Exposure to indoor allergens in different residential settings and its influence on IgE sensitization in a geographically confined Austrian cohort

Short title: Indoor allergen exposure and IgE sensitization

Teresa Stemeseder[1], Bettina Schweidler[1], Patrick Doppler[2], Eva Klinglmayr[1], Stephanie Moser[3,4], Lisa Lueftenegger[5,6], Martin Himly[1], Roland Lang[5], Joerg Zumbach[2], Gertie J. Oostingh[6], Thomas Hawranek[5], Arne C. Bathke[2], Gabriele Gadermaier[1]

[1]Department of Molecular Biology, University of Salzburg, Austria

[2]Department of Mathematics, University of Salzburg, Austria

[3]School of Education, University of Salzburg, Austria

[4]TUM School of Education, Technical University of Munich, Germany

[5]Department of Dermatology, Paracelsus Medical University Salzburg, Salzburg, Austria

[6]Biomedical Sciences, Salzburg University of Applied Sciences, Puch/Salzburg, Austria

Gabriele Gadermaier

Department of Molecular Biology, University of Salzburg

Hellbrunnerstraße 34, A-5020 Salzburg, Austria

Telephone: 0043-662-8044-5974, Fax: 0043-662-8044-183

Gabriele.Gadermaier@sbg.ac.at

Abstract

Background: Exposure to indoor allergens is crucial for IgE sensitization and the development of allergic symptoms. Residential settings influence the allergen levels in house dust and hence allergic sensitization. Within this study, we investigated allergen exposure and molecule-based IgE levels in a geographically confined region and evaluated the impact of living area, housing, pets and household cleanliness.

Methods: A non-selected cohort of 501 adolescents from the Salzburg area, Austria, participated in this cross-sectional study. House dust samples were obtained using a standardized procedure, and concentrations of major mite, cat, dog, and mold allergens were determined by a multiplex array for indoor allergens. Specific IgE to dust mite Der p 1 & Der p 2, cat Fel d 1, dog Can f 1 and mold Alt a 1 was analyzed in serum samples of participants using the ImmunoCAP ISAC. Information on allergies, living areas, dwelling form, pets, and household cleanliness was provided through completion of a questionnaire and linked with allergen exposure and IgE sensitization data.

Results: In the house dust samples of investigated homes, the levels of cat allergen were highest while the prevalence of mold allergens was the lowest. Prevalence of IgE sensitization to Der p 1 was 13.2% and to Der p 2 was 18.2%; reactivity to Fel d 1, Can f 1 and Alt a 1 was 14.4%, 2.4% and 2.0%, respectively. Reduced amounts of mite allergens were found in alpine regions, which correlated to reduced IgE levels to these allergens. Generally, a trend for increased sensitization prevalence from rural to alpine to urban regions was noted. Living on farms resulted in lower sensitization prevalence to mite and cat allergens, even though the exposure to mites was significantly elevated. The presence of cats was associated with a lower sensitization rate and lower IgE levels to cat and mite allergens, and a reduced rate of reported allergies. Cleaning did not impact allergen amounts, while IgE reactivity to mites and reported allergies were higher in participants living in cleaner homes.

Conclusion: Allergen exposure and IgE sensitization to indoor allergens showed considerable variability depending on the environmental setting of homes. Together with other factors, allergen

exposure is crucial for the development of IgE and allergic symptoms. Cross-sectional studies like this one contribute to the identification of risk factors and prevention measures to counteract the epidemic character of allergic diseases.

Introduction

Exposure to house dust is one of the basic features for the development of allergic symptoms to inhalant indoor allergens [1,2]. The most common allergens found in house dust originate from mites, animal dander, molds and cockroach [3]. Major allergens typically represent the most relevant IgE-binding molecules of an allergen source and are involved in triggering allergic symptoms [4]. The dominant allergens of the most common house dust mite species (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae)* belong to mite group 1 (Der p 1 and Der f 1) and mite group 2 (Der p 2 and Der f 2) allergens. These molecules account for IgE sensitization in more than 80% of mite-allergic subjects and a high level of cross-reactivity between mite species exists [3].

Fel d 1 is the major allergen from cat (*Felis domesticus*) and more than 80% of the total IgE reactivity to cat allergens is directed against Fel d 1 [5]. It accounts for a large proportion of allergens from animal dander in house dust, but large variations in allergen concentrations have been noted in households of different countries [6,7]. The second most important animal allergen found in house dust is Can f 1 from dog (*Canis familiaris*) with a sensitization prevalence of 75% among dog-allergic patients [8]. Similar to cat allergens, Can f 1 is frequently found in European households [6].

Alternaria alternata represents one of the most common indoor molds and its main allergen Alt a 1 is recognized by >90% of *Alternaria*-sensitized patients [9,10]. Due to perennial mold exposure and the association with asthma and respiratory allergies, investigating the relevance of exposure and IgE sensitization is of special interest [3,6]. Allergen exposure to cockroach can be high in inner-city areas of metropolises, while very low levels were found in Central Europe due to the inadequate habitat for the insects [11,12].

Exposure to allergens is a prerequisite for initiating an allergic sensitization followed by the production of allergen-specific IgE antibodies [13]. Exposure to indoor allergens has been linked to IgE sensitization and development of allergic symptoms in a number of studies. However, there are controversial findings regarding the kind of correlation, ranging from a protective effect upon high exposure to certain allergens [14-16] to a negatively influencing effect [17-19], whereas other studies found no effect at all [20,21]. Also, exposure itself is influenced by different factors such as pet ownership, infrastructural characteristics or altitude. These influences are widely discussed in the literature and strong differences in exposure levels between groups [6,21-23] as well as no effects [24] from specific influencing factors are found. In order to assess allergen exposure, it is well established to measure allergen concentrations in house dust samples using antibody-based detection systems [3]. Usually 2-4 sites within a household are sampled for the assessment of allergen exposure, typically including mattresses, bedding, carpets, and sofas [2,25]. Since IgE antibodies are a prerequisite for the development of allergies, linking IgE antibodies with allergen exposure in households can provide valuable information regarding risk factors and to suggest prevention measurements, thus contributing to diminish the progress of allergic diseases [26]. So far, no study investigated the association between indoor allergen exposure and allergic sensitization determined as IgE reactivity to single allergenic molecules in a large non-selected cohort.

The aim of this study was to analyze the exposure and IgE sensitization to five major indoor allergens originating from mite, cat, dog and mold. Therefore, allergen concentrations of Der p 1, mite group 2, Fel d 1, Can f 1 and Alt a 1 were measured in house dust of homes. IgE sensitization to corresponding purified allergens was analyzed in sera of 501 adolescent study participants and the relevance of different living areas, dwelling forms, pets or household cleanliness was evaluated. Allergen exposure was also evaluated with respect to reported allergies.

Methods

Study design and participants

This cross-sectional study was conducted in a non-selected cohort as described in detail elsewhere (Stemeseder *et al*., under review). Shortly, 501 pupils from schools in different geographic regions in the district of Salzburg, Austria were engaged in this study. Participants were recruited from school grades 8-13 (expected age 13-19 years) and sampling took place in the time between October 2013 and May 2014. Written informed consents from the participating subjects themselves and their legal guardian (if they were underage) were obtained. The study was conducted according to common ethical principles and approved by the local ethics committee of Salzburg, Austria, No. 415-E/1669/6-2013.

Assessment of allergen exposure in house dust samples

House dust samples were collected from participants' homes with a commercially available DUSTREAM® Collector (Indoor Biotechnologies, Charlottesville, VA, USA) attached to a household vacuum cleaner following a detailed protocol from Indoor Biotechnologies. Four areas each sized 20 x 30 cm on mattress (head and foot area), bedroom carpet and living room couch were sampled for 30 s per area. Dust extracts were prepared by dissolving 100 mg of fine dust in 2 ml of phosphate-buffered saline pH 7.4, 0.05% Tween-20 (PBS-T). Samples <10 mg of fine dust were not considered for further analysis. Proteins were extracted by shaking for 2 h at room temperature following a centrifugation step at 1,380 x g for 20 min. Supernatants were stored at -20 °C until further processing.

For allergen content analysis, the dust extracts were thawed and centrifuged again. Supernatants were diluted 1:10, 1:100 and 1:10,000 and examined in a Multiplex Array for Indoor Allergens (MARIA®, Indoor Biotechnologies, Charlottesville, VA, USA) using xMAP® Technology (Luminex, Austin, TX, USA) [27]. The array uses fluorescently labeled beads conjugated to monoclonal antibodies specific for purified allergen molecules. The allergen concentration of Der p 1 and mite group 2 allergens (house dust mites allergens), Fel d 1 (cat allergen), Can f 1 (dog allergen) and Alt a 1 (mold allergen) was investigated. A 12 point standard curve executed in duplicates was used to quantify the results. Additionally, quality controls provided with the test kit were applied. Measurements of fluorescence were performed in a Luminex200IS (Luminex, Austin, TX, USA) with Luminex100IS software (build 2.3). Raw data were imported to Masterplex QT v4.0 software (Hitachi Solutions America Ltd., San Bruno, CA, USA) for further analysis. Lower limit of detection (LLOD) was set to 3 x SD + mean of blank values and the standard curve was calculated using a five parameter logistics curve fit. Concentrations of allergens were calculated as mean of the three dilution-adjusted measurements per sample. Sample values with a bead count lower than 50 beads per analyte as well as values below the LLOD were excluded for mean calculation.

Blood sampling and specific IgE analysis using an allergen multiplex array

Capillary blood samples were obtained from the fingertip and incubated at room temperature for 15 min. After centrifugation at 14,000 rpm, serum was separated from the blood cells. Serum samples were subsequently stored at 4 °C for transport and -20 °C until further analysis. Analysis of sera for specific IgE to single purified allergens was done by means of ImmunoCAP ISAC® (Thermo Fisher Scientific, Uppsala, Sweden). According to the manufacturer's protocol, the test was performed with 30 µl of serum (Protocol No. 20-01-02-6). The resulting fluorescent signals were measured with a confocal laser scanner (LuxScan-10K, CapitalBio, Beijing, China). Data were analyzed in Phadia Microarray Image Analyzer (MIA) software and transformed into semi-quantitative ISAC Standardized Units (ISU). Specific IgE values ≥0.3 ISU were considered positive. Participants with a positive value to any of the 112 allergens on the ImmunoCAP ISAC® were considered as sensitized.

Assessment of personal and demographic data

Participating pupils filled out an in-house developed written questionnaire which was anonymous and linked to the IgE data and the dust sample using a number code. Demographic data such as gender and age were gathered. Furthermore, subjects reported on their living area *i.e.* urban (city of Salzburg), rural, or alpine (>800 m above sea level), their dwelling form *i. e.* house, flat, or farm, and the presence of pets within their homes. Additionally, they reported on self-assessed household cleanliness considering frequency of mattress and bedsheet exchange as well as vacuum cleaning and comparison of their homes to a very sterile environment. Cleanliness was assessed by values ranging from 1 to 5, with 5 indicating the highest cleanliness. All study participants were asked to report if they had any doctor-diagnosed allergy confirmed by a clinician.

Statistical analysis

In order to detect a nonparametric relative effect of 0.65 with the two-sided two-sample rank sum "Mann-Whitney" test, the approximate minimal sample size necessary to obtain power=0.9 at alpha=0.05 is n=61 per group, using Noether's formula. This sample size was always exceeded in our pairwise comparisons, where the smallest group had n=71.

Statistical analysis was performed with R in RStudio [28] and GraphPad Prism 5 for Windows (GraphPad Software, Inc., La Jolla, CA, USA). Correlations between ISU levels and allergen concentrations in house dust were calculated as Spearman's rank correlation. Comparisons of ISU levels or allergen concentrations between groups were performed using Mann-Whitney tests. Odd's ratios were calculated from Fisher's exact test for count data. P-values <0.05 were considered as statistically significant. P-values are reported without multiplicity adjustment throughout the manuscript and were categorized as follows: $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), $p<0.0001$ (****).

Results

Study cohort

501 pupils participated in the study by donating blood samples and returning the questionnaire. Dust samples from 96.0% of participants were considered for further analysis. Of 501 pupils, 71 were living in an urban region, 264 in rural regions and 165 in alpine regions. Participants also stated in which kind of dwelling they lived: 113 lived in flats, 310 in houses and 74 on a farm. Regarding pets, 340 (67.9%) reported to have pets, 236 (47.1%) had a cat and 88 (17.6%) had a dog in their home. Self-assessed household cleanliness was reported with a mean value of 2.94 assessed on a scale from 1 to 5, where 1 corresponded to very low cleanliness and 5 was highest cleanliness.

Indoor allergen exposure

Indoor allergen concentrations in house dust samples were measured by the MARIA system (Fig 1). The major cat allergen Fel d 1 was detected in 97.9% of investigated homes, independent of the presence of a cat. It represented the predominant allergen in the house dust (median 0.76 ng/mg fine dust) and reached values >343.7 ng/mg fine dust in 5% of the homes. Median concentrations of mite allergen Der p 1, mite group 2 and dog allergen Can f 1 were 0.03 ng/mg, 0.16 ng/mg and 0.06 ng/mg, respectively. Alt a 1 was detected in 3.3% of homes only and allergen levels were insignificantly low and could thus not be considered for further statistical analyses. Levels of house dust mite allergens (Der p 1 and mite group 2) were highly correlating in the samples ($p<0.0001$, $rho=0.7$). Notably, mite group 2 allergens were detected more frequently and at higher concentrations than Der p 1. A slight correlation ($p<0.0001$, $rho=0.2$) was also found for Can f 1 and Fel d 1. No significant correlations were found between the other allergens.

Fig 1. Allergen concentrations found in investigated house dust samples and respective detection rates.

Boxes indicate 25th and 75th percentile, horizontal line represents median, whiskers indicate 5th and 95th percentile.

The concentrations of indoor allergens were additionally analyzed with respect to different living areas (Fig 2A). Considerably lower levels of mite allergen Der p 1 as well as of mite group 2 allergens were found in alpine households. Cat allergen Fel d 1 was found to be less prevalent in urban homes in comparison to rural and alpine homes. Lower levels of Can f 1 were identified in alpine homes. Likewise, we observed differences in allergen concentrations in different forms of dwellings, *i. e.* flats, houses and farms (Fig 2B). Increased levels of mite allergens Der p 1 and mite group 2 were found in house dust samples of farms. The level of cat allergen Fel d 1 was higher in houses while no significant differences could be found for the dog allergen Can f 1.

Fig 2. Allergen concentrations in house dust samples collected in different housing settings. **(A)** Allergen concentrations in different living areas (urban, rural and alpine). **(B)** Allergen concentrations in different dwelling forms (flat, house and farm). Boxes indicate 25th and 75th percentile, horizontal line represents median, whiskers indicate 5th and 95th percentile. *, $p<0.05$; ***, $p<0.001$; ****, $p<0.0001$ for pairwise comparisons.

Differences in allergen levels were also identified between homes with and without pets. In house dust samples of participants with a pet in their home, lower concentrations of mite allergen Der p 1 but higher levels of Fel d 1 and Can f 1 were found (Fig 3A). In house dust of cat owners, significantly higher concentrations of the cat allergen Fel d 1 was found (Fig 3B). Likewise, in homes of dog owners, considerably higher levels of the dog allergen Can f 1 were present (Fig 3C). No statistically significant differences were found for other allergen levels with respect to pet, cat, or dog present in the home. No correlation of allergen concentration and self-reported household cleanliness was found for any of the investigated allergens.

Fig 3. Allergen concentrations in house dust samples collected in households with and without pets.

(A) Allergen concentrations in homes with and without a pet. **(B)** Allergen concentrations in homes with and without a cat. **(C)** Allergen concentrations in homes with and without a dog. Boxes indicate 25^{th} –75^{th} percentile, horizontal line represents median, whiskers indicate 5^{th} and 95^{th} percentile. *, p<0.05; ****, p<0.0001 for pairwise comparisons.

IgE sensitization to indoor allergens

IgE sensitization to indoor allergen molecules was evaluated by means of ImmunoCAP ISAC®. As previously reported, a general sensitization rate of 53.5% was found in the study population (Stemeseder *et al*., under review). IgE levels of the mite group 2 allergens Der p 2 and Der f 2 correlated strongly (p<0.0001, rho=0.99), therefore only Der p 2 was used for subsequent statistical analyses. IgE sensitization rates ranged from 13.2% to 18.2% for mite allergens Der p 1 and Der p 2 and cat allergen Fel d 1 (Fig 4). Low sensitization rates were detected for Can f 1 (2.4%) from dog as well as for the mold allergen Alt a 1 (2.0%). Highest IgE levels were found for the mite allergen Der p 2 (mean: 3.0 ISU). Although the sensitization prevalence was low with a mean of 0.33 ISU, Alt a 1-positive individuals frequently showed rather high IgE levels.

Fig 4. Dot plot of the specific IgE levels to indoor allergens and respective sensitization rates.
Dots represent individual measurements, lines indicate mean values and whiskers the standard deviation.

Weak but statistically significant positive correlations between allergen concentrations in house dust and IgE levels were found for mite group 2 allergen concentration and IgE levels to Der p 1 (p<0.05, rho=0.11) as well as for Can f 1 dog allergen concentration and Can f 1 IgE levels (p<0.05, rho=0.11). Interestingly, concentrations of Fel d 1 showed a slightly negative correlation with IgE levels to Der p 2 (p<0.05, rho=-0.12).

Residential settings and their impact on IgE sensitization

Potential influences of different residential settings on IgE sensitization were investigated. The overall sensitization rate did not differ significantly between the three investigated living areas but a trend of increased sensitization from rural (51.5% sensitized) to alpine (55.2%) to urban (57.7%) regions was observed. However, pupils living in alpine areas showed a significantly decreased sensitization rate and decreased IgE levels to mite allergens Der p 1 and Der p 2 (Fig 5A). No statistically significant difference between living areas was found for Fel d 1 from cat. Increased Can f 1 IgE levels were observed for pupils living in urban regions, but case numbers were rather low.

Fig 5. IgE levels to indoor allergens in sera of pupils living in different settings.
(A) IgE levels of pupils living in different areas (urban, rural, alpine). **(B)** IgE levels of pupils living in different dwelling forms (flat, house, farm). Dots represent individual measurements, lines indicate mean values and whiskers the standard deviation. *, $p<0.05$; **, $p<0.01$; ****, $p<0.0001$.

With regard to different dwelling forms, a significant difference ($p<0.05$) was found in the overall sensitization rate with an increase from farm (41.9% sensitized) to house (53.9%) to flat (60.2%). Living on a farm was associated with a decreased odd's ratio of 0.577 (95% CI: 0.337-0.978, $p<0.05$) for general IgE sensitization. Pupils living in flats showed a higher sensitization rate and IgE levels to the mite allergen Der p 2 (Fig 5B). Pupils living on farms showed decreased prevalence and IgE levels to cat allergen Fel d 1 compared to pupils living in houses.

Generally, a slightly lower sensitization rate of 52.4% was found for pupils living with any pet in their home compared to 55.5% for those not having a pet. Decreased IgE levels to cat allergen Fel d 1 but slightly higher IgE levels to dog allergen Can f 1 were found in pet owners (Fig 6A). For cat owners, a significantly decreased general sensitization rate of 47.9% was found ($p<0.05$) which also translated into a decreased odd's ratio of 0.658 (95% CI: 0.453-0.952, $p<0.05$). In addition, IgE levels to the mite allergen Der p 2 and the cat allergen Fel d 1 were lower for cat owners (Fig 6B). No differences in either sensitization rates or IgE levels to single allergens were found between dog owners and non-dog owners (Fig 6C). A slight positive correlation was found between self-reported household cleanliness and IgE levels to mite allergen Der p 1 ($p<0.01$, rho=0.12); no statistically significant association was observed with other allergens.

Fig 6. IgE levels to indoor allergens of participants living with or without pets.

(A) IgE levels of participants living with or without any pet. **(B)** IgE levels of participants living with or without a cat. **(C)** IgE levels of participants living with or without a dog. Dots represent individual measurements, lines indicate mean values and whiskers the standard deviation. *, $p<0.05$; **, $p<0.01$.

Residential settings and reported allergies

Within the study population, 21.8 % of participants reported to have any allergy that was diagnosed by a medical doctor. Thus, allergen exposure was additionally linked to reported allergies. Interestingly, significantly decreased levels of Der p 1 mite allergen concentration were observed for those pupils who reported any allergy compared to those who did not have any allergy ($p<0.01$).

With respect to living areas, a trend for a higher prevalence of reported allergies was found for urban regions (25.4%) compared to rural (21.6%) and alpine regions (21.0%). For different dwelling forms however, a significantly decreased ($p<0.01$) rate of allergies was found in participants living on farms (9.6%), compared to higher rates for participants living in flats (28.2%) or houses (22.7%). While no difference in allergy prevalence could be found between pet owners and non-pet owners, a slightly decreased but statistically not significant rate could be found for cat owners (18.6% for cat owners *vs.* 24.3% for non-cat owners). For dog owners on the other hand the rate of reported symptoms was slightly increased with a diagnosis rate of 25.0% as compared to non-dog owners (20.7%). Pupils reporting any allergy also reported to live in a cleaner environment compared to those without symptoms ($p<0.0001$).

Discussion

The presence of allergy eliciting molecules is a prerequisite for allergies and was shown to influence IgE sensitization as well as allergic symptoms such as asthma. We therefore investigated indoor allergen exposure and linked the results with IgE sensitization and reported allergy in 501 non-selected study participants from Austria. Moreover, we investigated the impact of different living and dwelling forms, pet ownership and household cleanliness. This cross-sectional study represents the first investigating exposure and molecule-based IgE sensitization to 5 major indoor allergens within a geographically confined area of Central Europe. Dust sampling and detection of indoor allergens as well as participants' specific IgE was conducted with commercially available multiplex kits, thus being highly reproducible and also comparable with other studies [27] (Stemeseder *et al*., under review).

Indoor allergen exposure and effect on IgE sensitization

Major allergens from the indoor allergen sources cats, house dust mites, and dogs were detected in more than 84% of investigated homes using a commercially available multiplex allergen detection assay. We found particularly high levels of Fel d 1, while detection of Can f 1 was significantly lower which probably reflects 47.1% of cats but only 17.6% of dogs in our investigated households. However, we noticed a slight correlation of Fel d 1 and Can f 1 which might reflect the generally high abundance of these animal allergens in the environment [6]. Similar to previous studies, the presence of mite group 1 and 2 allergens highly correlated [29]. While mite group 2 allergens were detected more frequently and with higher concentrations in our cohort as well as in a multicenter study using the same multiplex array technology, other investigations showed a higher prevalence of Der p 1 [27,29]. In our study cohort, the mold allergen Alt a 1 was rarely detected and present at very low levels and was therefore not subjected to further statistical analyses. Using Alt a 1 as sole marker might however be misleading since insufficient release due to suboptimal growth conditions was observed [30]. Generally, recent changes in constructions of buildings might favor the increase of prevalence of mold allergens and thus allergies thereof [31].

Similar to other studies, we found high IgE sensitization rates to mite allergens Der p 1 (13.2%), Der p 2 (18.2%) and cat allergen Fel d 1 (14.4%) [32-34]. Dog allergen Can f 1 was however only tested positive in 2.4% of our sera while a prevalence rate of 5% was found in a similar cohort [34] and 16.2% were found positive by skin prick tests in adults [33]. The low prevalence of 2% of Alt a 1 sensitization found in our study correlates well with results from skin prick testing in a Swedish cohort of 16-30 y olds [33], while a US-based study found a prevalence of almost 8% in the general population. These differences may result from different environmental conditions as well as divergent test methods using extracts for skin prick tests or single molecules. In general, we found high sensitization rates to indoor allergens in this adolescent cohort, who can thus be considered at risk for the development of allergic symptoms [34,35].

In order to investigate the relation between allergen exposure and IgE sensitization, we correlated allergen concentrations and IgE levels. Within this study, we showed a positive correlation between exposure to mite group 2 allergens and sensitization to Der p 1. Several studies have shown an increasing risk of dust mite sensitization with increasing Der p 1 exposure [36-38]. On the other hand, one study reported a bell-shaped, non-linear relation between mite exposure and sensitization, where intermediate levels of mite allergen showed highest sensitization rates [39]. A similar pattern was found for the risk of sensitization to pets being highest at moderate exposure levels [36]. In our study, we found a low but positive correlation between Can f 1 exposure and sensitization, analogous to a study by Williams *et al.* [40]. Differences between studies might originate from investigating more pre-selected cohorts like populations with farming or anthroposophic lifestyle or varying exposure measurements. Additionally, we found reduced Der p 1 exposure in participants reporting to suffer from allergies. Hence, we conclude from our study that there is a reversed correlation between allergen exposure and IgE sensitization. However, additional influences such as type of allergen, allergen dose or genetic background of subjects might need to be investigated in more detailed studies to get a clearer picture on this relation [1].

Effect of living area on exposure and allergic sensitization

Allergen exposure as well as IgE sensitization and allergies were shown to vary in different residential settings. The influence of living in urban, rural, and alpine regions in the province of Salzburg was therefore analyzed. A tendency for higher mite allergen exposure was found in rural areas compared to urban regions, but was not as pronounced as in a previous study conducted in Poland [21]. A reason might be that the urban area of Salzburg is not fully comparable to large industrialized towns. However, we found significantly reduced Der p 1 and mite group 2 concentrations in alpine regions, which also translated into reduced IgE levels to mite allergens. The data are fitting well with several previous studies showing the relation of mite allergen level and altitude [41,42], and also a decreased sensitization rate in schoolchildren living in alpine regions of France was noted [43]. A recent study did not find a correlation between altitude and house dust mite allergens in an alpine region of Germany and Austria [24]. In this study, several different types of buildings, e. g. private residences, taverns and mountain huts were investigated which may not be comparable with our study cohort, solely investigating allergens levels in homes of pupils. However, also this study showed lower Der f 1 levels at elevated altitude levels [24]. Relevant amounts of house dust mite allergens were also found at high altitude in Quito, Ecuador (2800 m a.s.l.), but study outcomes may be difficult to compare due to divergent climatic conditions [44].

Within this work a decreased amount of cat allergen in urban homes but, interestingly, a trend for higher IgE levels to Fel d 1 in urban study participants was found. A similar decreasing gradient of cat sensitization from city to rural inhabitants during childhood exposure was recently shown by Elhom *et al.* [45]. In our study, only 22.5% of urban homes but >50% in the two other living areas have a cat at home, which might contribute to the low allergen exposure level [46]. These findings however, seem to further underline the discussed protective effect of cat exposure in several studies [36,45,47]. Regarding dog allergens, decreased amounts of Can f 1 in alpine homes were found, while IgE levels to Can f 1 were also higher in urban participants. We can hence not confirm a protective effect of dog exposure from our results.

Notably, when investigating the general sensitization prevalence in the three different living areas, a trend for an increased sensitization rate towards urban regions was shown. Studies conducted in the United States, Denmark and Poland showed the same pattern, presenting differences in sensitization rates between urban and rural populations of 10, 20 and 40 percentage points, respectively [32,45,48]. The increased sensitization rate in urban regions also translated to a trend for an increased number of reported allergies in our study. However, the lower numbers of reported allergies in rural areas might also be influences by other socioeconomic factors, which might be prevalent in rural areas such as a higher threshold to go to a doctor, a lower frequency of available doctors or a difference in education levels. To summarize, there is an increased risk for developing a sensitization when being exposed to an urban environment, which might be particularly influential during childhood and adolescence.

Effect of dwelling forms on exposure and allergic sensitization

As clear differences were found between the living areas, the relevance of different dwelling forms (flat, house or farms) with respect to allergen exposure and sensitization was determined. A significantly increased mite allergen exposure in house dust samples taken in farms was found, while at the same time significantly decreased IgE levels to Der p 2 were observed in these study participants. Other studies also found increased amounts of house dust mite allergens on farms, which might be linked to storage mites typically present in a farming environment [37,49,50]. The PARSIFAL study conducted in 5 European countries did not find differences in group 1 allergen levels, but also demonstrated lower sensitization prevalence to mite allergens in farm-children compared to non-farm children [39].

Furthermore, a significant increase in Fel d 1 levels in samples taken from houses was found. This might relate to more limited possibilities of keeping a cat in a flat, as only 27.7% of flats have a cat while 49.0% of houses have one. On the other hand, this could be due to a different habit of pet keeping on farms, where cats often do not directly enter the living areas but live in the surrounding buildings. Again, farm-living participants showed decreased IgE levels to Fel d 1, a finding previously also obtained in the cross-sectional PARSIFAL study [39]. Different dwelling forms did not have an effect on exposure or IgE sensitization levels to Can f 1 from dogs. Notably, we detected decreased sensitization prevalence as well as lower IgE levels to investigated indoor allergens among participants living on farms.

In addition, we found a decreased rate of reported allergies for participants living on farms. A lower prevalence of atopy in farming environments was previously shown [39,51]. This finding was also clinically relevant, as farm children with regular exposure to the farming environment had the lowest prevalence rates of wheezing and atopic diseases [52]. Hence our findings support the protective effect of living on a farm in terms of a lower risk of developing IgE sensitization. As a tendency for increasing IgE levels from farm to house to flat was observed, it could be speculated that participants living in houses tend to have a more regular contact to farming environments than pupils living in flats. They might however also be surrounded by more green areas which was also shown to have a protective effect on allergic diseases [52,53].

Effect of pet ownership on exposure and sensitization

As allergens from pets, in particular cats and dogs, are of high allergenic relevance, the presence of any pets, cats or dogs, regarding allergen exposure and sensitization was investigated. In line with previous studies, significantly higher levels of cat (248-fold) and dog allergens (193-fold) were found when respective animals were present in the homes [6,38,54]. In our study cohort, a high prevalence of cats (47.1%) and dogs (17.8%) was noted, which was significantly elevated compared to a study investigating 12 European birth cohorts with 7.2-35% (average 14.9%) cats and 5.4.-35% (average 12%) dogs in homes [55].

Especially cat ownership seemed to have a protective effect, translating into decreased total sensitization rates and lower mean IgE levels to cat and mite allergens. In addition, a slightly reduced rate of reported allergies was found among cat owners. If the cat owners were stratified by different living areas, to rule out a possible confounding effect of the protection from living in rural areas, decreased sensitization rates for cat owners in all three living areas were found. However, only for rural regions the effect was statistically significant ($p<0.05$). Yet, earlier studies showed similar results, where an inverse relationship between having a positive skin test to cat allergen and having ever lived in a cat-owning household was found [47]. Another study showed an association between cat ownership and decreased sensitization prevalence to cat and dog, while no such impact was found for mite or grass pollen sensitization [36]. However, there is literature stating that pet exposure does not influence sensitizations or even showed an increased but not sustainable risk of IgE responses after early cat allergen exposure [20,56]. Our results indicate that existing exposure to cats is associated with lower IgE sensitization in this group of adolescents. Still we cannot completely exclude an overlapping protective effect of living in rural areas. Exposure in early childhood might also have additional influences on a potential protective effect, which was not investigated in this study.

Effect of household cleanliness on exposure and sensitization

As an association between allergen exposure and IgE sensitization was shown, the effects of household cleanliness as an influencing variable was of interest. No correlation between allergen occurrence in the dust samples and self-estimated cleanliness of homes could be verified. However, we found slight correlations between household cleanliness and IgE levels to single allergens from mites. Analogously, subjects reporting to have any allergy lived in a cleaner household, while no significant difference in household cleanliness was found between households of subjects with allergic family members and those without (Stemeseder *et al.*, manuscript in preparation). This suggests that cleanliness could play a role in development of allergy.

Although the reported household cleanliness in our study considered the frequency of changing bedsheets, we could not confirm the result of a recent study performed in France reporting an increased Der f 1 amount when changing the bedsheets less often [57]. The negative effect of cleaning regarding sensitization could on the one hand relate to household chemicals for cleaning, as *e. g.* propylene glycol and glycol ethers abundance in indoor air was shown to be associated with an increased risk of allergic symptoms [58]. On the other hand, this could relate to findings by Schram-Bijerk *et al*. [39], where the highest sensitization rates to mite allergens were found at intermediate allergen exposure levels. Thus, the effect of cleaning might just be enough to shift from very high exposure to intermediate exposure and hence cause higher sensitization rates.

In conclusion, we could show that indoor allergen exposure has an effect on the development of IgE sensitization. The amount of allergens in homes is influenced by geographically different locations or dwelling forms and pet keeping, but not substantially with household cleanliness. Changes in exposure could be associated with significant differences in IgE sensitization to investigated indoor allergens. These findings were, however, not strictly limited to indoor allergens, as living areas and housing as well as cats and household cleanliness also influenced IgE levels to allergens from grass and birch pollen (Stemeseder *et al.*, manuscript in preparation data). In combination with other factors, such as genetic predisposition or lifestyle, our data combined with data from other studies will enable the determination of risk factors and prevention measurements to efficiently counteract the burden of allergic diseases.

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Author contribution

Conceptualization: GG, MH, JZ, SM

Methodology: TS, SM, MH, EK, GG

Formal analysis: TS, ACB, PD

Investigation: TS, BS, EK, LL

Resources: RL, TH, GJO

Writing original draft: TS, GG

Visualization: TS

Supervision: RL, TH, GJO

Project administration: TS, GG

Funding acquisition: GG, MH, JZ, SM

All authors read and approved the final manuscript.

Conflict of interest

GG received lecture fees from Thermo Fisher Scientific. All other authors declared no conflict of interest.

527 # Figures

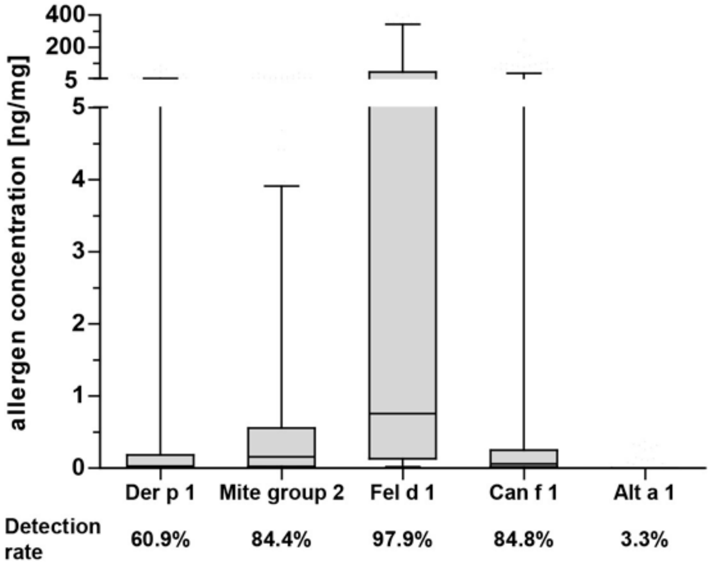


Fig 1

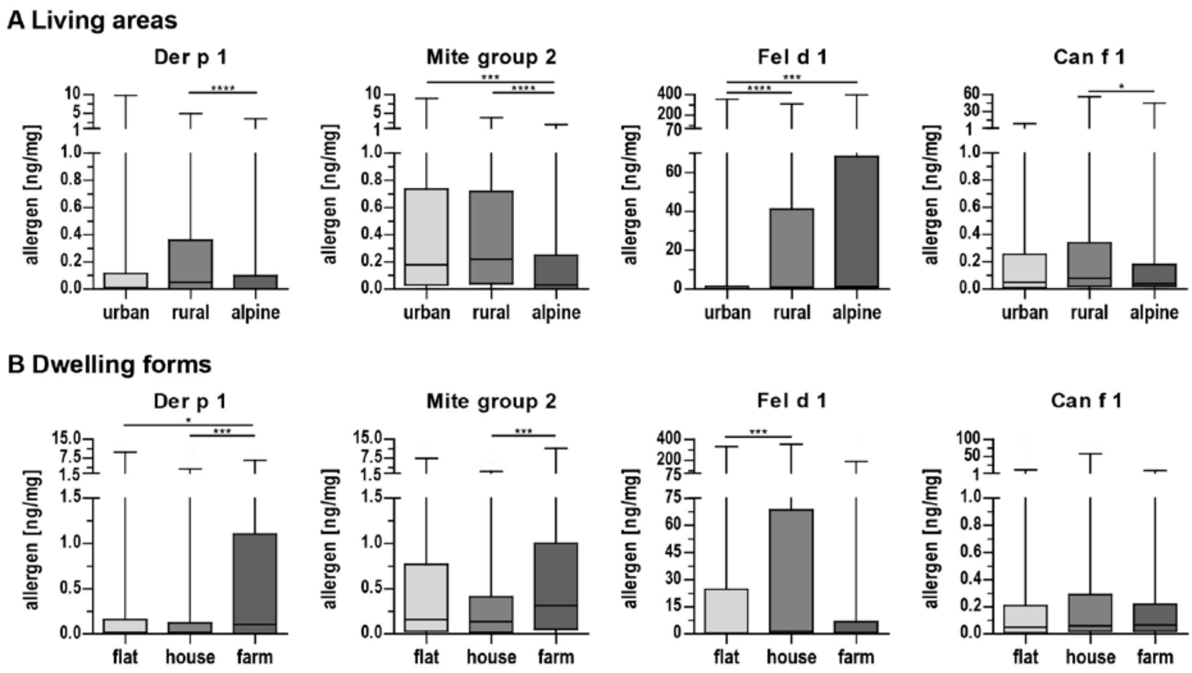
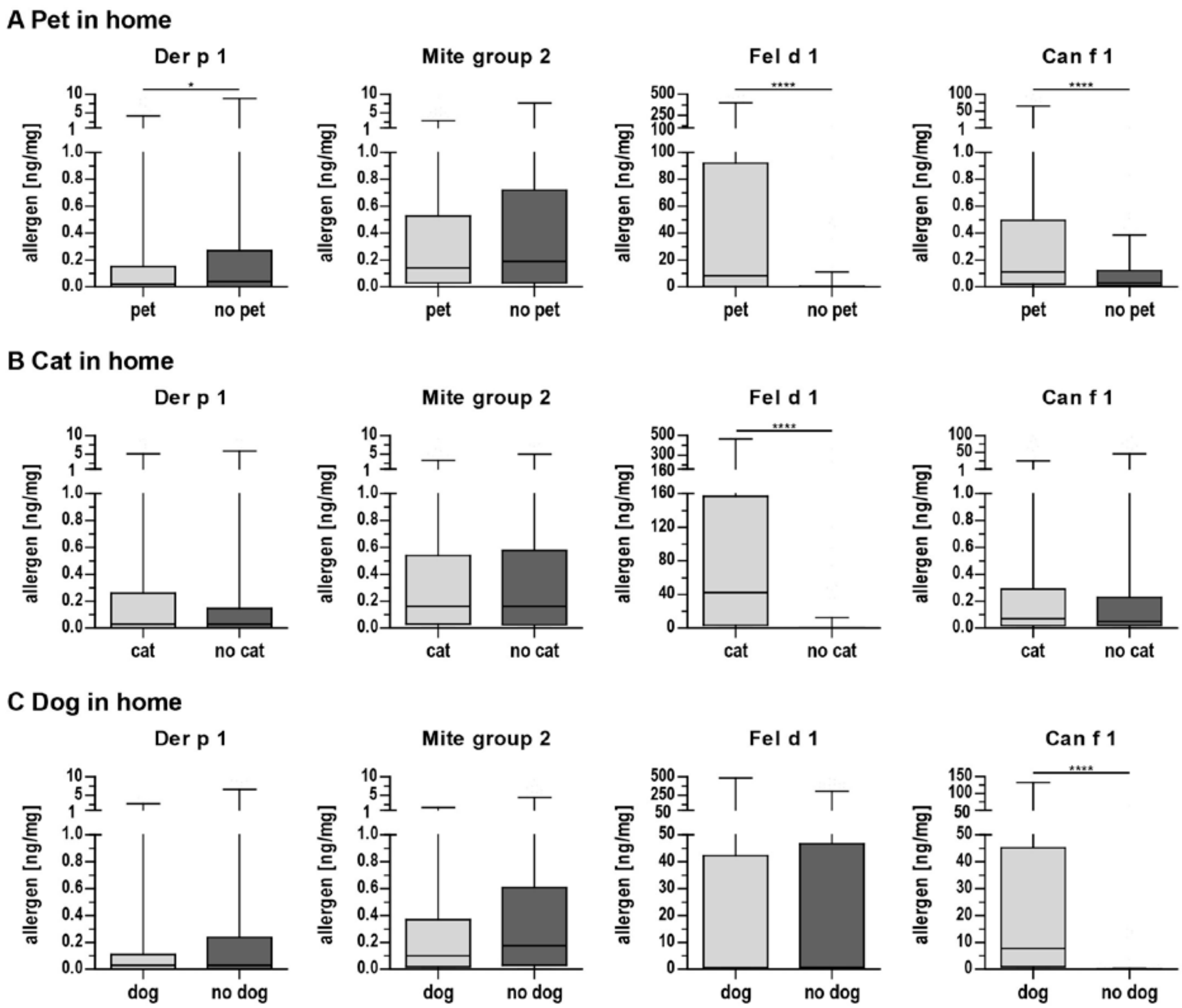

A Living areas
Der p 1
Mite group 2
Fel d 1
Can f 1
allergen [ng/mg]
urban
rural
alpine
B Dwelling forms
Der p 1
Mite group 2
Fel d 1
Can f 1
allergen [ng/mg]
flat
house
farm

Fig 2

534



535 Fig 3

536

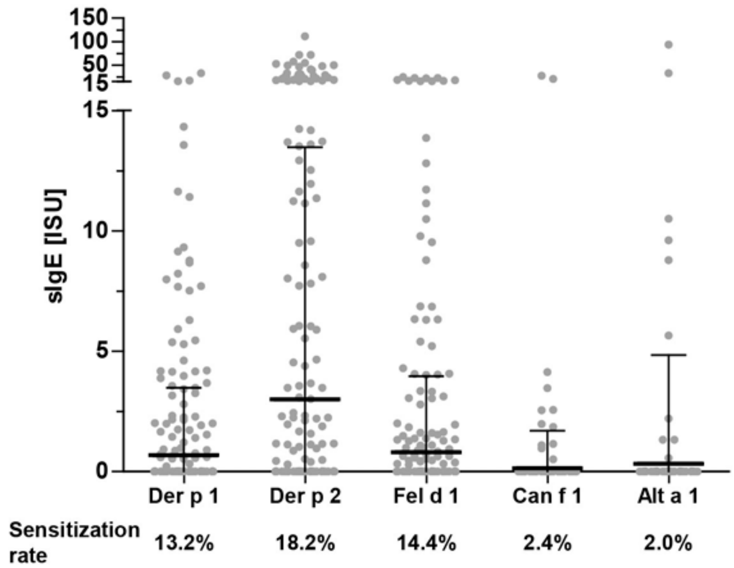

150
100
50
15
15
10
5
0
sIgE [ISU]
Der p 1
Der p 2
Fel d 1
Can f 1
Alt a 1
Sensitization rate
13.2%
18.2%
14.4%
2.4%
2.0%

Fig 4

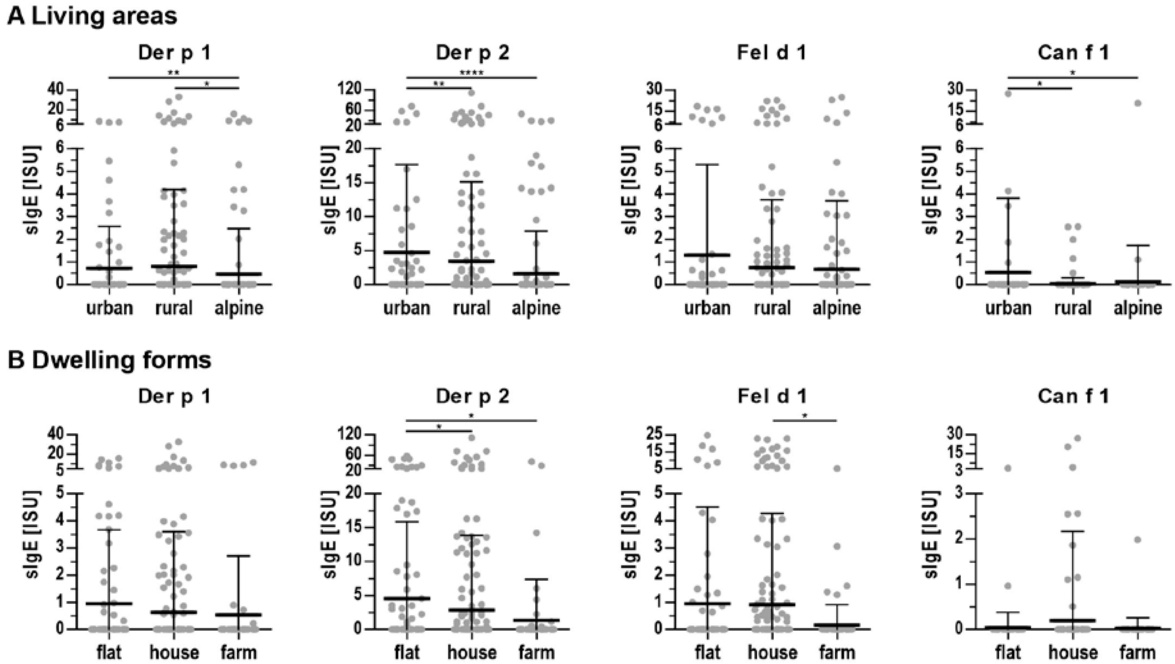

A Living areas
Der p 1
Der p 2
Fel d 1
Can f 1
sIgE [ISU]
urban
rural
alpine
B Dwelling forms
flat
house
farm

Fig 5

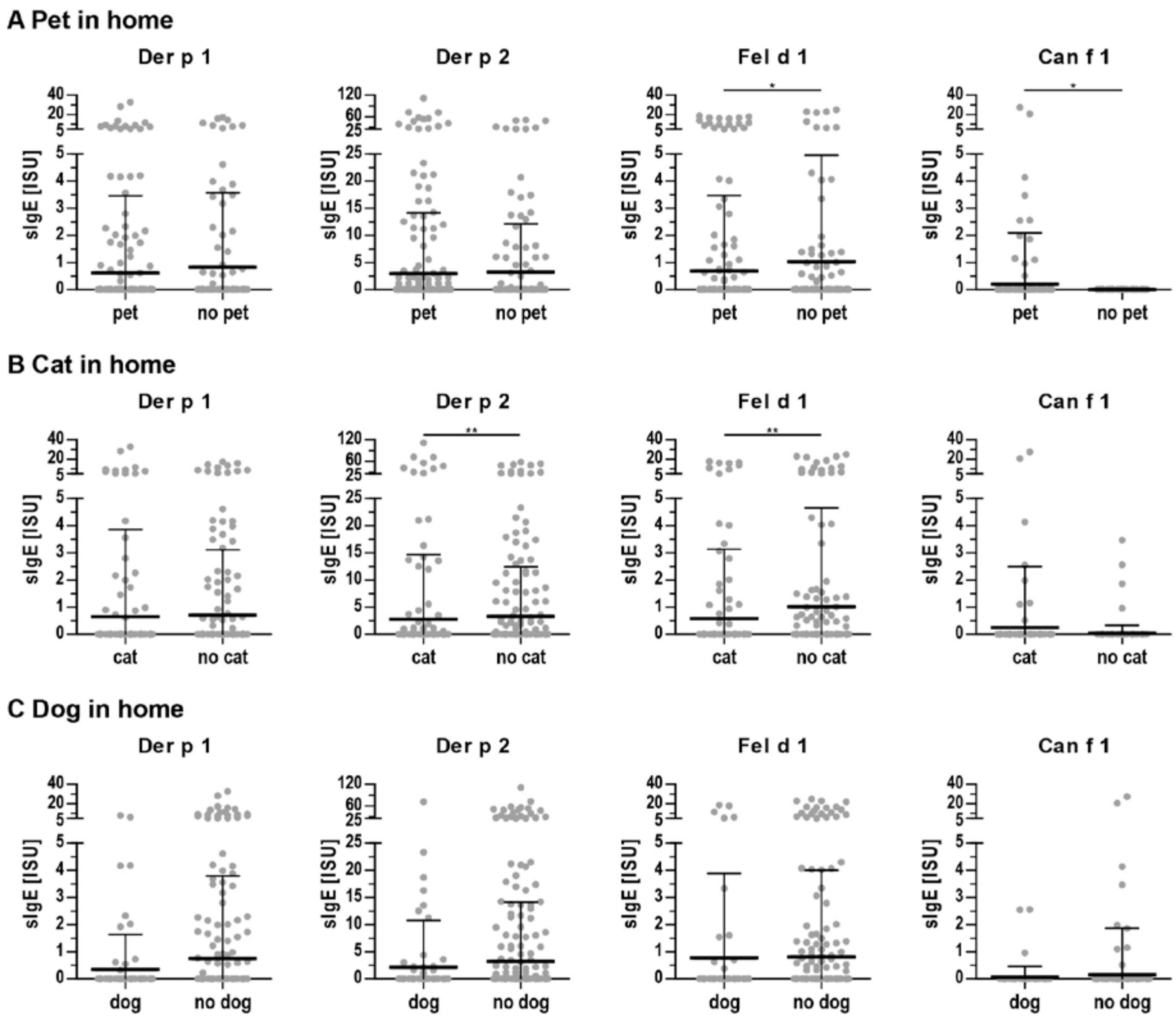

A Pet in home
B Cat in home
C Dog in home
Der p 1
Der p 2
Fel d 1
Can f 1
sIgE [ISU]
pet
no pet
cat
no cat
dog
no dog
*
**

Fig 6

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