32, 33). Many reports have confirmed the original observation, but with significant differences between the effects of cat and dog ownership (18, 20, 34). Several studies have not found a protective effect of cat ownership or have implied that the observed results could be explained by allergic families choosing not to own animals (35, 36). Two meta-analyses on the effects of pet ownership came to slightly different conclusions but neither found evidence that cat ownership would increase the risk of cat sensitization or asthma (32, 33). It is well established that cat allergen is present in schools as well as in homes without a cat (37-39). Moreover, recent evidence suggests that the quantity of allergen in homes without a cat or in schools is a function of the proportion of homes with an animal in the community (31, 39, 40). Interestingly, if some allergic families choose not to own a cat, this would only cause an increase in sensitization among families without a cat, if their children were still just as likely to become sensitized to cat allergens. In previous studies of the same cohort, at age 12 years, we found that 80% of the cat allergic children had never lived in a house with a cat (37), and new onset of allergic sensitization was not related to having a cat at home (41). In the present study, 39 out of 46 (85%) cat sensitized individuals with current asthma were not living in a house with a cat, and 22 of them had neither a cat nor dog at home. The implication is clearly that sufficient exposure to induce symptoms is occurring in homes without a cat, in schools, or in other public places. Equally it suggests that cat or dog sensitization should be considered relevant in patients even if they do not have an animal at home.

Several long term cohorts studying asthma have found that a large proportion of the cases developed the disease early in life (42). In addition it is generally found that allergic

sensitization is an important factor favoring persistence of the condition (22). On the other hand, there are cases that present in the teenage years, and there is also good evidence for an increase in the proportion of female cases at this age (12, 43). In at least one study the cases that presented around puberty were less allergic (44). In our cohort, despite a significant increase in the number of cases between age 12 and 19 there was only a modest change in the ratio of female to male cases. The 63 cases who had a diagnosis of asthma at age 19, but had not had that at age 12 had a strikingly lower prevalence of sensitization than those with persistent asthma. This was particularly clear for those who were no longer symptomatic at age 19: 16 out of 25 (64%) had no sensitization (Table 3).

When it first became possible to monitor exposure to dust mite allergens, it was generally assumed that the child's exposure was primarily in their own home (7, 45). Several developments over the last 15 years have shed doubt on the simple relationship. Firstly, it is clear that the highest exposure to cat or dog allergens, i.e., an animal in the house, is not associated with higher prevalence of sensitization or with higher titers of IgE ab. Secondly, with increased awareness of the relevance of mite allergen some European countries have had a decrease in the range of mite exposure levels. Thirdly, an important mite avoidance study in Manchester, UK found that despite careful avoidance measures in the children's homes they still became sensitized to mite (46). What matters here is that the subjects in the present study did not become sensitized to mite allergens. The logical explanation for the results is that the children can become sensitized to perennial allergens from exposure to both mite and cat allergens outside their own house. The contrast between Manchester UK and Norrbotten is that in Norrbotten none of the schools or other houses in the community would have mite allergens (14, 22).

In the original criteria for assessing the evidence for the causality of a dust exposure in a lung disease, a dose response relationship between exposure and the disease was an important factor (9, 47). However, those criteria were written about inorganic dusts where individual sensitization was not considered to be relevant. It is now clear that the relationship between individual exposure and sensitization is too complex to use the individual's home exposure as a surrogate for overall exposure. Furthermore, it is increasingly likely that the presence of an animal, particularly an outdoor dog, can further lower the risk of sensitization of the child (48, 49). Thus, we consider that sensitization has to be the primary characteristic used to examine the relationship between allergens and asthma.

Using stepwise logistic regression analyses, we further examined the relevance of IgE ab to components and extracts as risk factors for different asthma outcomes [See Table E7 online]. The results were strikingly different using the older dog epithelium assay (e2) compared to dog dander (e5). We believe this result confirms our view that IgE responses to different mammals are collinear, and that the real conclusion is that the asthma risk in Norrbotten is dominated by IgE ab to mammalian allergens [see Table 2]. In particular, this relates to the role of high titer IgE ab to mammalian allergens, and the risk of current persistent asthma.

There are several limitations to the present study. Firstly, we do not have details on animal ownership from birth, and we did not sample all the houses in this cohort. However, it is increasingly clear that measurements of allergen in dust from an individual's home alone cannot be taken as an adequate model of exposure. Secondly, there have been significant changes in the extracts used for *in vitro* testing of sensitization over the period of this study. Indeed the dog dander assay (e5) gave twice as many positive results as the earlier dog epithelium assay (e2) (See Table 1). Thirdly, there are more component assays available today than there were as

recently as five years ago. Nonetheless, neither the individual component assays nor the microchip assays can provide a complete analysis of sensitization to the extract. However, a large population based cohort with high participation rate and the longitudinal study design contribute to the validity of the results. Furthermore, the diagnosis of asthma in the cohort has been validated and showed high specificity (50).

In conclusion, the presence of a cat or dog in the house was not a significant predictor of sensitization or the risk of asthma in a geographic area where animal dander dominated the sensitization to perennial allergens. Nonetheless, IgE Ab, and particularly high titer IgE Ab was strongly associated with the prevalence, severity and persistence of asthma. Among the individuals with asthma who had been diagnosed before age 12 and were still symptomatic at age 19, high titer IgE Ab to cat or dog allergen was associated with asthma with an odds ratio of 13 [6.9-25]. Furthermore, 85% of the individuals in this group did not have a cat at home and 48% had neither cat nor dog. The results are in keeping with a model where the allergens of cat and dog are present throughout the community at levels sufficient to contribute to both sensitization and asthma symptoms. Equally, the results strongly support a direct role for IgE Ab in asthma morbidity.

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Figure Legends

Fig 1. Probability of wheeze related to the titer of IgE antibodies specific for cat dander, dog dander, dust mite, or birch pollen. Unadjusted logistic regression lines are depicted with 95% C.I. in gray. $P<0.001$, $P<0.001$, $P=0.17$, $P<0.001$, for cat dander, dog dander, dust mite and birch pollen, respectively.

Fig 2. Relationship between IgE antibodies to any mammal and asthma severity assessed by frequency of wheezing attacks [A] or medication usage [B]. The number of subjects in each of the seven groups shown was: 716 negative; and 30, 63, 80, 40, 15, and 19 ≥ 100 IU/mL. Using chi square test for trend the p value was <0.001 for wheezing attacks or medication usage.

Fig 3. Relationship between sensitization and asthma diagnosis among subjects with persistent asthma ($n=68$); late onset asthma ($n=63$), and no reported asthma ($n=672$) [A]. The same analysis for subjects with current asthma which was persistent ($n=50$) or late onset ($n=38$), or no reported asthma ($n=672$) [B]. For each group the prevalence of sensitization is shown for any allergen (≥ 0.35 IU/mL), any mammalian allergen, high titer mammalian allergen (≥ 17.5 IU/mL), or no sensitization.

Fig 4. Relationship between IgG specific for Fel d 1 and cat ownership Both low titer (150-500 Units/mL) and high titer (>500 Units/mL) were significantly high among cat owners ($n=279$) (** $p<0.01$). By contrast, any IgE to cat dander was less common among cat owners (* $p<0.05$). The approximate range of cat allergen exposure is shown for cat owners and non-cat owners ($n=684$) (See Ref 37).

Table 1. Univariable analysis of asthma based on physician diagnosis for different levels of IgE antibodies to inhalant allergens.

Allergen	IgE Titer by Class in Students with/without [§] Asthma [^]			Odds Ratios for Asthma (Based on Physician Diagnosis)		
	Negative	Class 1-3	Class 4-6	Any IgE Ab >0.35 IU/ml (≥Class 1)	IgE Ab 0.36-17.5 IU/ml (Class 1-3)	IgE Ab >17.5 IU/ml (Class 4-6)
Cat Dander	80/664	46/119	23/31	3.8 [2.6-5.5]***	3.2 [2.1-4.8]***	4.6 [2.6-8.2]***
Dog Dander	83/689	45/115	21/10	4.4 [3.0-6.4]***	3.2 [2.1-4.9]***	13.2 [6.1-28.7]***
Dog Epithelium	116/763	26/46	7/5	4.3 [2.6-6.9]***	3.7 [2.2-6.2]***	8.0 [2.5-25.5]***
Horse	101/720	35/81	13/13	3.6 [2.4-5.5]***	3.1 [2.0-4.8]***	5.9 [2.7-13.0]***
Any Mammal	70/646	45/128	34/40	4.3 [3.0-6.2]***	3.2 [2.1-4.9]***	5.7 [3.5-9.4]***
Dust Mite	137/751	12/63	0/0	1.0 [0.5-2.0], p=0.88	1.0 [0.5-2.0], p=0.88	-
<i>Alternaria</i>	145/803	4/11	0/0	2.0 [0.6-6.4], p=0.27	2.0 [0.6-6.4], p=0.27	-
Birch tree	89/661	40/107	20/46	2.9 [2.0-4.2]***	2.8 [1.8-4.2]***	2.6 [1.5-4.5]***
Timothy grass	97/647	37/115	15/52	2.1 [1.4-3.0]***	2.1 [1.4-3.3]***	1.6 [0.9-3.0], p=0.10

Footnotes:

§ The numbers before and after the slash indicate students with and without asthma, respectively

[^] As indicated by a physician diagnosis of asthma

*** p<0.001

Table 2. Multivariable binary logistic regression of the relationship of physician diagnosis of asthma to IgE antibodies to: cat dander, grass pollen, and birch pollen [A]; dog dander, grass pollen, and birch pollen [B]; any mammal and both pollens [C]; and any mammal and any pollen [D].

A.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Cat Dander	3.0 (1.9-4.7)***	3.9 (2.2-7.1)***
Grass Pollen	0.93 (0.57-1.5)	1.2 (0.61-2.3)
Birch Pollen	1.7 (1.0-2.9)*	1.8 (0.99-3.4)
B.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Dog Dander	3.5 (2.2-5.6)***	11.4 (5.2-25.1)***
Grass Pollen	0.98 (0.60-1.6)	1.2 (0.59-2.3)
Birch Pollen	1.5 (0.89-2.5)	1.8 (0.95-3.4)
C.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Any Mammal	3.7 (2.4-5.8)***	5.1 (3.0-8.6)***
Grass Pollen	0.91 (0.56-1.5)	1.1 (0.56-2.1)
Birch Pollen	1.4 (0.86-2.4)	1.6 (0.85-3.0)
D.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Any Mammal	3.8 (2.4-5.8)***	4.9 (2.9-8.1)***
Any Pollen	1.3 (0.83-2.0)	1.8 (1.1-3.0)*

Footnotes:

* p<0.05

*** p<0.001

Table 3. Prevalence and effect of IgE antibody titer among subject with persistent or late onset current asthma in relation to reported medication use.

A. Persistent Current Asthma (n=50)								
Medication Use:	Never / Sometimes			Often / Daily			Total	% Female
IgE Sensitivity	N=	Class 1-3	Class 4-6	N=	Class 1-3	Class 4-6		
None	5	0	0	3	0	0	8*	50%
Mammal[‡]	17	10	7	20	7	13	37*	54%
Non-Mammal Only	4	1	3	1	1	0	5	20%

B. Late Onset Current Asthma (n=38)								
Medication Use:	Never / Sometimes			Often / Daily			Total	% Female
IgE Sensitivity	N=	Class 1-3	Class 4-6	N=	Class 1-3	Class 4-6		
None	7	0	0	7	0	0	14*	79%
Mammal[‡]	10	8	2	6	2	4	16*	56%
Non-Mammal Only	4	4	0	4	2	2	8	38%

Footnotes:

**Prevalence of non-allergic subjects:* The ratio of non-allergic subjects to subjects with IgE to any mammalian allergen for persistent current asthma was 8:37 and for late onset current asthma was 14:16 ($p<0.01$). The ratio non-allergic subjects to subjects with IgE to any allergen for persistent current asthma was 8:42 and for late onset current asthma was 14:24 ($p=0.05$).

[‡]*Effect of titer of IgE antibodies on severity:* The ratio of subjects with low titer (Class 1-3) to high titer (Class 4-6) IgE to mammalian allergen among subject reporting no medication use and some use of medication was 18:9, whereas the ratio for those reporting frequent or daily medication use was 9:17 ($p<0.05$). The ratio of subjects with low titer (Class 1-3) to high titer (Class 4-6) IgE to any allergen among subject reporting no medication use and some use of medication is 23:12, whereas the ratio for those reporting frequent or daily medication use is 12:19 ($p<0.05$).

Table 4. IgE antibodies to cat- and dog-related components: univariable analysis of asthma based on current asthma for different levels of IgE antibodies to allergen components of cat and dog dander [A], and multivariable binary logistic regression of the relationship of current asthma to IgE antibodies to: Fel d 1 and Can f 1 (I), Fel d 1 and Can f 2 (II) and Fel d 1 and Can f 5 (III) [B].

A.

IgE Titer by Class in Students with Current/No ^s Asthma				Odds Ratios for Asthma (Based on Current Asthma)	
Allergen	Negative	Class 1-3	Class 4-6	Any IgE Ab >0.35 IU/ml (≥Class 1)	IgE Ab >17.5 IU/ml (Class 4-6)
Fel d 1	51/677	33/104	19/29	5.2 (3.4-8.0)***	8.7 (4.6-17)***^
Fel d 2	89/785	6/12	2/2	5.0 (2.1-12)**^	8.8 (1.2-63)^
Fel d 4	75/746	19/48	5/4	4.6 (2.7-7.9)***	12 (3.3-47)***^
Fel d 5w	41/99	0/1	0/0	---	---

IgE Titer by Class in Students with Current/No ^s Asthma				Odds Ratios for Asthma (Based on Current Asthma)	
Allergen	Negative	Class 1-3	Class 4-6	Any IgE Ab >0.35 IU/ml (≥Class 1)	IgE Ab >17.5 IU/ml (Class 4-6)
Can f 1	75/770	12/31	12/5	6.8 (3.9-12)***	25 (8.5-72)***^
Can f 2	85/787	7/18	6/1	6.3 (3.0-13)***^	55 (6.5-461)***^
Can f 3	89/787	3/9	5/4	5.9 (2.3-15)***^	15 (3.5-63)***^
Can f 5	68/741	23/61	8/4	5.2 (3.2-8.5)***	21 (6.4-74)***^

B.

I.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Fel d 1	3.3 (2.0-5.6)***	3.2 (1.5-7.0)**
Can f 1	2.7 (1.4-5.2)**	11 (3.2-35)***
II.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Fel d 1	4.0 (2.5-6.5)***	4.8 (2.4-9.5)***
Can f 2	2.4 (1.1-5.3)*	24 (2.6-225)**
III.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Fel d 1	3.3 (1.9-5.6)***	4.8 (2.4-9.4)***
Can f 5	2.2 (1.2-4.0)**	7.7 (2.2-27)***

Footnotes for Tables 4A & 4B:

§The numbers before and after the slash indicate students with current asthma and without asthma at age 19 (*i.e.*, no current asthma), respectively

* $p < 0.05$

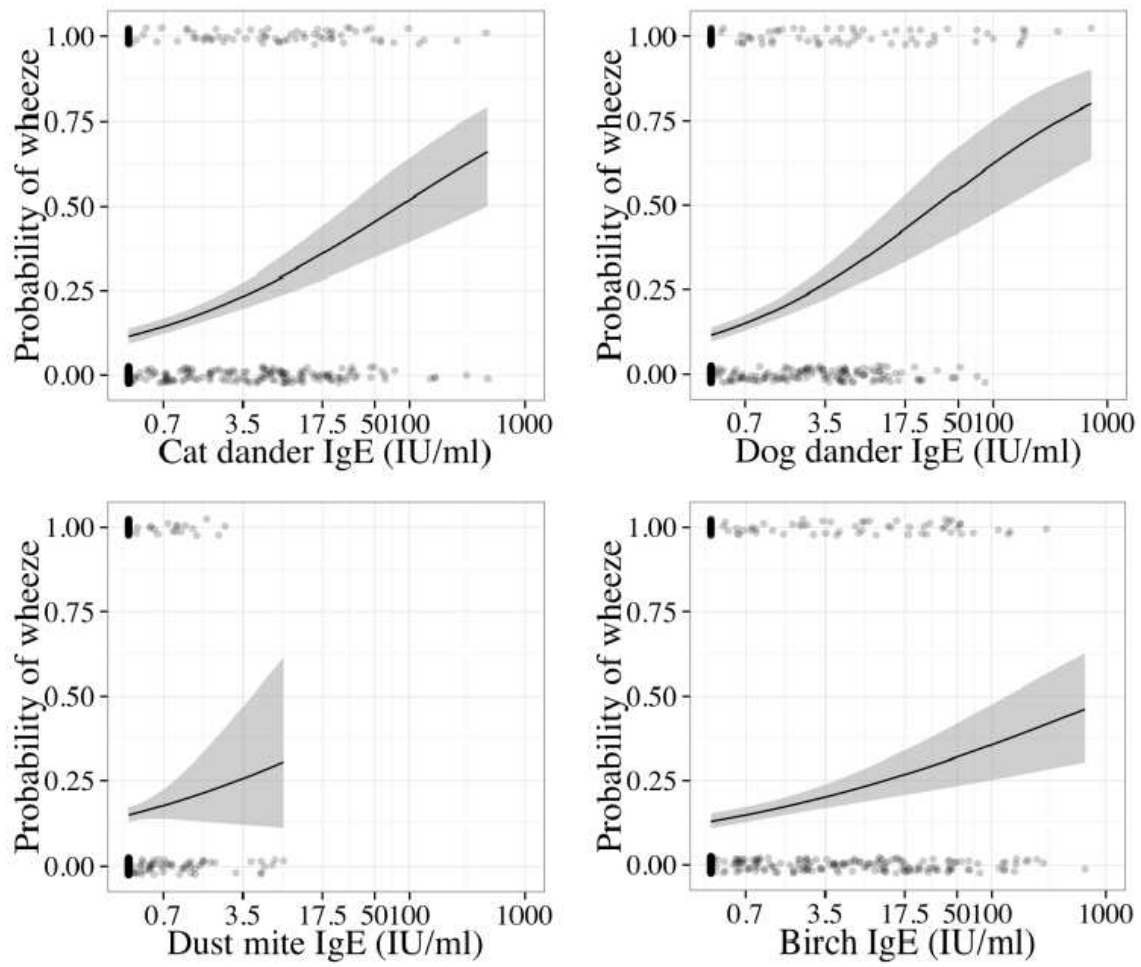
** $p < 0.01$

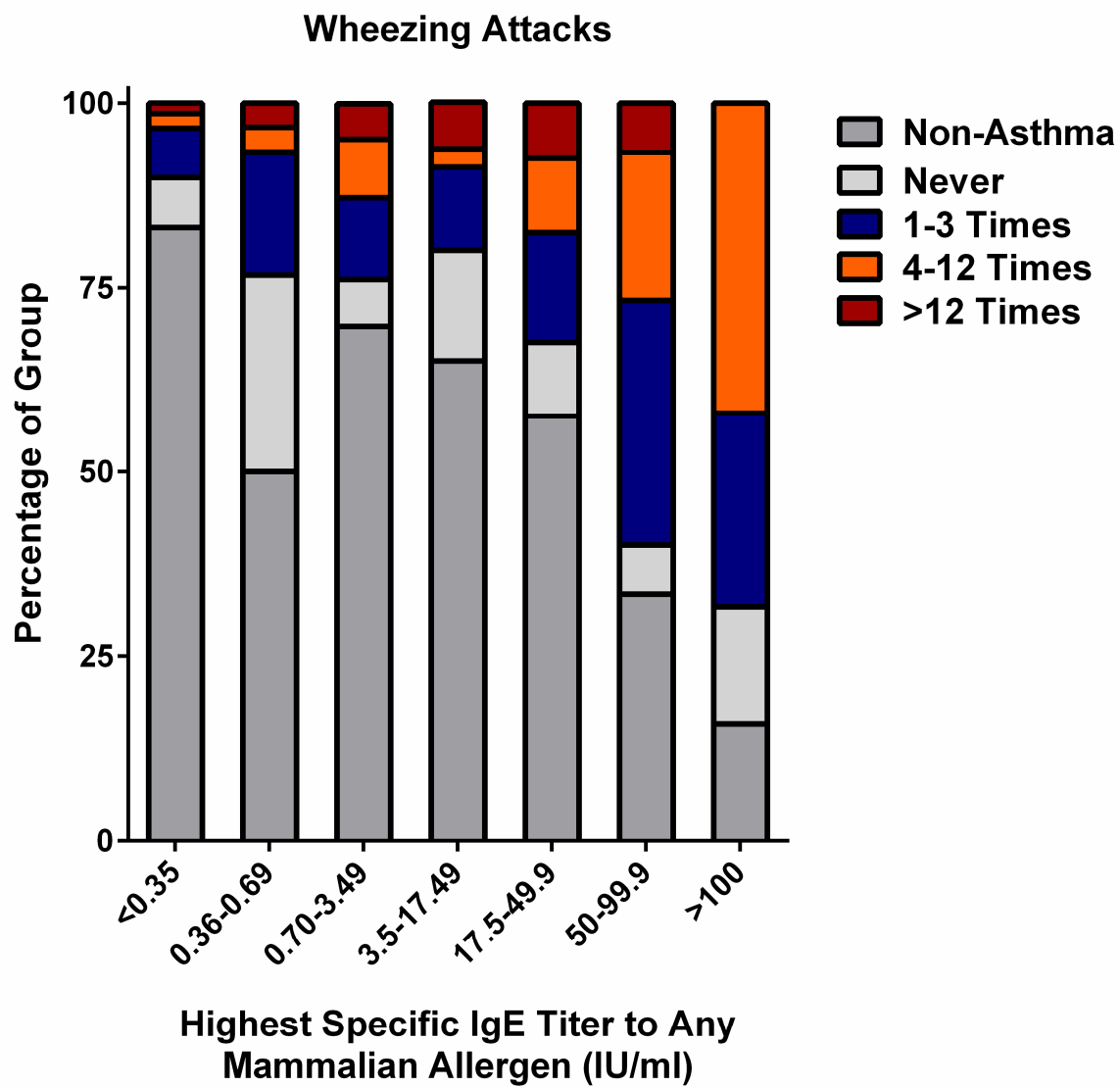
*** $p < 0.001$

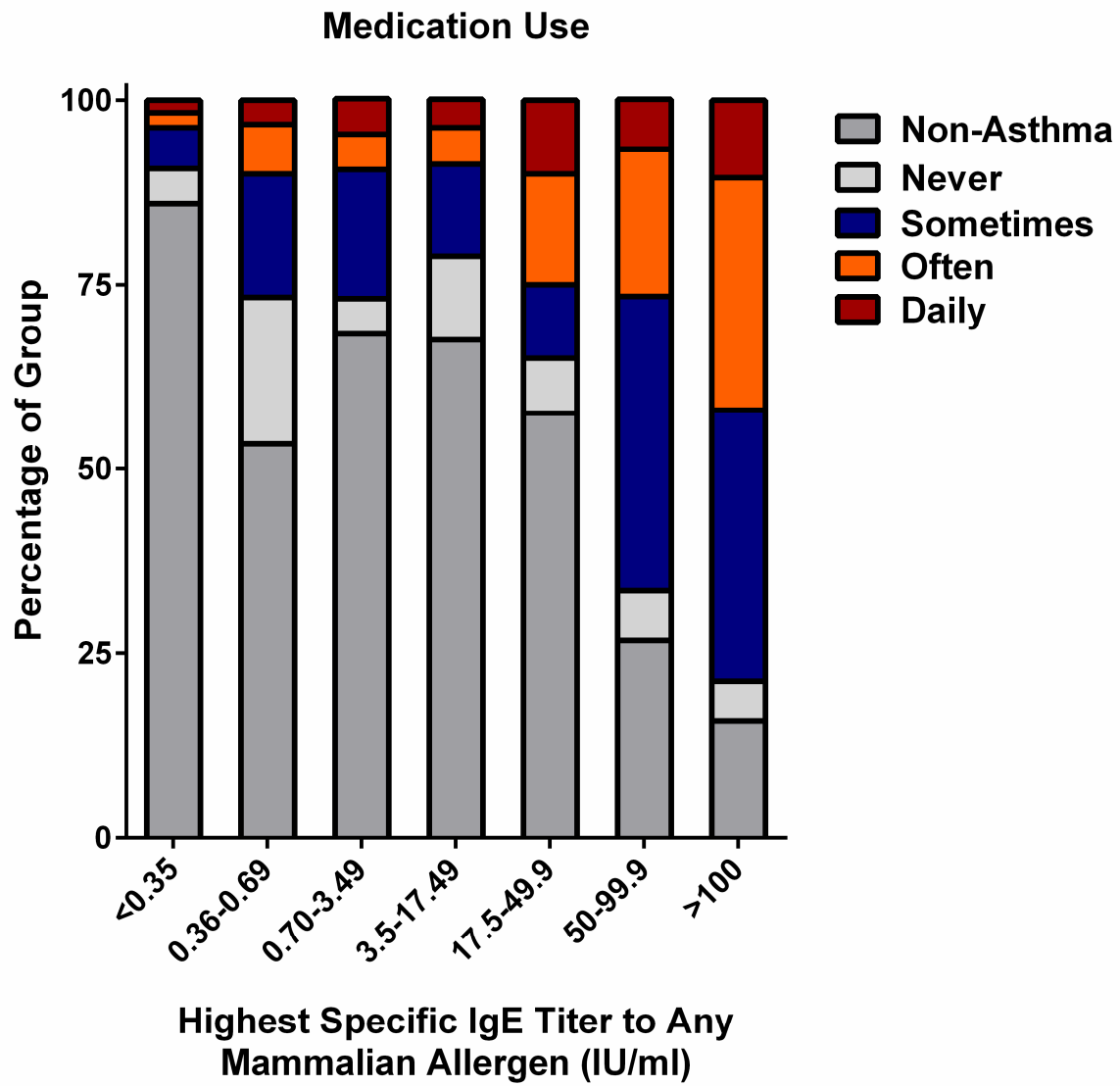
^Statistical analysis of odds ratios performed with Fisher's exact test

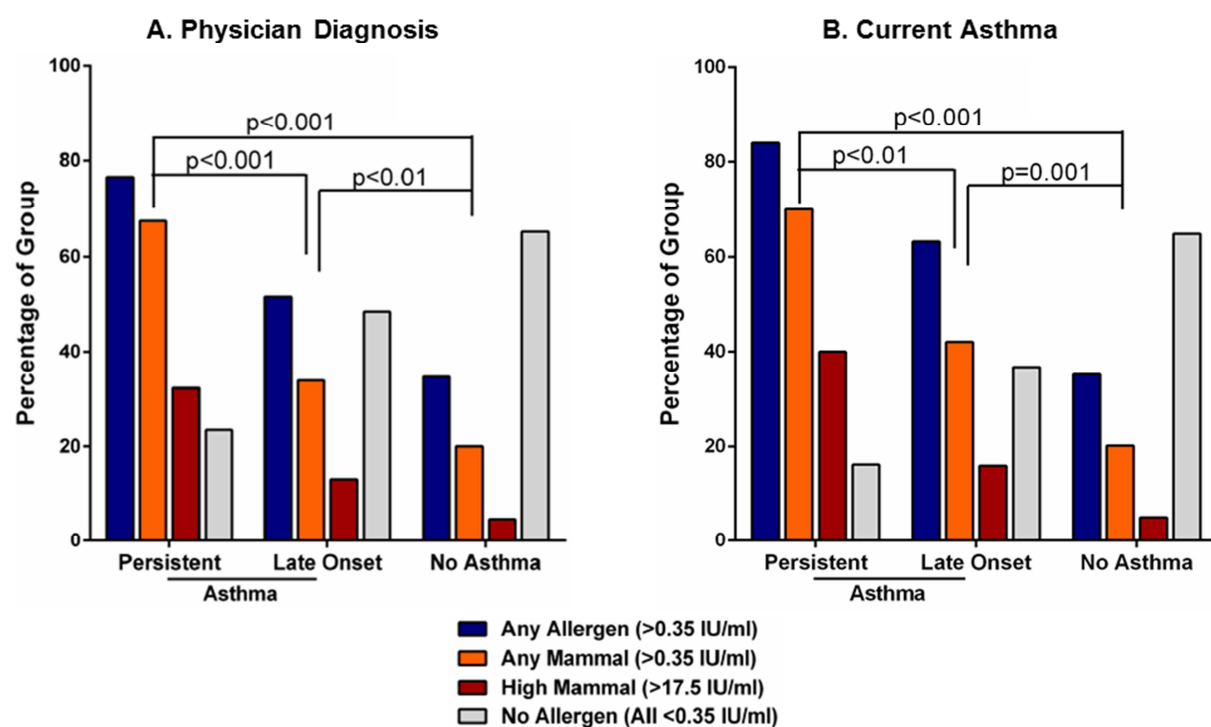
Table 5. A matrix of intercorrelations [with 95% confidence intervals] that was calculated from a cross-tabulation of serum IgE variables, including specific IgE antibodies to cat and dog danders and the representative allergen components and that shows the degree of correlation between the serum IgE variables

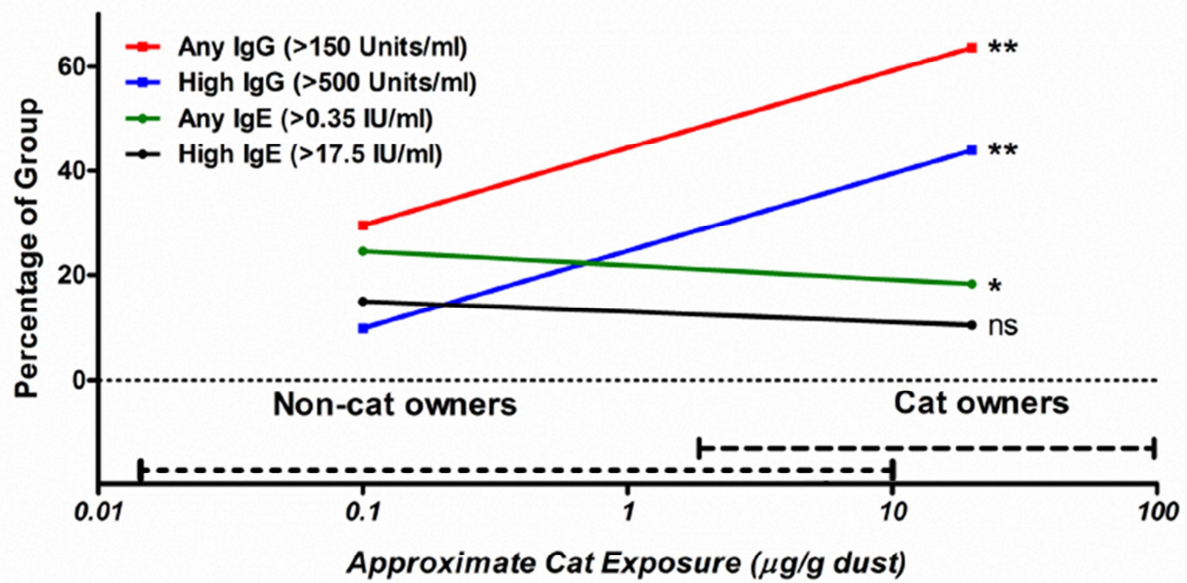
	Dog Dander	Can f 1	Can f 2	Can f 3	Can f 5	Fel d 1	Fel d 2	Fel d 4	Cat Dander
Dog Dander	1.00	--	--	--	--	--	--	--	--
Can f 1	0.80 [0.74-0.85]	1.00	--	--	--	--	--	--	--
Can f 2	0.86 [0.82-0.89]	0.87 [0.83-0.90]	1.00	--	--	--	--	--	--
Can f 3	0.31	0.29	0.26	1.00	--	--	--	--	--
Can f 5	0.57 [0.47-0.68]	0.45	0.48	0.15	1.00	--	--	--	--
Fel d 1	0.15	0.15	0.06	0.22	0.05	1.00	--	--	--
Fel d 2	0.08	0.12	0.06	0.76 [0.69-0.81]	0.02	0.26	1.00	--	--
Fel d 4	0.20	0.37	0.17	0.23	0.05	0.33	0.19	1.00	--
Cat Dander	0.12	0.15	0.03	0.33	0.02	0.94 [0.92-0.95]	0.46	0.40	1.00











Supplementary Material for:

Relevance of Specific IgE Antibody Titer to the Prevalence, Severity and Persistence of Asthma among 19-year-Olds in Northern Sweden

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Contents:

- I. Definitions of Population Studied (Tables E1A, E1B)
- II. Specificity of Assays used for IgE antibodies (Table E2)
- III. Methods for IgG and IgG4 assays.
- IV. Additional statistical analysis of specific IgE to dog and cat-derived allergens
- V. Additional Tables in relation to results.
- VI. Figures E1, E2 and E3 with Legends.

Definitions and Details of the Cohort: For further details see Tables E1A and E1B

Asthma: a report of physician – diagnosed asthma.

Late onset Asthma: Asthma at age 19 but not at age 7 or 12 (see Table E1).

Persistent Asthma: Asthma at age 7, 11 and 19 or at age 11 and 19.

Current Asthma: Asthma diagnosis at age 19 with concurrent symptoms or medication use.

Never Asthma: No report of asthma at age 7, 12 or 19 years.

Wheeze: Reported wheezing during the last 12 months. Number of attacks was also reported.

Asthma Medication: Use of asthma medication during the last 12 months, frequency of use was also reported.

Details of Methods for IgG and IgG4 Radioimmunoprecipitation assays:

Purified natural Fel d 1 (Indoor Biotechnologies, Charlottesville, VA) was radiolabeled with ¹²⁵I using the Chloramine T technique. The assays were carried out using established techniques (14,

18, 25, 26). Briefly, for IgG, the serum was diluted and incubated with radiolabeled Fel d 1 for 3 hours. The IgG was subsequently precipitated using goat anti-human IgG overnight. Sera with positive levels of IgG to Fel d 1 (>150 Units/ml) were also assayed for IgG4 specific for Fel d 1. For IgG4, the assay involved initial incubation of the serum dilutions with radiolabeled Fel d 1 for 2 hours, followed by the addition of a monoclonal mouse anti-human IgG4 Ab (clone HP-6023; Sigma Aldrich, St. Louis, MO) and normal mouse serum (Chemicon International, Temecula, CA). Finally, the mouse IgG was precipitated using goat anti-mouse IgG (Chemicon International, Temecula, CA). The anti-mouse IgG was absorbed repeatedly over a human IgG column to remove antibodies cross reactive with human IgG. In each assay, the precipitates were washed three times and counted with a gamma counter; these assays were carried out in parallel with a control curve to give unitage (14, 18, 25, 26). The unit for IgG Ab has previously been estimated to represent 0.1 ng of Fel d 1 bound (18). For the IgG4 assays, 50 sera, which were negative for IgG Ab to Fel d 1 were assayed and all were negative. The background was set at >2 times the standard deviation of the radioactivity bound with sera that were negative in the IgG assay.

Details on Additional Statistical Analysis of Specific IgE to Dog and Cat-Derived Allergens

A matrix of intercorrelation was developed in order evaluate the degree of correlation between independent serum IgE variables, including: dog dander, Can f 1, Can f 2, Can f 3, Can f 5, cat dander, Fel d 1, Fel d 2, and Fel d 4. This analysis returns intercorrelation values between 0 (i.e., statistically dissimilar) and 1 (i.e., statistically similar); variables with correlation coefficients greater than 0.5 were considered to lack sufficient independence to be included as independent variables in the same logistic regression model.

Using results from assays of IgE to both extracts and components we carried out multivariable stepwise logistic regression analyses, including cat and dog as separate variables. In keeping with the known cross-reactivity between lipocalins, albumins and other mammalian proteins there was clear evidence for interaction between cat and dog. Thus, the analyses were different using the results from the more recently introduced dog dander assay (e5) compared to the results with dog epithelium (e2) [Tables E7B and E7C]. In multivariable analysis IgE antibodies to cat dander were highly significant compared to dog epithelium as predictors of physician diagnosis of asthma. By contrast, dog dander was highly significant compared to cat in the same analysis. In addition, the results were different in analysis of these relationships with current asthma or current persistent asthma [Tables E7E, E7F and E7I]. Using multivariable analysis with the component results, we observed strong results with Fel d 1, Can f 1 and Can f 5 [Tables E7A, E7D, E7G, E7H and E7J]. In keeping with the results shown in Table 5, we found strong association with both Fel d 1 and Can f 5 ($p < 0.001$ in each case) for the analysis of for high titer IgE ab and current persistent asthma [see Table E7J].

Our conclusion is that the relationships between the mammalian allergens cat, dog and horse are too close for simple evaluation. The results are strongly influenced by different forms of the extract assays, by the nature of the component assays used, and by the asthma classification used.

Legends to Online Figures

Fig E1: Relationship of the titers of IgE Ab to cat dander (e1) [A and B] or dog dander (e5) [C and D] to the frequency of wheezing attacks [A and C] or medication usage [B and D].

Fig E2: The percent of all subjects with active wheezing (as judged by treatment requirement) [A], or only those subjects who reported the use of asthma medication “often” or “daily” [B], in relation to both IgE Ab to cat and the presence of low titer IgG Ab to Fel d 1 [150-500 units/ml] and high titer IgG Ab [≥ 500 IU/ml]. In each category the cat owners are indicated..

Fig E3: Serum IgG4 Ab to Fel d 1 in IgE Ab negative and IgE Ab positive subjects were assayed in sera where the IgG Ab assays were positive. Values are shown for cat owners [triangles] and non-cat owners [circles]. Wheezing subjects are shown as red symbols. The numbers of subjects shown include those of the IgG negative sera that were not assayed. In the IgE positive group and the IgE negative group, the odds ratios for a positive IgG4 assay with cat ownership were 18 (7.9-41) and 17 (6.3-48), respectively ($p < 0.001$ in each case).

Table E1. Cohort details: population used for primary analysis, including symptoms and treatment [A]; and population used for analysis of persistent versus late onset asthma [B].

A.

Group	Definition	N=	Physician Diagnosis of Asthma at Age 19
Total Population	Serum and full questionnaire data at age 19	963	Yes or No
Asthma	POSITIVE answer to asthma diagnosis at age 19	146	Yes
No Asthma	NEGATIVE answer to asthma diagnosis at age 19	817	No
Current Asthma	POSITIVE answer to asthma diagnosis at age 19 with POSITIVE answer to wheeze/medication use in 19 th year	103	

B.

Group	Definition	N=	Physician Diagnosis of Asthma			
			N'=	Age 7	Age 11	Age 19
Persistent Asthma*	POSITIVE answer to asthma diagnosis at ages 7 and/or 11, and POSITIVE answer to asthma diagnosis at age 19	68	30	Yes	Yes	Yes
			9	Yes	No Response	Yes
			29	No	Yes	Yes
Late Onset Asthma*	NEGATIVE answer to asthma diagnosis at ages 7 or 11, and POSITIVE answer to asthma diagnosis at age 19 only	63	62	No	No	Yes
			1	No Response	No	Yes
Ambiguous Asthma	Either persistent asthma or late onset asthma phenotype	15	14	No	No Response	Yes
			1	No Response	No Response	Yes
Recurring Asthma	POSITIVE answer to asthma diagnosis at age 7 and at age 19, and NEGATIVE answer to asthma diagnosis at age 11	4	4	Yes	No	Yes
Resolved Asthma	POSITIVE answer to asthma diagnosis at ages 7 and/or 11, and NEGATIVE asthma diagnosis at age 19	15	4	Yes	Yes	No
			5	Yes	No	No
			1	Yes	No Response	No
			5	No	Yes	No
No Asthma*	NEGATIVE answer to asthma diagnosis at any age	798	672	No	No	No
			19	No Response	No	No
			105	No	No Response	No
			2	No Response	No Response	No

Footnotes:

*Groups used for secondary analysis

Table E2. Summary of serum assays performed for IgE antibodies

Allergen Assayed	IgE Assays		Number of Sera Assayed
	Extract	Specific Protein	
Total IgE	a-IgE		963
Common silver birch pollen	t3		963
Timothy grass pollen	g6		963
<i>Dermatophagoides pteronyssinus</i>	d1		963
<i>Alternaria alternata</i>	m6		963
Cat dander	e1		963
Fel d 1 (uteroglobin)		z19^	243
Fel d 2 (cat serum albumin)		e220	245
Fel d 4 (lipocalin)		e228	221
Fel d 5w (cat IgA)		z5^	179
Dog Dander	e5		963
Can f 1 (lipocalin)		e101	272
Can f 2 (lipocalin)		e102	272
Can f 3 (dog serum albumin)		e221	244
Can f 5 (prostatic kallikrien)		e226	272
Dog Epithelium	e2		963
Horse Dander	e3		963
German cockroach	i6		202
Pork	f26		215

Footnotes:

^Assays performed using biotinylated allergen coupled to a streptavidin ImmunoCAP.

Additional IgE assays to allergen extracts: 20 sera were assayed for fish (cod; f3), 19 sera were assayed for chicken (f83), 46 sera were assayed for *Aspergillus fumigatus* (m3), and 50 sera were assayed for mouse epithelium (e71).

Additional IgE assays to specific proteins: 23 sera were assayed for horse lipocalin (Equ c 1; e227), pig serum albumin (n Sus s; e222) and bovine serum albumin (Bos d 6; e204).

Table E3. Univariable analysis of asthma based on prevalence and severity for different levels of IgE antibodies to cat and dog dander, as well as pollen. Any allergen includes values of specific IgE greater than 0.35 IU/mL, and high titer allergen includes values of specific IgE that are greater than 17.5 IU/mL.

	IgE Antibodies					Odds Ratios Compared with Non-Asthmatics				
	Any Allergen [^]	Any Mammal	High Mammal	Any Pollen	High Pollen	Any Allergen	Any Mammal	High Mammal	Any Pollen	High Pollen
Persistent / Current Asthma	42/50	35/50	20/50	31/50	13/50	9.7 [4.5-21]	9.2 [4.9-17]	13 [6.9-25]	4.7 [2.6-8.5]	3.2 [1.6-6.2]
Late Onset / Current Asthma	24/38	16/38	6/38	18/38	9/38	3.2 [1.6-6.2]	2.9 [1.5-5.6]	3.7 [1.4-9.2]	2.6 [1.3-5.0]	2.8 [1.3-6.1]
Non-Asthmatic	281/798	161/798	39/798	206/798	80/798	---	---	---	---	---
Unclassified / Current Asthma	9/15	9/15	4/15	8/15	4/15	---	---	---	---	---
Unclassified / Not Current Asthma	11/19	10/19	3/19	9/19	1/19	---	---	---	---	---
Persistent / Not Current Asthma	11/18	10/18	3/18	6/18	3/18	---	---	---	---	---
Late Onset / Not Current Asthma	9/25	6/25	2/25	7/25	4/25	---	---	---	---	---

Table E4. Geometric mean total serum IgE[§] (IU/ml) in relation to sensitization (specific IgE Ab >0.35 IU/ml) and physician **diagnosis** of asthma.

Sensitization								
Allergen	Positive				Negative			
	N	Asthma	N	No Asthma	N	Asthma	N	No Asthma
Cat Dander	69	191 (147-248)	150	139 (115-168)	80	28.9 (21.1-39.5)	664	25.0 (22.5-27.7)
Dog Dander	66	192 (145-253)	125	167 (136-205)	83	30.8 (22.6-41.9)	689	25.7 (23.2-28.5)
Any Mammal	79	177 (136-231)	168	130 (109-157)	70	24.0 (17.7-32.5)	646	24.2 (21.8-26.9)
Any Allergen [^]	97	148 (116-188)	289	107 (93.3-124)	52	16.9 (12.1-23.6)	525	18.3 (16.4-20.3)

Footnotes:

§ Geometric means $\pm$ 95% confidence intervals

[^] Allergens assayed include: cat dander, dog dander, dog epithelium, horse dander, dust mite (*D. pt.*), silver birch, timothy grass, and *Alternaria*.

Table E5. Effect of cat and/or dog ownership on sensitization to cat and dog dander among individuals with persistent or late onset current asthma [A] and among all individuals in the cohort [B].**A.**

Cat IgE:		Class 6/5	Class 4	Class 3	Class 2/1	Any	High Titer	Negative
Cat Owners	17	1	1	2	3	7	2	10
with dog	9	1	0	2	1	4	1	6
without dog	8	0	1	0	2	3	1	4
Non-Cat Owners	71	6	10	12	10	38	16	32
with dog	33	2	3	6	6	17	5	16
without dog	38	4	7	6	4	21	11	16

Dog IgE:		Class 6/5	Class 4	Class 3	Class 2/1	Any	High Titer	Negative
Dog Owners	42	3	4	2	10	19	7	23
with cat	9	1	0	0	2	3	1	6
without cat	33	2	4	2	8	16	6	17
Non-Dog Owners	46	6	7	3	9	25	13	21
with cat	8	0	1	0	3	4	1	3
without cat	38	6	6	3	6	21	12	18

B.

Cat IgE:		Class 6/5	Class 4	Class 3	Class 2/1	Any	High Titer	Negative
Cat Owners	279	6	6	17	22	51	12	228
with dog	176	5	3	7	17	32	8	144
without dog	103	1	3	10	5	19	4	84
Non-Cat Owners	684	11	31	60	66	38	42	516
with dog	330	3	13	34	26	17	16	254
without dog	354	8	18	26	40	21	26	262

Dog IgE:		Class 6/5	Class 4	Class 3	Class 2/1	Any	High Titer	Negative
Dog Owners	506	6	6	34	46	92	12	414
with cat	176	2	2	9	11	24	4	152
without cat	330	4	4	25	35	68	8	262
Non-Dog Owners	457	8	13	32	49	102	21	355
with cat	103	0	2	5	7	14	2	89
without cat	354	8	11	27	42	88	19	266

Table E6. Relationship of current cat ownership to the prevalence of IgE antibodies specific for cat dander and cat proteins.

Allergen	Cat Ownership	IgE Antibody Titers (IU/ml)			Odds Ratio for IgE >0.35 IU/ml*
		Negative (<0.35)	0.36-17.5 (Class 1-3)	>17.5 (Class 4-6)	
Cat Dander	Cat Owners, n [%]	228 [82%]	39 [14%]	12 [4.3%]	0.69, p = 0.035
	Non-Cat Owners, n [%]	516 [75%]	126 [18%]	42 [6.1%]	
Fel d 1	Cat Owners, n [%]	236 [85%]	34 [12%]	9 [3.2%]	0.62, p = 0.012
	Non-Cat Owners, n [%]	528 [77%]	112 [16%]	42 [6.2%]	
Fel d 2	Cat Owners, n [%]	269 [97%]	6 [2.2%]	2 [0.7%]	1.16, p = n.s.
	Non-Cat Owners, n [%]	665 [97%]	15 [2.2%]	2 [0.3%]	
Fel d 4	Cat Owners, n [%]	258 [94%]	11 [4.0%]	4 [1.5%]	0.54, p = 0.034
	Non-Cat Owners, n [%]	612 [90%]	61 [9.0%]	5 [0.7%]	

Footnotes:

*Odds ratios for prevalence of specific IgE antibodies in each group comparing cat owner to non-cat owners

Table E7A. Multivariate logistic binomial regression of the relationship of physician diagnosis of asthma to IgE antibodies to: Fel d 1, Can f 1, Can f 2, and Can f 5.

A.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Fel d 1	2.2 (1.4-3.6)**	2.3 (1.1-4.9)*
Can f 1	2.0 (0.92-4.4)	3.8 (0.90-16)
Can f 2	0.83 (0.31-2.2)	2.9 (0.20-43)
Can f 5	1.9 (1.1-3.6)*	4.5 (1.1-19)*
B.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Fel d 1	2.5 (1.6-4.0)***	3.2 (1.7-6.2)***
Can f 5	2.3 (1.4-4.0)**	8.2 (2.3-29)***
C.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Can f 1	3.0 (1.4-6.5)**	7.2 (1.9-27)**
Can f 2	0.71 (0.27-1.9)	2.0 (0.14-27)
Can f 5	2.8 (1.6-5.0)***	5.0 (1.2-21)*
D.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Can f 1	2.6 (1.4-5.1)**	8.6 (2.7-27)***
Can f 5	2.7 (1.6-4.8)***	5.5 (1.4-22)*

Table E7B. Multivariate logistic binomial regression of the relationship of physician diagnosis of asthma to IgE antibodies to: cat dander, dog dander, grass pollen, and birch pollen.

A.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Cat Dander	1.5 (0.74-2.9)	1.7 (0.81-3.6)
Dog Dander	2.7 (1.4-5.5)**	9.0 (3.7-22)***
Grass Pollen	0.95 (0.57-1.6)	1.2 (0.58-2.3)
Birch Pollen	1.4 (0.85-2.4)	1.7 (0.87-3.2)
B.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Dog Dander	4.5 (3.1-6.6)***	14 (6.4-30)***

* p<0.05

**p<0.01

*** p<0.001

Table E7C. Multivariate logistic binomial regression of the relationship of physician diagnosis of asthma to IgE antibodies to: cat dander, dog epithelium, grass pollen, and birch pollen.

A.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Cat Dander	2.5 (1.5-4.0)***	3.3 (1.7-6.3)***
Dog Epithelium	1.7 (0.97-3.1)	2.4 (0.65-8.7)
Grass Pollen	0.95 (0.58-1.6)	1.2 (0.62-2.3)
Birch Pollen	1.7 (0.99-2.7)	1.8 (0.97-3.3)

Table E7D. Multivariate logistic binomial regression of the relationship of current asthma to IgE antibodies to: Fel d 1, Can f 1, Can f 2, and Can f 5.

A.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Fel d 1	2.9 (1.6-5.0)***	3.2 (1.4-7.0)**
Can f 1	2.0 (0.89-4.6)	5.0 (1.2-21)*
Can f 2	1.1 (0.41-3.0)	3.5 (0.23-53)
Can f 5	1.7 (0.85-3.3)	3.6 (0.77-16)
B.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Fel d 1	3.3 (2.0-5.6)***	3.2 (1.5-7.0)**
Can f 1	2.7 (1.4-5.2)**	11 (3.2-35)***
C.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Can f 1	3.0 (1.3-6.9)**	12 (3.1-44)***
Can f 2	1.1 (0.39-2.9)	2.1 (0.16-29)
Can f 5	2.7 (1.4-5.1)**	4.1 (0.91-19)
D.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Can f 1	3.1 (1.5-6.4)**	14 (4.5-46)***
Can f 5	2.7 (1.4-5.0)**	4.6 (1.1-19)*

Table E7E. Multivariate logistic binomial regression of the relationship of current asthma to IgE antibodies to: cat dander, dog dander, grass pollen, and birch pollen.

A.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Cat Dander	2.2 (1.0-4.6)*	1.7 (0.75-4.0)
Dog Dander	1.9 (0.87-4.0)	13 (5.1-31)***
Grass Pollen	0.98 (0.56-1.7)	1.3 (0.58-2.8)
Birch Pollen	1.7 (0.94-3.1)	1.7 (0.81-3.6)

Table E7F. Multivariate logistic binomial regression of the relationship of current asthma to IgE antibodies to: cat dander, dog epithelium, grass pollen, and birch pollen.

A.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Cat Dander	2.7 (1.5-4.8)***	3.8 (1.9-7.6)***
Dog Epithelium	1.8 (0.98-3.4)	3.3 (0.89-12)
Grass Pollen	0.99 (0.56-1.7)	1.3 (0.64-2.8)
Birch Pollen	1.8 (1.0-3.2)*	1.8 (0.92-3.7)

B.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Cat Dander	3.4 (2.1-5.6)***	4.9 (2.6-9.2)***
Birch Pollen	1.9 (1.1-3.1)*	2.0 (1.1-3.9)*

Table E7G. Multivariate logistic binomial regression of the relationship of physician diagnosis of asthma to IgE antibodies to: Fel d 1, Can f 5, grass pollen, and birch pollen.

A.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Fel d 1	2.2 (1.3-3.7)**	2.8 (1.4-5.5)**
Can f 5	2.0 (1.1-3.6)*	7.4 (2.1-26)**
Grass Pollen	0.98 (0.59-1.6)	1.2 (0.59-2.3)
Birch Pollen	1.5 (0.87-2.6)	1.8 (0.95-3.4)

Table E7H. Multivariate logistic binomial regression of the relationship of current asthma to IgE antibodies to: Fel d 1, Can f 5, grass pollen, and birch pollen.

A.	Any Positive (>0.35 IU/ml)	High Titer (>17.5 IU/ml)
Fel d 1	2.8 (1.6-4.9)***	4.1 (2.0-8.2)***
Can f 5	1.8 (0.97-3.4)	6.9 (2.0-24)**
Grass Pollen	0.97 (0.54-1.7)	1.2 (0.57-2.7)
Birch Pollen	1.8 (0.95-3.3)	1.8 (0.86-3.6)

Table E7I. Multivariate logistic binomial regression of the relationship of current persistent asthma to IgE antibodies to: cat dander, dog dander, grass pollen, and birch pollen.

A.	Any Positive (>0.35 IU/ml)
Cat Dander	3.9 (1.4-11)**
Dog Dander	1.8 (0.66-5.1)
Grass Pollen	0.64 (0.30-1.3)
Birch Pollen	1.8 (0.84-4.0)
B.	Any Positive (>0.35 IU/ml)
Cat Dander	6.0 (3.0-12)***
Grass Pollen	0.62 (0.30-1.3)
Birch Pollen	2.0 (0.95-4.3)
C.	Any Positive (>0.35 IU/ml)
Cat Dander	5.6 (2.8-11)***
Birch Pollen	1.6 (0.83-3.2)
D.	Any Positive (>0.35 IU/ml)
Cat Dander	7.2 (3.9-13)***

A.	High Titer (>17.5 IU/ml)
Cat Dander	0.93 (0.29-3.0)
Dog Dander	33 (12-97)***
Grass Pollen	1.0 (0.32-3.2)
Birch Pollen	1.5 (0.51-4.1)
B.	High Titer (>17.5 IU/ml)
Cat Dander	0.93 (0.29-3.0)
Dog Dander	33 (12-97)***
Birch Pollen	1.5 (0.54-3.9)
C.	High Titer (>17.5 IU/ml)
Dog Dander	32 (14-72)***
Birch Pollen	1.5 (0.55-3.8)
D.	High Titer (>17.5 IU/ml)
Dog Dander	35 (16-76)***

Table E7J. Multivariate logistic binomial regression of the relationship of current persistent asthma to IgE antibodies to: Fel d 1, Can f 5, grass pollen, and birch pollen.

A.	Any Positive (>0.35 IU/ml)
Fel d 1	3.6 (1.6-7.9)**
Can f 5	3.2 (1.4-7.3)**
Grass Pollen	0.71 (0.33-1.6)
Birch Pollen	1.4 (0.61-3.3)
B.	Any Positive (>0.35 IU/ml)
Fel d 1	3.8 (1.7-8.3)***
Can f 5	3.6 (1.7-7.9)***
Grass Pollen	0.83 (0.41-1.7)
C.	Any Positive (>0.35 IU/ml)
Fel d 1	3.5 (1.7-7.5)***
Can f 5	3.6 (1.7-7.7)***
D.	Any Positive (>0.35 IU/ml)
Can f 5	7.9 (4.3-15)***

A.	High Titer (>17.5 IU/ml)
Fel d 1	3.9 (1.6-9.7)**
Can f 5	12 (3.4-42)***
Grass Pollen	0.97 (0.32-3.0)
Birch Pollen	1.5 (0.58-4.1)
B.	High Titer (>17.5 IU/ml)
Fel d 1	3.9 (1.6-9.5)**
Can f 5	12 (3.4-42)***
Birch Pollen	1.5 (0.59-4.0)
C.	High Titer (>17.5 IU/ml)
Fel d 1	4.3 (1.8-10)***
Can f 5	13 (3.7-45)***
D.	High Titer (>17.5 IU/ml)
Can f 5	25 (8.0-77)***

