Journal of Asthma and Allergy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Associations of Protein Classes With Cross-Reactivity and Cross-Sensitization in Furry Animal Allergens: A Component-Resolved Diagnostics Study

Jiancai Lu[1,*], Huiqing Zhu[2,*], Qingqing Yang[1], Yunjian Xu[1], Zhifeng Huang[1], Baoqing Sun[1]

[1]Department of Clinical Laboratory, National Center for Respiratory Medicine, National Clinical Research Center for Respiratory Disease, State Key Laboratory of Respiratory Disease, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong, 510120, People's Republic of China; [2]Department of Clinical Laboratory, Huizhou Central People`s Hospital, Huizhou, Guangdong, 516001, People's Republic of China

*These authors contributed equally to this work

Correspondence: Baoqing Sun; Zhifeng Huang, Department of Clinical Laboratory, National Center for Respiratory Medicine, National Clinical Research Center for Respiratory Disease, State Key Laboratory of Respiratory Disease, Guangzhou Institute of Respiratory Health, the First Affiliated Hospital of Guangzhou Medical University, 151 Yanjiang Road, Guangzhou, Guangdong, 510120, People's Republic of China, Tel +86 020 81568020, Email sunbaoqing@vip.163.com; zfhuang_gzhmu@163.com

Objective: This study aimed to investigate the molecular sensitization patterns of cats, dogs, and horses in patients with cat and/or dog sensitization and the IgE cross-reactivity with other furry animals.

Methods: In 95 patients diagnosed with allergic diseases and sensitized to cats and/or dogs (confirmed by specific Immunoglobulin E (sIgE) ≥ 0.35 kU_A/L to crude cat and/or dog dander extracts), sIgE levels of cat components (Fel d 1/2/4), dog components (Can f 1/2/3/5), horse dander (Equ c 1), as well as allergens from cow, guinea pig, mouse, rat, rabbit, and chicken, were measured. Sensitization profiles and cross-reactivity were analyzed. Inhibition tests using serum albumin (SA) and lipocalin proteins were performed.

Results: Sensitization rates of crude extracts from other furry animals ranged from 16.8% to 49.5%. A strong positive correlation between cat and dog serum albumin (Fel d 2 and Can f 3) and rabbit epithelium, mouse epithelium, guinea pig epithelium and rat epithelium (rs: 0.66–0.87, all $P < 0.05$), while the lipocalin family (Fel d 4, Can f 1, Can f 2 and Equ c 1) only had a low to moderate correlation with the epithelial allergens of the above four animals (rs: 0.36–0.65, all $P < 0.05$). Simultaneous sensitization to SA and these four furry animal allergens accounted for 42.4%. sIgE levels of furry animal extracts were significantly higher in SA-positive groups (all $P < 0.05$) The results of the inhibition test showed that Fel d 2 and Can f 3 had high inhibition rates of four epithelial allergens, ranging from 66.5% to 91.8% and 75.8% to 91.9%, respectively. When lipocalin family components were used as inhibitors, the sIgE inhibition rates of these furry animal extracts were almost all lower than 50%.

Conclusion: SA is the primary driver of cross-sensitization between cats, dogs, and other furry animals, rather than lipocalins.

Keywords: animal allergen, sIgE, component-resolved diagnosis, CRD, serum albumin, cross-reactivity

Introduction

Immunoglobulin E (IgE)-mediated allergic diseases caused by furry mammals are increasingly common, with sensitization to multiple animal allergens posing significant diagnostic and therapeutic challenges.[1] Accurate identification of the primary sensitizing components is crucial for targeted prevention and treatment.[2] While hair and dander from cats and dogs remain the primary sources of animal allergens, exposure to other furry animals, such as rabbits, rodents, and horses, is also prevalent, particularly in occupational or domestic settings.[3]

Cross-reactivity among animal allergens is largely driven by shared protein families, such as serum albumin (SA) and lipocalins, which exhibit high structural and sequence homology across species.[4] Proteins with greater than 62%

Received: 6 November 2024
Accepted: 25 February 2025
Published: 5 March 2025

sequence identity to human homologs rarely cause sensitization; however, SA and lipocalins are exceptions, as their high homology increases the risk of cross-reactivity.[5] For example, SA from cats (Fel d 2) and dogs (Can f 3) share significant sequence identity, leading to IgE cross-reactivity with SA from other mammals, such as horses, mice, and cattle.[6] Similarly, lipocalins, a diverse family of proteins, are major allergens in cats (Fel d 4), dogs (Can f 1/2), and horses (Equ c 1), with cross-reactivity observed due to conserved structural motifs.[7]

Component-resolved diagnostics (CRD) has revolutionized allergy diagnosis by enabling the identification of specific sensitizing proteins and distinguishing true IgE-mediated allergy from cross-reactivity.[8] Studies have demonstrated that sensitization to SA and lipocalins is associated with varying clinical outcomes, highlighting the importance of understanding the molecular basis of cross-reactivity.[9] For instance, sensitization to Fel d 2 and Can f 3 has been linked to polysensitization to multiple furry animals, while lipocalins are often associated with more severe respiratory symptoms.[10]

Despite these advances, the mechanisms underlying cross-reactivity and the clinical relevance of specific protein families remain incompletely understood. This study aims to investigate the molecular sensitization patterns of cats, dogs, and horses in patients with cat and/or dog sensitization and explore the IgE cross-reactivity with other furry animals. By analyzing the associations of SA and lipocalins with cross-sensitization and employing inhibition assays to assess homology-driven cross-reactivity, we aim to provide new insights into the molecular mechanisms of animal allergy.

Experimental Details

Subjects

Ninety-five patients diagnosed with allergic diseases such as allergic rhinitis (AR), asthma, or allergic rhinitis combined with asthma (AR&Asthma) were randomly selected from the preliminary epidemiological survey.[11] These patients were sensitized to cats and/or dogs (confirmed by Hycor system detection of sIgE $\geq$ 0.35 kU_A/L).

Allergen Specific IgE Measurement

The allergen sIgE levels in patients' serum samples were quantified using the ALLEOS 2000™ allergen detection system (Hycor Biomedical, USA), which employs a chemiluminescent magnetic micro-particle method. Briefly, horseradish peroxidase (HRP) was conjugated directly to the detection antibody, which interacted with biotinylated allergens and streptavidin-coated magnetic beads. Following the antigen-antibody reaction, the streptavidin-biotin complex formed an immune complex, which, upon addition of a chemiluminescent substrate, emitted a signal. The intensity of this signal was proportional to the concentration of total IgE in the sample. Allergen-specific IgE results were expressed in kU_A/L, with a dynamic detection range of 0.10–100 kU_A/L. For this study, an sIgE concentration of ≥0.10 kUA/L was considered positive, encompassing even low-level sensitizations to animal allergens. While a threshold of ≥0.35 kUA/L was used for sample inclusion, we placed particular emphasis on low-level detection for allergen components and less common animal allergens to ensure a comprehensive analysis. Conversely, IgE levels <0.10 kUA/L were classified as negative. Positive sIgE results were further stratified into seven classes: Class 1 (0.10–0.35 kU_A/L), Class 2 (0.35–0.70 kU_A/L), Class 3 (0.70–3.50 kU_A/L), Class 4 (3.50–17.50 kU_A/L), Class 5 (17.50–50.00 kU_A/L), Class 6 (50.00–100.00 kU_A/L), and Class 7 (≥100.00 kU_A/L).

SA and lipocalins are two major protein families implicated in allergen cross-reactivity. SA includes allergens such as Fel d 2 (cat) and Can f 3 (dog), while the lipocalin family comprises allergens such as Fel d 4 (cat), Can f 1 and Can f 2 (dog), and Equ c 1 (horse). These proteins are known for their structural similarities, which contribute to cross-reactivity among different furry animal allergens. If the patient is sensitized to cat dander (cat d) and/or dog dander (dog d) allergens, the patient's serum is further tested for cat's allergen components (Fel d 1/ 2/ 4), dog's allergen components (Can f 1 / 2 / 3 / 5), horse dander (horse d), and horse's allergen components (Equ c 1), as well as for the other common animal allergens: cow dander, guinea pig epithelium, mouse epithelium, mouse urine, rat epithelium, rabbit epithelium, and chicken feather sIgE concentrations.

Allergen Component-Specific IgE Inhibition Test

For the IgE-based inhibition tests with animal allergen components, we developed a methodology for sIgE-inhibition testing on the fully automated Hycor Alleos 2000. This methodology has been validated and is a well-established technique in our team, as demonstrated in previous studies.[12,13]

Allergen component antigens used for the inhibition assays were sourced from INDOOR Biotechnologies Inc. (USA). In the inhibition assay, specific allergen components, including Fel d 2, Can f 3, Fel d 4, Can f 1, Can f 2, and Equ c 1, were diluted in phosphate-buffered saline (PBS) to a final concentration of 100 μg/mL to serve as inhibitor solutions. For determination of serum sIgE levels, sensitized patient serum was preincubated with each allergen component inhibitor in a 1:1 ratio for 2 hours at 37°C. As a control, patient serum was preincubated with PBS under identical conditions (1:1 ratio, 37°C, 2 hours). The percentage of inhibition was calculated using the following formula:

$$\%\,\text{Inhibition} = \frac{\text{sIg E}_{\text{PBS}} - \text{sIg E}_{\text{inhibit}}}{\text{sIg E}_{\text{PBS}}} \times 100\%$$

sIgE_{PBS} represents the sIgE levels detected in patient serum preincubated with PBS under identical conditions (1:1 ratio, 37°C, 2 hours).

$\text{sIgE}_{\text{inhibit}}$ represents the sIgE levels detected in patient serum preincubated with each allergen component inhibitor under the same conditions (1:1 ratio, 37°C, 2 hours).

Statistical Analysis

The test results were integrated using Excel 2019 (Microsoft ® Excel®2019), and analyzed using GraphPad Prism 8.0.2 (©1992-2019 GraphPad Software, Inc)., R Studio 2022.07.2 (©2009-2022 RStudio, PBC), and IBM SPSS Statistics 26.0 (Chicago, IL). Image editing and integration were performed using Adobe Illustrator CC 2015.0.0 (Adobe Inc). Parametric quantitative data were presented as the mean ± standard deviation, and nonparametric quantitative data were presented as the median (interquartile range). Categorical data were reported as percentages. The *t*-test or Mann–Whitney *U*-test was used to compare numerical data between groups, the one-way analysis of variance (ANOVA) or Kruskal–Wallis *H*-test was used to compare distribution variances among multiple groups. Spearman correlation analysis was used to assess the correlation between non- normally distributed quantitative data. The strength of the correlation was interpreted as follows: 0.00–0.19 (very weak), 0.20–0.39 (weak), 0.40–0.59 (moderate), 0.60–0.79 (strong), and 0.80–1.00 (very strong). Bar graphs were used to display percentages. Upset plots, generated using UpSetR in R,[14] visualized co-sensitization patterns between SA, lipocalins, and other animal allergens. The Venn diagram was a visual display of the co-sensitization between different allergens. A $P < 0.05$ was considered statistically significant.

Results

Characteristics of the Object

Of the 95 patients included, 59 (62.1%) were males and the median age was 9 (6, 23) years (median (Q1-Q3)). The patients were divided into 2 groups according to age: 63 (66.3%) in the <18 years group and 32 (33.7%) in the ≥18 years group. This included 22 (23.2%) patients with allergic rhinitis, 42 (44.2%) patients with allergic asthma, and 31 (32.6%) patients with allergic rhinitis combined with asthma. Basic information of the 95 patients was shown in Table 1.

Specific IgE Sensitization Profile of Furry Animal Crude Extracts and Components

The sensitization rate to cat and dog dander was 96.8% among the patients included in this study. For the cat component allergens, the positivity rates were 90.5%, 23.2%, and 40.0% for Fel d 1, Fel d 2, and Fel d 4, respectively. For the dog component allergens, the positivity rates were 46.3% for Can f 1, 17.9% for Can f 2, 24.2% for Can f 3, and 25.3% for Can f 5. In comparison, the positivity rates for horse dander and its major component Equ c 1 were 49.5% and 15.8%, respectively. Among patients sensitized to cats and/or dogs, sensitization to other common furry animal allergens, including cow dander, guinea pig epithelium, mouse epithelium, mouse urine, rat epithelium, rabbit epithelium, and chicken feathers, was lower, with rates ranging from 16.8% to 47.4% (Figure 1A). Further analysis of component-specific IgE revealed that 92.3%, 23.9%, and 41.3% of cat sIgE-

Table 1 Basic Information of Research subjects

Patients	Numbers
Total, (n)	95
Gender, n (%)	
Male	59 (62.1)
Female	36 (37.9)
Age, n (%)	
Median (IQR)	9 (6, 23)
<18 years	63 (66.3)
≥18 years	32 (33.7)
Diagnosis, n (%)	
AR	22 (23.2)
Asthma	42 (44.2)
AR&Asthma	31 (32.6)

Abbreviations: n, Sample size; AR, Allergic rhinitis.

positive patients exhibited sIgE positivity for the cat components Fel d 1, Fel d 2, and Fel d 4, respectively (Figure 1B). Among dog sIgE-positive patients, 34.8% were not sensitized to any of the dog components (Can f 1, Can f 2, Can f 3, or Can f 5) (Figure 1C). In contrast, among equine sIgE-positive patients, only 31.9% were sensitized to Equ c 1, the primary sensitizing component in horses (Figure 1D).

Analysis of Potential Associations Between Furry Animal Components and Other Multiple Allergens

Correlation and optimal scaling analyses were conducted on 10 furry animal allergen extracts, as well as cat, dog, and horse components, to investigate potential associations. The results of the correlation analysis (Figure 2A) revealed a strong correlation between cat dander and its major allergenic component, rFel d 1 (r spearman' rho = 0.93), while the correlation with Fel d 4 was notably lower (r spearman' rho = 0.31). No significant correlation was observed between rFel d 1 and other furry animal extracts and components ($P > 0.05$). The correlation coefficients between dog extracts and dog components (Can f 1/2/3) ranged from 0.59 to 0.81, with no significant correlation detected for Can f 5. Similarly, the correlation coefficient between horse dander and its primary component, Equ c 1, was 0.60. Additionally, there was a strong positive correlation between serum albumins (nCan f 3 and Fel d 2) and extracts from rabbit epithelium, mouse epithelium, guinea pig epithelium, and rat epithelium (r spearman' rho 0.66–0.87), with a particularly high correlation observed between nCan f 3 and Fel d 2 (r spearman' rho = 0.87). In contrast, allergens from the lipocalin family (Fel d 4, Can f 1, Can f 2, and Equ c 1) showed only low to moderate correlations (r spearman' rho 0.36–0.65) with the aforementioned epithelial-derived allergens. No statistically significant correlation was found between Fel d 4 and the four epithelial allergens (data not shown). Furthermore, optimal scaling analysis indicated that serum albumins were potentially associated with mouse epithelium, guinea pig epithelium, rat epithelium, rabbit epithelium, and chicken feathers in fur-bearing animals (Figure 2B).

Cross-Sensitization Analysis of Serum Albumin and Lipocalin From Cats and Dogs With Other Furry Animal Extracts

An Upset plot was used to visualize cross-sensitization patterns between SA (Fel d 2 and nCan f 3) and lipocalin (rCan f 1, Can f 2, Fel d 4, and Equ c 1) with other animal allergens (Figure 3). The results indicated that the most frequent cross-sensitization involved SA and rabbit epithelium, mouse epithelium, rat epithelium, and guinea pig epithelium, accounting for 42.4% (14/33) of the cases. In total, sensitization to SA in combination with any of the aforementioned epithelial-derived allergens constituted 63.6% (21/33) of the sensitization cases (Figure 3A). In contrast, simultaneous

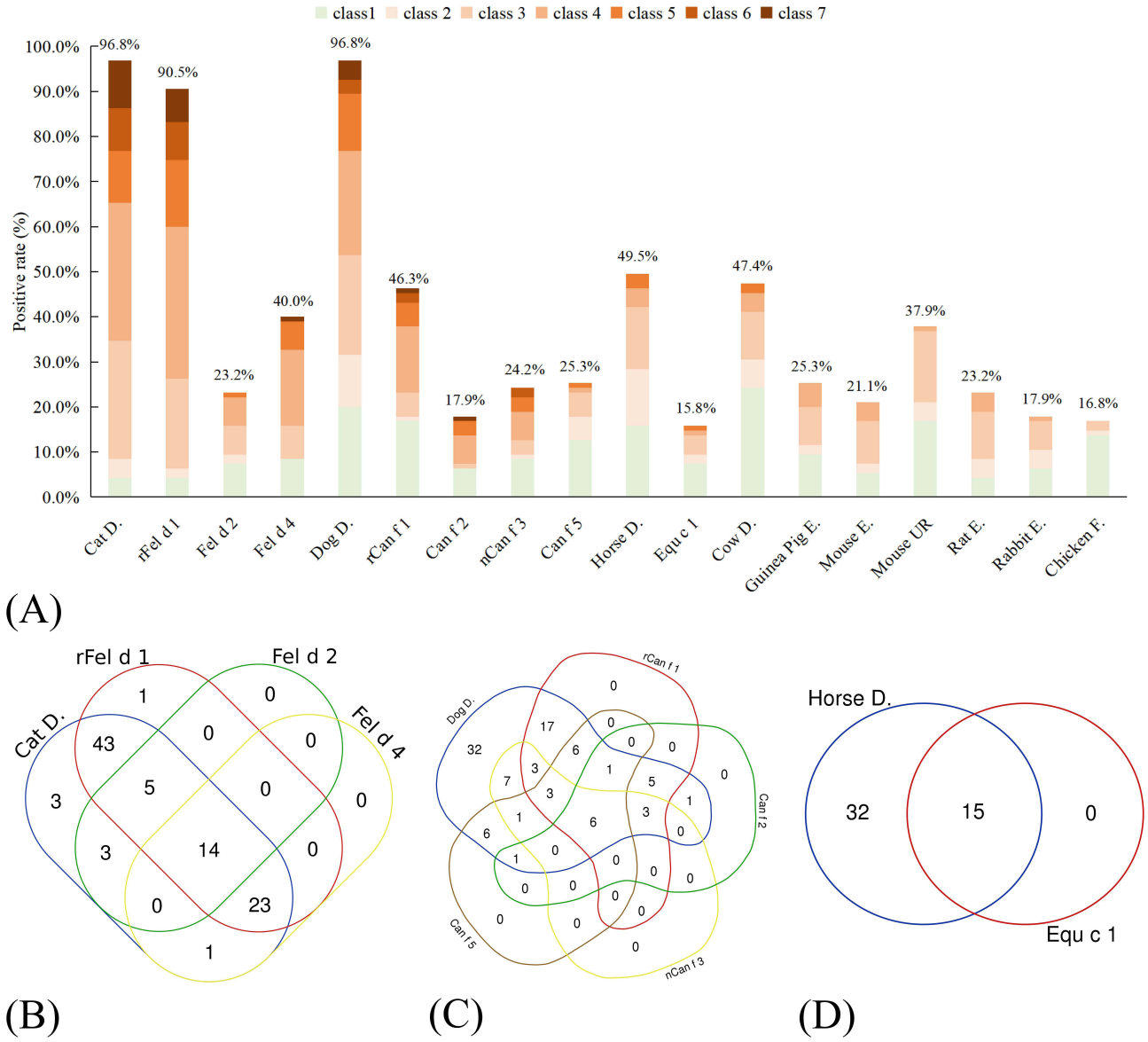

Figure 1 Positive rates of allergen extracts and components in furry animals (**A**), and overlap positivity rates for cat D. (**B**) dog D. (**C**), and horse D. (**D**) with their respective components. D.- dander, E- epithelium, Fel d 1/ 2/ 4- Components of cat (*Felis domesticus*), Can f 1/ 2/ 3/ 5- Components of dog (*Canis familiaris*), Equ c 1- Component of horse (*Equus caballus*).

sensitization to SA and allergens from chicken feathers, mouse urine, cattle dander, or horse dander was less common (Figure 3B), comprising only 7.2% (5/69) of the cases. In contrast to SA, lipocalin proteins were less frequently co-sensitized with rabbit epithelium, mouse epithelium, rat epithelium, and guinea pig epithelium, accounting for only 17.9% (12/67) of cases (Figure 3C). Similarly, only 6.4% (5/78) of lipocalin proteins were co-sensitized with chicken feathers, mouse urine, cow dander, and horse dander (Figure 3D).

Impact of Sensitization to Serum Albumin or Lipocalin on the Levels of Sensitization to Other Furry Animal Allergens

To further investigate the potential association of SA and lipocalin with other furry animal allergens, we compared the differences in sIgE levels for various animal allergens between SA- and lipocalin-positive and -negative groups. In the Fel d 2-positive group, significantly higher sIgE levels were observed for dog dander [15.21 (1.87, 70.06) kU_A/L vs

https://doi.org/10.2147/JAA.S505066

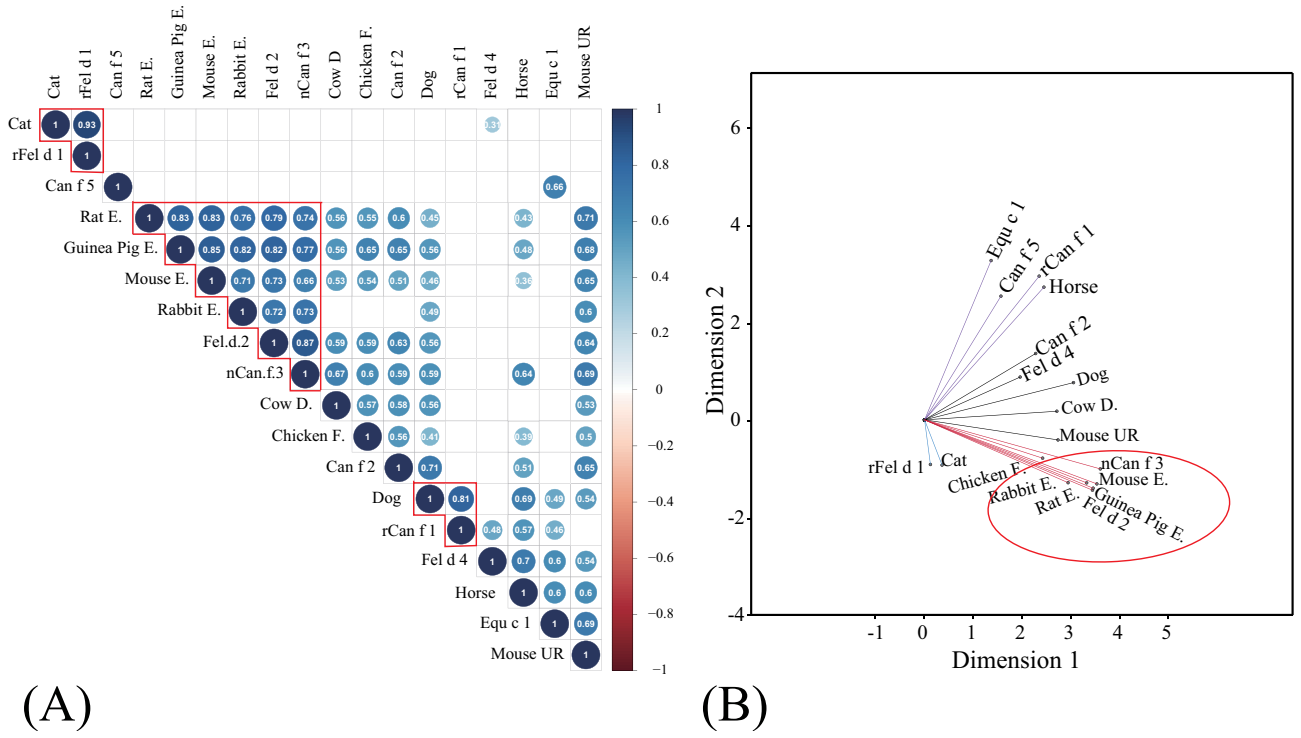

Figure 2 Spearman-rho correlation analysis (**A**) with optimal scale analysis (**B**) between all furry animal allergens (crude extracts and fractions). Note: Values are not shown when there is no significant difference in correlation between two allergens (P >0.05), and the depth of the color between the two allergens represents the strength of the correlation; optimal scale analysis was performed using specific IgE levels as a categorical variable, with the closer the to points of an allergen being to each other, the higher the correlation. D.- dander, E- epithelium, Fel d 1/ 2/ 4- Components of cat (*Felis domesticus*), Can f 1/ 2/ 3- Components of dog (*Canis familiaris*), Equ c 1- Component of horse (*Equus caballus*).

0.99 (0.28, 4.78) kU_A/L], horse dander [0.85 (0.23, 3.34) kU_A/L vs 0.05 (0.04, 0.19) kU_A/L], cow dander [0.95 (0.12, 4.02) kU_A/L vs 0.07 (0.03, 0.14)kU_A/L], guinea pig epithelium [1.34 (0.21, 3.10) kU_A/L vs 0.03 (0.02, 0.05)kU_A/L], mouse epithelium [1.27 (0.18, 3.15) kU_A/L vs 0.03 (0.02, 0.04) kU_A/L], mouse urine [0.39 (0.13, 1.43) kU_A/L vs 0.02 (0.02, 0.11) kU_A/L], rat epithelium [0.89 (0.16, 2.18) kU_A/L vs 0.02 (0.02, 0.04) kU_A/L], rabbit epithelium [0.33 (0.07, 1.53) kU_A/L vs 0.02 (0.02, 0.03)kU_A/L], and chicken feathers [0.10 (0.06, 0.18) kU_A/L vs 0.04 (0.03, 0.06)kU_A/L] compared to the Fel d 2-negative group (all $P < 0.001$) (Figure 4A). Similarly, sIgE levels for these nine animal allergens were significantly higher in the nCan f 3-positive group, compared to the nCan f 3-negative group (all $P <$ 0.05) (Figure 4B).

We defined patients as lipocalin-positive (Lipocalin Po) if they tested positive for any of Fel d 4, rCan f 1, Can f 2, or Equ c 1, and as lipocalin-negative (Lipocalin Ne) if they tested negative for all these allergens. In the lipocalin-positive group, sIgE levels for dog dander [5.14 (2.04, 24.30) kU_A/L vs 0.58 (0.20, 1.02) kU_A/L] and horse dander [0.23 (0.05, 1.55) kU_A/L vs 0.04 (0.03, 0.09) kU_A/L] were significantly higher than in the lipocalin-negative group (all $P < 0.001$). Regarding other animal allergens, although the overall sIgE levels were relatively low in both groups, there were significant differences between the groups for guinea pig epithelium [0.05 (0.03, 0.16) kU_A/L vs 0.03 (0.02, 0.05) kU_A/L, $P = 0.034$], rabbit epithelium [0.03 (0.02, 0.06) kU_A/L vs 0.02 (0.02, 0.04) kU_A/L, $P = 0.041$], and chicken feathers [0.05 (0.03, 0.08) kU_A/L vs 0.03 (0.02, 0.06) kU_A/L, $P = 0.011$] (Figure 5).

Inhibitory Effects of Serum Albumin and the Lipocalin on sIgE Responses to Animal Allergens

In allergen component sIgE inhibition assays, SA (Fel d 2 and nCan f 3), and lipocalin proteins (Fel d 4, rCan f 1, Can f 2, and Equ c 1) were used as allergen inhibitors at a concentration of 100 μg/mL. The results showed that the mean self-

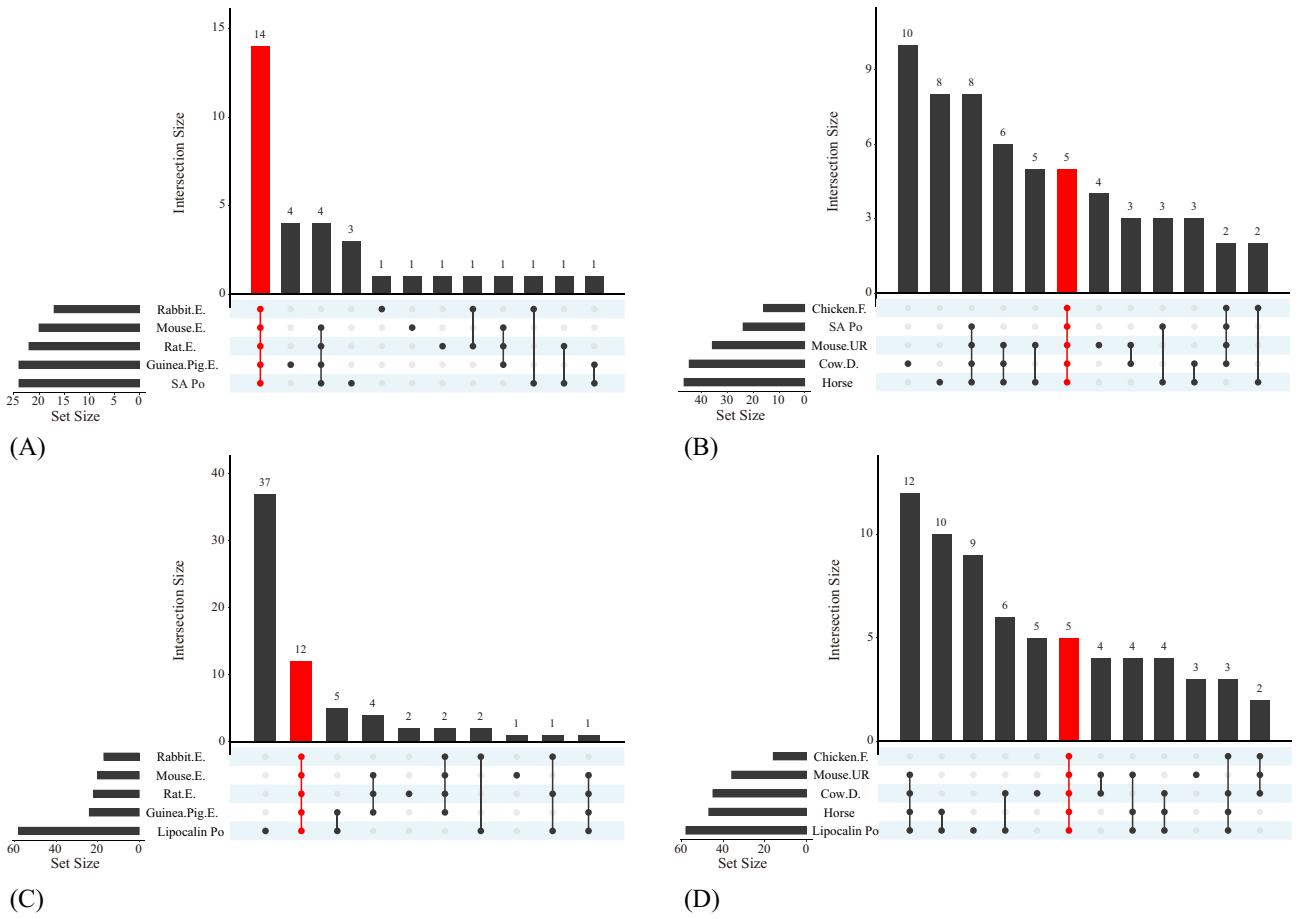

Figure 3 Upset plots of serum albumin (SA) and lipocalins against other furry animal extracts (**A-D**), showing cross-sensitization patterns with rabbit, mouse, rat, Guinea pig epithelium (**A** and **C**), and chicken feathers, mouse urine, cow dander, and horse dander (**B** and **D**). Note: The bars in the lower left corner show the number of positives for each allergen, the black color in the lower right corner indicates that data are available at that location, the different black dots connecting the lines indicate that there is an intersection between the allergens, and the bars above indicate the number of instances contained in each type of set. SA Po refers to Can f 3 and/or Fel d 2 sensitization; Lipocalin Po indicates sensitization to one or more of Fel d 4, rCan f 1, Can f 2, or Equ c 1 allergens. Upset Plot shows only the first 12 sets with higher frequencies. D.- dander, E- epithelium.

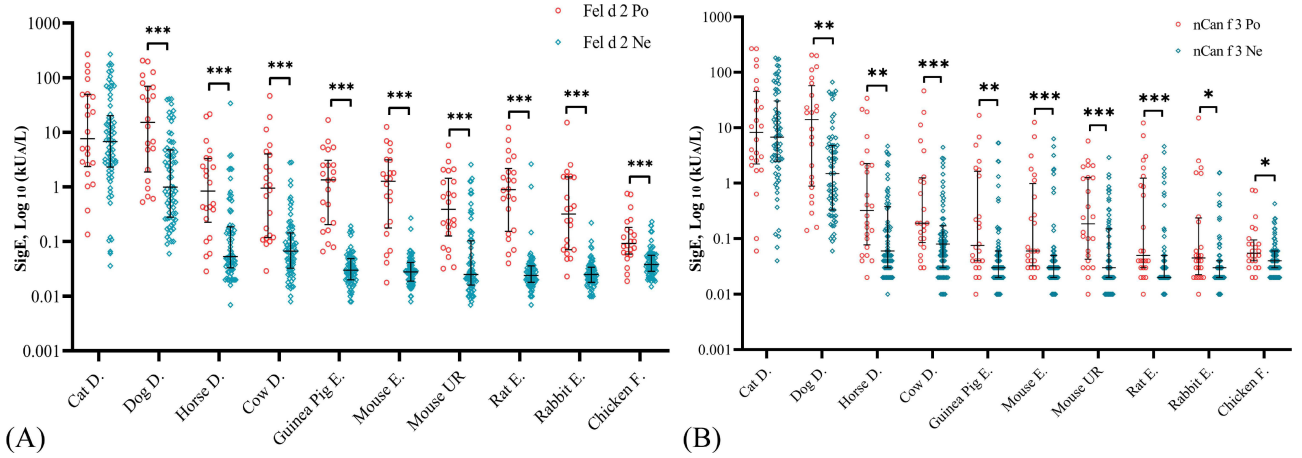

Figure 4 Differences in sIgE levels in serum albumin (SA) positive (Po) or negative (Ne) groups for each animal allergen extract: Fel d 2-positive (n=22) vs Fel d 2-negative (n=73) (**A**), and Can f 3-positive (n=23) vs Can f 3-negative (n=72) (**B**). Po- Positive. Ne- Negative. D.- dander, E- epithelium, Fel d 2- Component of cat (*Felis domesticus*), Can f 3- Component of dog (*Canis familiaris*), Equ c 1- Component of horse (*Equus caballus*).*P < 0.05, **P < 0.01, ***P < 0.001.

https://doi.org/10.2147/JAA.S505066

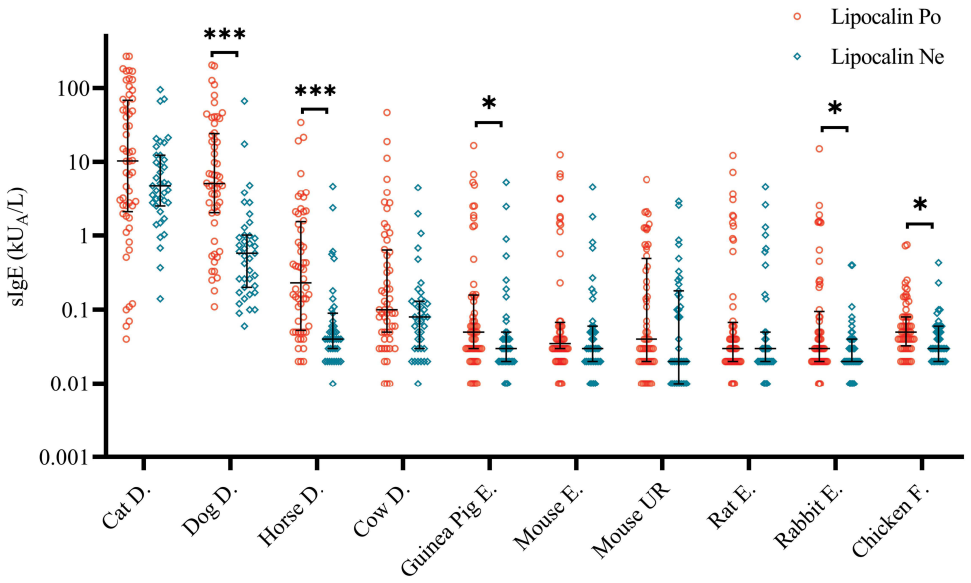

Figure 5 Differences in sIgE levels in Lipocalin-positive or negative groups for each animal allergen extract. Lipocalin Po (n=56) indicates sensitization to one or more of Fel d 4, rCan f 1, Can f 2, or Equ c 1 allergens. Lipocalin Ne (n=39) indicates sensitization to none of Fel d 4, rCan f 1, Can f 2, or Equ c 1 allergens. D.- dander, **E**- epithelium. *P < 0.05, ***P < 0.001.

inhibition rates for Fel d 2, nCan f 3, Fel d 4, rCan f 1, Can f 2, and Equ c 1 were 96.4%, 93.3%, 66.8%, 90.2%, 99.1%, and 75.4%, respectively, indicating near-complete inhibition (Figure 6A). The Fel d 2 inhibitor demonstrated an average inhibition rate of 76.4% against Can f 3, another serum albumin, while the nCan f 3 inhibitor exhibited a higher average inhibition rate of 90.4% against Fel d 2 (Table 2, Figure 6B and 6D). Both Fel d 2 and nCan f 3 inhibitors showed strong inhibitory effects on sIgE binding to guinea pig epithelium, mouse epithelium, rat epithelium, and rabbit epithelium, with inhibition rates ranging from 66.5% to 91.8% and from 75.8% to 91.9%, respectively (Table 2, Figure 6C and 6E). In contrast, the four lipocalin inhibitors exhibited much lower inhibition rates against these furry animal extracts, with most inhibition rates falling below 50%. Specifically, the inhibition of sIgE binding to guinea pig epithelium, mouse epithelium, rat epithelium, and rabbit epithelium by lipocalin proteins ranged from 25.3% to 43.2% (Table 2 and Figures S1A-H).

Discussion

Sennsitization patterns of cats, dogs, and horses in patients with cat and/or dog sensitization and explored IgE cross-reactivity with other furry animals. Our results revealed that serum albumin (SA) is the primary driver of cross-sensitization between cats, dogs, and other furry animals, rather than lipocalins. Sensitization rates to other furry animals ranged from 16.8% to 49.5%, with strong correlations observed between SA (Fel d 2 and Can f 3) and rabbit, mouse, guinea pig, and rat epithelium (rs: 0.66–0.87, all P < 0.05). Inhibition tests demonstrated high inhibition rates for SA (66.5–91.9%) but low rates for lipocalins (<50%). These findings provide new insights into the molecular mechanisms underlying cross-reactivity among furry animal allergens.

Furry animals, particularly pets such as cats and dogs, are significant sources of indoor allergens and are considered major risk factors for allergic rhinitis and asthma.[15] Studies have reported that cat allergies affect 7% to 25% of the population, and pet allergies have become an increasingly serious public health concern.[16–18] In Western countries, up to 60% of households have pets, and similarly, cats and dogs are the most popular pets in China.[17] In addition, small mammals and farm animals are also common sources of indoor and outdoor inhalant allergens to which individuals are frequently exposed in daily life or at work.[3] Studies have demonstrated that animal allergens can easily be disseminated to public spaces such as schools, daycare centers, public transportation, and the homes of non-pet owners.[3] Data from a European study based on skin prick testing (SPT) indicated that in 2009, 26% and 27% of adults were allergic to cats and dogs, respectively.[19] In our previous multicenter study, the prevalence of sensitization to cats, dogs, and horses

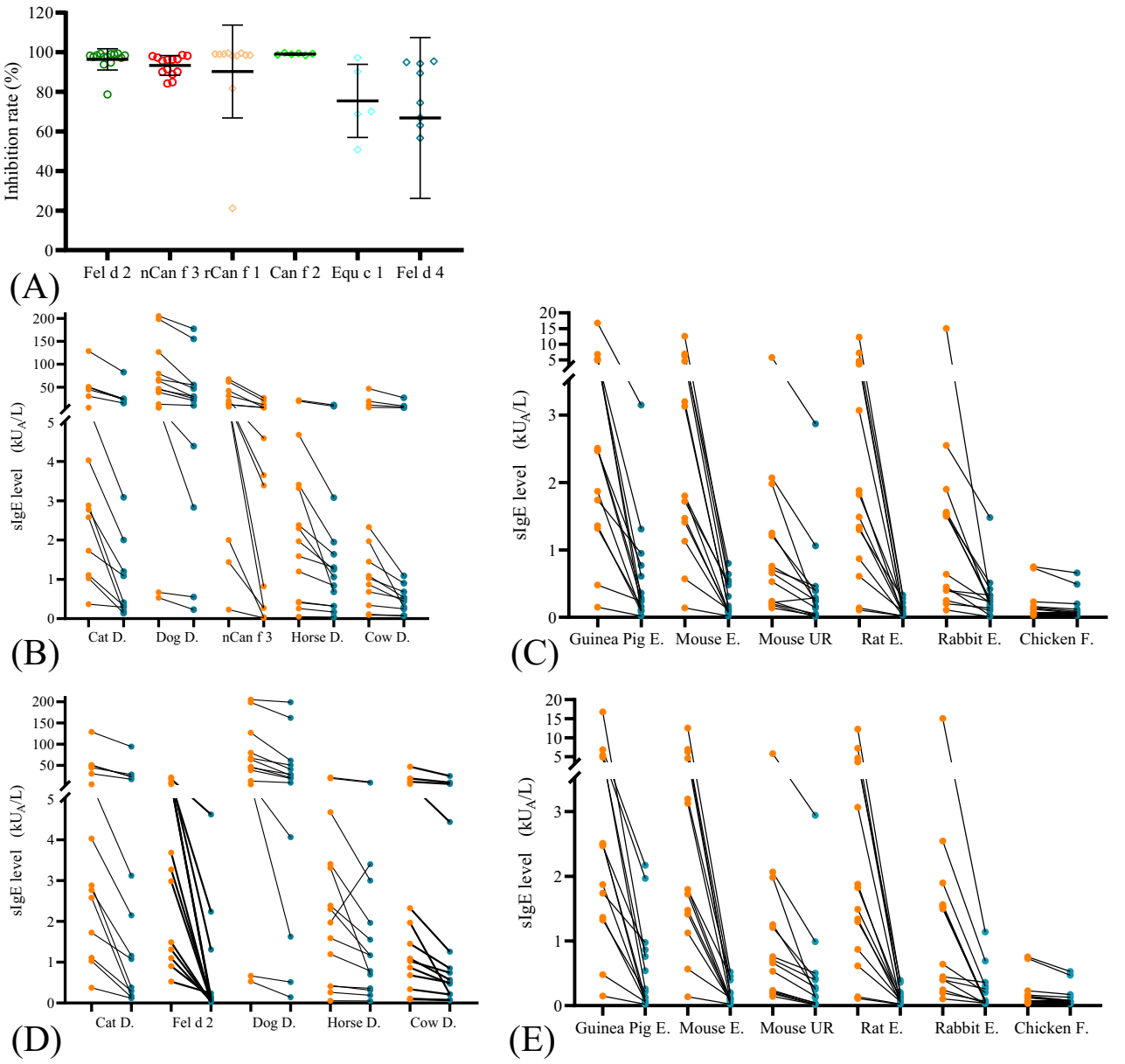

Figure 6 Self-inhibition rates of individual allergen components (**A**). Changes in sIgE concentrations before and after inhibition using serum albumin Fel d 2 as the inhibitor for nCan f 3 and various allergen extracts (**B** and **C**); changes in sIgE concentrations before and after inhibition using serum albumin Can f 3 as the inhibitor for Fel d 2 and various allergen extracts (**D** and **E**). The lines connecting the data points represent pre- and post-inhibition values for the same patient. Yellow dots indicate pre-inhibition concentrations, while blue dots indicate post-inhibition concentrations. D.- dander, E- epithelium, Fel d 1/ 2/ 4- Components of cat (*Felis domesticus*), Can f 1/ 2/ 3- Components of dog (*Canis familiaris*), Equ c 1- Component of horse (*Equus caballus*).

among Chinese patients with suspected allergic diseases was 14.9%, 9.3%, and 5.5%, respectively.[11] Sensitization prevalence varied by age, increasing steadily throughout childhood, peaking in adolescence, and subsequently declining.[11] Given that animal allergies can significantly diminish patients' quality of life, allergists often advise avoiding contact with the offending animal. As a result, such allergies may restrict interactions between individuals and their pets, potentially affecting the human-animal bond. Thus, further investigation into allergic diseases triggered by furry animals is critical for the prevention, accurate diagnosis, and effective treatment of animal allergies.

In this study, we recruited 95 patients with AR and/or asthma to examine the CRD-based sensitization profiles of various crude extracts and allergenic components from furry animals, and to identify the major proteins responsible for cross-reactivity between these animal allergens. A key advantage of CRD is the ability to identify major sensitizing

https://doi.org/10.2147/JAA.S505066

Table 2 Inhibition of Serum Albumin and Lipocalin Proteins Against Each Allergen

Allergens	SA Inhibitor		Lipocalin Inhibitor			
	Fel d 2	Can f 3	Fel d 4	rCan f 1	Can f 2	Equ c 1
Cat D.	55.3%	57.4%	36.5%	31.6%	30.2%	42.5%
Fel d 2	96.4%	90.4%				
Fel d 4			66.8%	46.5%	47.0%	45.3%
Dog D.	35.9%	41.1%	31.8%	39.2%	36.1%	31.3%
rCan f 1			44.9%	90.2%	39.6%	39.7%
Can f 2			47.4%	45.3%	99.1%	42.5%
nCan f 3	76.4%	93.3%				
Horse D.	38.3%	28.9%	53.2%	41.4%	43.0%	71.8%
Equ c 1			67.3%	31.9%	45.6%	75.4%
Cow D.	39.5%	41.5%	48.7%	28.2%	30.8%	52.3%
Guinea Pig E.	81.6%	82.9%	42.7%	39.8%	41.7%	38.4%
Mouse E.	88.7%	91.2%	43.2%	42.7%	41.0%	40.6%
Mouse UR	61.1%	69.9%	45.9%	33.2%	23.7%	51.9%
Rat E.	91.8%	91.9%	41.1%	43.2%	37.1%	34.1%
Rabbit E.	66.3%	75.8%	39.0%	42.1%	32.9%	25.3%
Chicken F.	21.3%	29.2%	8.9%	22.1%	35.0%	2.8%

Notes: Inhibitor concentrations are all 100 μg/mL. The formula for the inhibition rate is given in the Methods section. The mean inhibition rate is the average of the inhibition rates of all samples. D.- dander, E.- epithelium, el d 1/ 2/ 4- Components of cat (*Felis domesticus*), Can f 1/ 2/ 3/ 5- Components of dog (*Canis familiaris*), Equ c 1- Component of horse (*Equus caballus*).

proteins with enhanced detection sensitivity. In this study, Fel d 1 was identified as the predominant cat allergen, with 92.4% of cat-sensitized individuals reacting to Fel d 1, consistent with previous literature.[20] Fel d 1, a secreted bead protein, accounts for 60–90% of IgE responses to cat dander and exhibits minimal cross-reactivity with other mammalian allergens.[20] Our correlation and optimal scaling analyses further demonstrated that Fel d 1 was strongly correlated with IgE responses to cat crude extracts, with no significant correlations observed with other animal allergens. Fel d 2 and Can f 3, SAs from cats and dogs, are considered minor allergens. Approximately 15–35% of cat- and dog-allergic individuals exhibit IgE reactivity to Fel d 2 and Can f 3.[4,20,21] In the present study, the sensitization rates for Fel d 2 and Can f 3 were 23.2% and 24.2%, respectively, which aligns with findings from previous studies. Moreover, a significant correlation was observed between the two allergens. We examined four lipocalin proteins from cats, dogs, and horses, which are major allergens in various furry animals and have been associated with disease severity.[8] However, the rates of lipocalin sensitization observed in our study were lower than those reported in previous studies. IgE reactivity to Fel d 4 has been reported in up to 63% of cat-sensitized individuals, with evidence of cross-reactivity to other lipocalin proteins such as Can f 6 and Equ c 1.[22] In this study, the positivity rate for Fel d 4 was only 40.0%, while those for Can f 1 and Can f 2 were 46.3% and 17.9%, respectively, all lower than the rates previously reported. Additionally, 88.2% of patients with Can f 2-specific antibodies also exhibited IgE reactivity to Can f 1. Prostatic kallikrein Can f 5 is a protein expressed exclusively in the prostate gland of male dogs, and it shows no significant cross-reactivity with other furry animal allergens.[23] Among equine-sensitized patients, only 31.9% recognized Equ c 1, the primary sensitizing component. This discrepancy may partly be attributed to the fact that patients in this study were sensitized solely to cats and/or dogs, and it is possible that some individuals with low-level sensitization did not exhibit clinical symptoms related to animal allergy. Additionally, the lower rate of Equ c 1 positivity may be influenced by several factors, including regional differences in allergen exposure, variations in the allergenic potency of Equ c 1 in different horse breeds, or the presence of other minor allergens in horse dander that may contribute to sensitization. Differences in diagnostic methods and patient populations across studies may also play a role.

Cross-reactivity is commonly observed among allergens within the same protein family, and this is particularly evident in serum albumins and lipocalins from furry animals. Research data indicate that exposure and sensitization to

common pets (cats/dogs) significantly increase the risk of sensitization to other furry animals (such as rabbits, hamsters, rats, mice, guinea pigs, horses, and cattle).[24] This may be attributed to cross-reactivity driven by lipocalins, especially in cases where there has been no direct or indirect contact with these animals.[25] In this study, both correlation and optimal scaling analyses indicated a significant positive association between SA and other mammalian allergens. The correlation coefficients between SA and the four furry mammalian allergens (mice, rats, guinea pigs, rabbits) ranged from 0.66 to 0.87. Differential analysis of sIgE levels in individuals sensitized or non-sensitized to SA or lipocalin also supported the conclusion that SA is the primary protein responsible for cross-sensitization between cats, dogs, and other mammalian allergens. Our results suggest that the increased risk of sensitization to other furry animals, due to sensitization to common pets, is largely attributable to cross-reactive SA proteins, rather than proteins from the lipocalin family. While previous studies have identified lipocalins as key drivers of cross-reactivity among furry animal allergens,[7,10] our findings highlight serum albumin (SA) as the primary contributor. SA demonstrated high inhibition rates (66.5–91.9%) for epithelial allergens, whereas lipocalins showed lower rates (<50%), likely due to structural diversity and limited sequence homology within the lipocalin family.[26] The restricted panel of lipocalin components tested (eg, Fel d 4, Can f 1, Can f 2, Equ c 1) and the precise IgE inhibition methodology of the Hycor Alleos 2000 system may also explain these differences. Additionally, dietary factors, such as exposure to dairy proteins, may influence lipocalin sensitization, as lipocalins in cow's milk (Bos d 5) share structural similarities with those in furry animals.[7,10] The high sequence homology between albumins from different species explains the broad cross-reactivity observed between patients' IgE antibodies and albumins from various species.[27] As a result, these IgE antibodies were strongly inhibited by albumins in inhibition assays.

In allergen-specific IgE inhibition assays, our data further confirmed that the cross-reactivity between common pet allergens and other mammalian allergens is primarily driven by SA, while lipocalins only partially inhibited the specific IgE response to mammalian extracts. When Can f 3 and Fel d 2 inhibitors were pre-incubated with the sera of individuals sensitized to guinea pig, mouse, rat, and rabbit epithelium, Can f 3 exhibited the strongest inhibition, ranging from 75.8% to 91.9%, while Fel d 2 also showed a high degree of inhibition of sIgE binding to these allergens. Regardless of the specific lipocalin protein, inhibition rates were generally between 20% and 50%. Nevertheless, the lipocalin protein family still plays a role in the cross-reactivity of animal allergens, as most mammalian allergens identified as major sensitizers belong to the lipocalin family. These include major allergens from horse (Equ c 1), cattle (Bos d 2), guinea pig (Cav p 1/2), rat (Rat n 1), and mouse (Mus m 1).[5,7] Additionally, there has been limited research on the IgE-mediated cross-reactivity between lipocalins from different allergen sources.[26] Among the four lipocalin proteins tested in this study, the mutual inhibition rates ranged from 31.9% to 67.3%, with Fel d 4 exhibiting the highest inhibitory potency. The characteristic of the lipocalin family is their highly similar three-dimensional structures and relatively low sequence homology, which likely explains the cross-reactivity observed among lipocalins.[6,28] Notably, whether using SA or lipocalin as inhibitors, the inhibition rates for chicken feather allergens were consistently low (2.8–35%), which aligns with the previously observed low correlation between chicken allergens and the components tested.

In our previous research, patients sensitized to SA exhibited higher nasal symptom scores compared to the non-sensitized group, along with poorer quality of life scores.[29] Additional studies have further supported a significant association between SA sensitization and the diagnosis of moderate to severe rhinitis and asthma.[30] Our findings suggest that SA is a key factor contributing to polysensitization to multiple mammalian allergens. For patients with common pet allergies, this cross-reactivity may pose a relevant risk, as incidental exposure to other furry animals could trigger allergic reactions due to shared SA epitopes. However, further studies are needed to directly assess the clinical impact of SA-mediated cross-reactivity on respiratory symptoms in these patients.

This study has several limitations. First, we lacked data on clinical symptoms, and therefore were unable to conduct an in-depth analysis of the relationship between sensitization to specific components and disease severity. Second, due to the unavailability of commercial sIgE test reagents for allergen components from other furry animals and some known cat and dog molecular allergens (eg, Fel d 3, Fel d 7, Can f 4, Can f 6), we were unable to further analyze allergens specific to rarer animals or fully capture the complete sensitization profiles. This limitation highlights the need for broader allergen panels and improved detection methods in future studies. Additionally, the patients included in this study were sensitized to cats and/or dogs. However, as the institutional ethics committee did not approve allergen challenge

tests, we were unable to confirm whether these patients were truly allergic to cats or dogs, especially in those with low sIgE levels.

Conclusion

In conclusion, our study utilized CRD to characterize the major sensitizing components of common pets and explored the correlations and potential sensitization clusters among these allergens. Furthermore, using a novel inhibition assay, we confirmed that SA is the primary protein responsible for cross-reactivity between common pet allergens and other animal allergens, and it can strongly inhibit their sIgE binding.

Abbreviations

AIT, Allergen immunotherapy; sIgE, Specific immunoglobulin E; CRD, Component Resolved diagnosis; SA, Serum albumin; AR, Allergic rhinitis; HRP, horseradish peroxidase; PBS, Phosphate-buffered saline; ANOVA, The one-way analysis of variance; IQR, Interquartile ranges; SPT, skin prick testing.

Ethics Approval

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (GYYY-2021-67). All participants provided written informed consent. For participants under the age of 18, written consent was obtained from their parents or legal guardians.

Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by the Guangdong Zhongnanshan Medical Foundation (ZNSXS-20220015) and the project of the First Hospital of Guangzhou Medical University (20221A011078).

Disclosure

The authors have no relevant financial or non-financial interests to disclose.

References

1. Gergen PJ, Mitchell HE, Calatroni A, et al. Sensitization and exposure to pets: the effect on asthma morbidity in the us population. *J Allergy Clin Immunol Pract*. 2018;6(1):101–107e102. doi:10.1016/j.jaip.2017.05.019
2. Asarnoj A, Hamsten C, Waden K, et al. Sensitization to cat and dog allergen molecules in childhood and prediction of symptoms of cat and dog allergy in adolescence: a BAMSE/MeDALL study. *J Allergy Clin Immunol*. 2016;137(3):813–821e817. doi:10.1016/j.jaci.2015.09.052
3. Zahradnik E, Raulf M. Respiratory allergens from furred mammals: environmental and occupational exposure. *Vet Sci*. 2017;4(3). doi:10.3390/vetsci4030038
4. Spitzauer S, Schweiger C, Sperr WR, et al. Molecular characterization of dog albumin as a cross-reactive allergen. *J Allergy Clin Immunol*. 1994;93(3):614–627. doi:10.1016/S0091-6749(94)70073-7
5. Dramburg S, Hilger C, Santos AF, et al. EAACI molecular allergology user's guide 2.0. *Pediatr Allergy Immunol*. 2023;34(Suppl 28):e13854. doi:10.1111/pai.13854
6. Saarelainen S, Rytkonen-Nissinen M, Rouvinen J, et al. Animal-derived lipocalin allergens exhibit immunoglobulin E cross-reactivity. *Clin Exp Allergy*. 2008;38(2):374–381. doi:10.1111/j.1365-2222.2007.02895.x
7. Virtanen T, Kinnunen T, Rytkönen-Nissinen M. Mammalian lipocalin allergens--insights into their enigmatic allergenicity. *Clin Exp Allergy*. 2012;42(4):494–504. doi:10.1111/j.1365-2222.2011.03903.x
8. Schoos AM, Nwaru BI, Borres MP. Component-resolved diagnostics in pet allergy: current perspectives and future directions. *J Allergy Clin Immunol*. 2021;147(4):1164–1173. doi:10.1016/j.jaci.2020.12.640
9. Tsolakis N, Malinovschi A, Nordvall L, et al. Sensitization to minor cat allergen components is associated with type-2 biomarkers in young asthmatics. *Clin Exp Allergy*. 2018;48(9):1186–1194. doi:10.1111/cea.13135
10. Hemmer W, Sestak-Greinecker G, Braunsteiner T, et al. Molecular sensitization patterns in animal allergy: relationship with clinical relevance and pet ownership. *Allergy*. 2021;76(12):3687–3696. doi:10.1111/all.14885

11. Zhu H, Huang Z, Liu T, et al. Sensitization to furry animals in patients with suspected allergic disease in China: a multicenter study. *J Asthma Allergy*. 2022;15:1701–1712. doi:10.2147/JAA.S390473
12. Liao C, Hou X, Wu L, et al. Major grass pollen allergen components and cross-reactive carbohydrate determinants in mugwort-sensitized child patients with allergic respiratory disease in Western China. *Front Pediatr*. 2022;10:816354. doi:10.3389/fped.2022.816354
13. Luo W, Huang H, Zheng P, et al. CCD inhibition test can improve the accuracy of the detection of pollen and seed food allergen-specific IgE in Southern China. *J Asthma Allergy*. 2021;14:439–447. doi:10.2147/JAA.S302920
14. Conway JR, Lex A, Gehlenborg N. UpSetR: an R package for the visualization of intersecting sets and their properties. *Bioinformatics*. 2017;33 (18):2938–2940. doi:10.1093/bioinformatics/btx364
15. Perzanowski MS, Ronmark E, Platts-Mills TA, et al. Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. *Am J Respir Crit Care Med*. 2002;166(5):696–702. doi:10.1164/rccm.2201035
16. Chan SK, Leung DYM. Dog and cat allergies: current state of diagnostic approaches and challenges. *Allergy Asthma Immunol Res*. 2018;10 (2):97–105. doi:10.4168/aair.2018.10.2.97
17. Davila I, Dominguez-Ortega J, Navarro-Pulido A, et al. Consensus document on dog and cat allergy. *Allergy*. 2018;73(6):1206–1222. doi:10.1111/all.13391
18. Suzuki S, Nwaru BI, Ekerljung L, et al. Characterization of sensitization to furry animal allergen components in an adult population. *Clin Exp Allergy*. 2019;49(4):495–505. doi:10.1111/cea.13355
19. Heinzerling LM, Burbach GJ, Edenharter G, et al. GA(2)LEN skin test study I: GA(2)LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. *Allergy*. 2009;64(10):1498–1506. doi:10.1111/j.1398-9995.2009.02093.x
20. Van Ree R, Van Leeuwen WA, Bulder I, et al. Purified natural and recombinant Fel d 1 and cat albumin in in vitro diagnostics for cat allergy. *J Allergy Clin Immunol*. 1999;104(6):1223–1230. doi:10.1016/S0091-6749(99)70017-5
21. Spitzauer S, Pandjaitan B, Söregi G, et al. IgE cross-reactivities against albumins in patients allergic to animals. *J Allergy Clin Immunol*. 1995;96(6 Pt 1):951–959. doi:10.1016/S0091-6749(95)70233-4
22. Smith W, Butler AJ, Hazell LA, et al. Fel d 4, a cat lipocalin allergen. *Clin Exp Allergy*. 2004;34(11):1732–1738. doi:10.1111/j.1365-2222.2004.02090.x
23. Mattsson L, Lundgren T, Olsson P, et al. Prostatic kallikrein: a new major dog allergen. *J Allergy Clin Immunol*. 2009;123(2):362–368. doi:10.1016/j.jaci.2008.11.021
24. Liccardi G, Passalacqua G, Salzillo A, et al. Is sensitization to furry animals an independent allergic phenotype in nonoccupationally exposed individuals? *J Investig Allergol Clin Immunol*. 2011;21(2):137–141.
25. Liccardi G, Bilò MB, Manzi F, et al. What could be the role of molecular-based allergy diagnostics in detecting the risk of developing allergic sensitization to furry animals? *Eur Ann Allergy Clin Immunol*. 2015;47(5):163–167.
26. Nilsson OB, Binnmyr J, Zoltowska A, et al. Characterization of the dog lipocalin allergen C an f 6: the role in cross-reactivity with cat and horse. *Allergy*. 2012;67(6):751–757. doi:10.1111/j.1398-9995.2012.02826.x
27. Pandjaitan B, Swoboda I, Brandejsky-Pichler F, et al. Escherichia coli expression and purification of recombinant dog albumin, a cross-reactive animal allergen. *J Allergy Clin Immunol*. 2000;105(2):279–285. doi:10.1016/S0091-6749(00)90077-0
28. Flower DR. The lipocalin protein family: structure and function. *Biochem J*. 1996;318(Pt 1):1–14. doi:10.1042/bj3180001
29. Huang Z, Zhu H, Lin R, et al. Serum albumin as a cross-reactive component in furry animals may be related to the allergic symptoms of patients with rhinitis. *J Asthma Allergy*. 2021;14:1231–1242. doi:10.2147/JAA.S334195
30. Uriarte SA, Sastre J. Clinical relevance of molecular diagnosis in pet allergy. *Allergy*. 2016;71(7):1066–1068. doi:10.1111/all.12917