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Sensitization to Furry Animals and Clinical Relevance of House Dust Mite-Induced Allergic Rhinitis in Guangzhou, China

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Keywords
Cross-sensitization · Co-sensitization · Furry animals · House dust mite · Allergic rhinitis

Abstract
Introduction: The impact of furry animal allergens on house dust mite (HDM)-induced allergic rhinitis (AR) is unclear. ***Objective:*** We aimed to investigate the co-sensitization and cross-sensitization of furry animal allergens and assess their clinical relevance with HDM-induced AR. ***Methods:*** We enrolled 268 patients with HDM-induced AR who were diagnosed with skin prick tests positive for dogs and/or cats. Specific immunoglobulin E (sIgE) for dogs (e1) and cats (e2), their components (Can f 1–5 and Fel d 1–2), and other uncommon furry animal extracts were measured. Symptoms and quality of life were assessed with a visual analog scale (VAS). ***Results:*** The VAS scores for the AR and asthma (AS; *n* = 166), moderate-to-severe persistent-AR (*n* = 132), and e1P (positive)-e2P (*n* = 89) groups were higher than those for single AR (*n* = 102), other AR classifications, and other AR sensitization profiles, respectively. The IgE positivity rates for components such as Can f 1–3 and Fel d 2 and those for rats, sheep, mice, cows, and horses were highest in e1P-e2P patients. Can f 1–4, Fel d 1, Fel d 2, or the combined allergens were positively correlated with VAS scores. AR combined with AS and sensitization to Can f 4, Fel d 1, or mice were risk factors for HDM-induced AR with VAS scores ≥5. ***Conclusions:*** Extensive cross-sensitization or co-sensitization was found between Can f 1–3, Fel d 2, or rat, sheep, mouse, cow, and horse extracts. Higher sIgE levels for Can f 1–4 and Fel d 1–2 or a higher number of furry animal allergens lead to more severe symptoms and a reduced quality of life. Combined with AS, sensitization to Can f 4, Fel d 1, or mice were risk factors for moderate-to-severe HDM-induced AR.

Introduction

House dust mites (HDMs) are the allergens most likely to cause allergic rhinitis (AR). A study in Guangzhou showed that >80% of patients with AR are allergic to HDM [1]. However, with the increase in the number of

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pets, more patients are developing allergies to furry animals [2]. Furry animal allergens are present in carpets, clothing, and other pollutants and can also be transferred through clothing shipped to places without animals [3]. Moreover, these allergens are difficult to avoid and eliminate because they remain airborne for long periods of time. While furry animal allergens can produce proteins that induce allergies, they can also carry other airborne allergens such as toxins, pollens, or fungal spores [3]. These harmful properties have attracted increased clinical attention.

Sensitization to furry animals, especially cats and dogs, is a major risk factor for the development of AR and asthma (AS) [4, 5]. In the United States, up to 72 and 36% of patients with allergies had a skin prick test (SPT) with a positive result for cats and dogs, respectively [6], compared with 26 and 27% adult patients in Europe [7]. In China, patients with allergies are often sensitized to cat and dog allergens in combination with other inhaled allergens and patients sensitized to only cats or dogs are very uncommon. Nevertheless, the positivity rates for cat and dog allergens are increasing [2]. A 4-year observational study in southern China reported cat and dog dander positivity rates of 4.21 and 4.26%, respectively [8]. Positivity rates for uncommon animals such as pigeons, parrots, sheep, mice, cows, and horses are currently unknown.

Sensitization to furry animals may aggravate allergic airway hyper responsiveness and airway inflammation [9], reduce the quality of life [10], and be associated with severe allergies [10]. Multiple sensitization and cross sensitization [11] to dogs, cats, and other furry animals are ubiquitous, often leading to more severe allergies [10]. Thus, the screening of furry animal allergens, particularly their components, is essential for identifying cross-sensitization and co-sensitization in patients with chronic allergic airway diseases and provides a basis for risk assessment of patients sensitized to furry animals. However, component-resolved diagnosis for furry animals is currently lacking in China.

Because HDMs are the main allergens in Guangzhou, this study included patients with HDM-induced AR with sensitization to cats and/or dogs. Disease severity and duration, visual analog scale (VAS) score of symptoms, and quality of life were evaluated. Specific immunoglobulin E (sIgE) levels of dog and cat extracts and components were measured along with sIgE levels of uncommon furry animal extracts (pigeons, parrots, ducks, chickens, sheep, rats, mice, geese, cows, and horses). This study also assessed the cross-sensitization and co-sensitization between these furry animals and analyzed the impact of furry animals and their components on HDM-induced AR in order to provide rational suggestions for the prevention and treatment of patients with HDM-induced AR with sensitization to dogs or cats.

Materials and Methods

Patients

This study was conducted from January 2016 to December 2018 in the Department of Allergy and Clinical Immunology and the Department of Pediatrics at the First Affiliated Hospital of Guangzhou Medical University. It was approved by the Independent Ethical Committee of First Affiliated Hospital of Guangzhou Medical University, and each participant or their statutory guardian provided written informed consent (GYYY-2016-73).

The inclusion criteria for patients enrolled in our study were those who (1) were diagnosed with AR with or without allergic asthma based on the Allergic Rhinitis and its Impact on Asthma (ARIA) recommendations [12] and the Global Initiative for Asthma guidelines (http://ginasthma.org/), (2) had positive SPTs to HDM *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae* and dogs and/or cats and were negative for other types of allergens such as pollens and molds, and (3) had allergic symptoms of rhinitis and/or asthma after exposure to *Dermatophagoides pteronyssinus*. The exclusion criteria were (1) history of specific allergen immunotherapy, (2) upper respiratory infection or chronic rhinosinusitis, and (3) the use of concomitant medications (e.g., antihistamines, intranasal corticosteroids) that could affect AR symptoms within 2 weeks before enrollment. AR classification was assessed according to the ARIA guidelines, and a VAS of 0–10 was used to evaluate nasal (sneezing, rhinorrhea, nasal congestion, nasal itching, and loss of sense of smell) and ocular (eye itching, conjunctival redness, watery eyes, and eyelid edema) symptoms and quality of life (impact on sleep, impact on work life, impact on social life, and physical activities). A response of 0 was "no" and 10 was "very severe." We defined age ≥18 and age <18 as "adult" and "minor," respectively, and a VAS score ≥5 as "moderate-to-severe (M/S)."

Tests for Allergen sIgE and Its Components for Furry Animals

SIgE levels for dogs (e1) and its components (Can f 1–5), cats (e2) and its components (Fel d 1–2), pigeons (e11), parrots (e91), ducks (e86), chickens (e85), sheep (e81), rats (e73), mice (e71), geese (e111), cows (e4), and horses (e3) were measured with the EUROIMMUN system (Euroline; EUROIMMUN, Lubeck, Germany). Reagent kits were kindly provided by EUROIMMUN, and the experiments were performed according to the manufacturer's instructions. SIgE levels were expressed in international units per milliliter (IU/mL) with the following range 0.35–100 IU/mL. Any measurement over the upper limit of the detected range was given a value of 100 IU/mL. Tests with sIgE levels <0.35 IU/mL were defined as sIgE negative and ≥0.35 IU/mL were defined as sIgE positive. SIgE-positive tests were categorized into the following 6 classes: class 1 (≥0.35 to <0.70 IU/mL), class 2 (≥0.70 to <3.50 IU/mL), class 3 (≥3.50 to <17.50 IU/mL), class 4 (≥17.50 to <50 IU/mL), class 5 (≥50 to <100 IU/mL), and class 6 (≥100 IU/mL).

Statistical Analysis

Descriptive parameters such as means and SDs for normally distributed continuous data and frequencies and percentages for categorical data were calculated. Nonnormally distributed data were expressed as medians and 25–75% interquartile ranges. Pearson X^2 or Fisher's exact tests were used to determine the association between categorical variables. The Mann-Whitney U test was used to compare numerical data between groups, and the Spearman rank test was used to assess correlations. A binary logistic regression was performed to evaluate possible risk factors for M/S AR (VAS ≥5). A p value <0.05 was considered statistically significant. PASW Statistics for Windows, version 19.0 (SPSS, Inc.,), was used for all statistical analyses.

Results

Patient Characteristics

A total of 268 patients were included; 38.06% of the patients were diagnosed with single AR and 61.94% had both AR and AS. The mean age was 16.32 ± 13.84 years and the mean VAS score was 3.84 ± 1.07. Patients enrolled in our study had mild intermittent AR (MI-AR) (13.81%), M/S intermittent AR (M/SI-AR; 29.10%), mild persistent AR (MP-AR; 7.84%), and M/S persistent AR (M/SP-AR; 49.25%) according to the ARIA guidelines [12]. Additionally, 39 (14.55%) patients had negative serum sIgE results for e1 and e2 (e1N-e2N), 82 (30.60%) were positive for e1 and negative for e2 (e1P-e2N), 58 (21.64%) were negative for e1 and positive for e2 (e1N-e2P), and 89 (33.21%) were positive for both e1 and e2 (e1P-e2P; Table 1).

Table 1. Clinical characteristics of the included patients with HDM-induced AR

Patients studied, *n*	268
Gender	
Male	163 (60.82)
Female	105 (39.18)
Age, years	
Mean ± SD	16.32±13.84
<18	182 (67.91)
≥18	86 (32.09)
Diagnosis	
AR	102 (38.06)
AR and AS	166 (61.94)
Classfication of AR according to ARIA	
MI-AR	37 (13.81)
M/SI-AR	78 (29.10)
MP-AR	21 (7.84)
M/SP-AR	132 (49.25)
Characteristics of cat and dog sensitization	
e1N-e2N	39 (14.55)
e1P-e2N	82 (30.60)
e1N-e2P	58 (21.64)
e1P-e2P	89 (33.21)
VAS scores, mean ± SD	3.84±1.07

Values are presented as *n* (%), unless otherwise stated. AR, allergic rhinitis; AR and AS, allergic rhinitis and asthma; ARIA, allergic rhinitis and its impact on asthma; MI, mild intermittent; M/SI, moderate-to-severe intermittent; MP, mild persistent; M/SP, moderate-to-severe persistent; e1, dog extract; e2, cat extract; N, negative; P, positive; e1N, dog allergen reactive negetive; e1P, dog allergen reactive positive; e2N, cat allergen reactive negetive; e2P, cat allergen reactive positive; VAS, visual analog scale; HDM, house dust mite.

Prevalence of Sensitization and sIgE Levels of Furry Animal Allergens in Different Groups

Positivity rate to Can f 5 was significantly higher in female patients than in male patients (18.10 vs. 4.29%, $p < 0.01$), and positivity rates to Can f 1, Can f 2, Can f 4, Can f 5, and horse extract (e3) were significantly higher in adults than in minors (32.56 vs. 10.44%, 15.12 vs. 3.85%, 15.12 vs. 4.40%, 23.26 vs. 3.30%, and 15.12 vs. 6.04%, respectively; $p < 0.05$).

The positivity rate of sIgE for dog extracts (e1) in all patients was 54.85%, while that for the main component, Can f 1 was only 17.54% and its main sIgE classes were high levels (classes 4–5). Another main component, Can f 5, had a positivity rate of only 9.70%, and its sIgE levels were low (classes 1–3). The positivity rate of sIgE for cat extracts (e2) was the highest at 63.81%, and its major component, Fel d 1, had positive rate of 61.19% with mainly high level sIgE classes. Among e1-positive patients, a total of 150 (91.46%) were only sensitized to Fel d 1. For other uncommon furry animals, positivity rates were below 15%; the highest was for cows (e4), with a positivity rate of 13.06%. The sIgE classes of these uncommon furry animal extracts were mainly concentrated at low levels (Fig. 1).

Compared to patients with single AR, AR and AS patients had higher positivity sIgE rates for e1 and components Can f 1, Can f 2, Can f 4, Can f 5 and e2 and components Fel d 1, and Fel d 2. SIgE for sheep (e81), rat (e73), mouse (e71), and horse (e3) extracts had higher positivity rates in patients with single AR; however, the difference was not statistically significant between the AR and AR and AS groups.

The positivity rates for e1 and its components, Can f 1–4, were significantly higher among patients with M/SP-AR than among those with MP-AR, M/SI-AR, and MI-AR ($p < 0.05$). Positivity rates for e2 and its components,

Table 2. Positive rate (percentage) of the 12 crude extracts and 7 components in different types of AR groups

	MI-AR, % (*n*)	M/SI-AR, % (*n*)	MP-AR, % (*n*)	M/SP-AR, % (*n*)	Chi-square value	*p* value	MI-AR	M/SI-AR	MP-AR	M/SP-AR
Can f	37.84 (14/37)	47.44 (37/78)	52.38 (11/21)	64.39 (85/132)	**10.962**	**0.012**	a	ab	ab	b
Can f 1	5.41 (2/37)	10.26 (8/78)	14.29 (3/21)	25.76 (34/132)	**12.665**	**0.004**	a	a	ab	b
Can f 2	0.00 (0/37)	3.85 (3/78)	0.00 (0/21)	12.88 (17/132)	**10.384**	**0.010**	a	a	a	a
Can f 3	0.00 (0/37)	1.28 (1/78)	0.00 (0/21)	14.39 (19/132)	**16.857**	**<0.001**	ab	a	ab	b
Can f 4	0.00 (0/37)	1.28 (1/78)	0.00 (0/21)	15.15 (20/132)	**18.197**	**<0.001**	ab	a	ab	b
Can f 5	2.70 (1/37)	8.97 (7/78)	4.76 (1/21)	12.88 (17/132)	3.636	0.288	a	a	a	a
Fel d	40.54 (15/37)	48.72 (38/78)	76.19 (16/21)	77.27 (102/132)	**28.121**	**<0.001**	a	a	ab	b
Fel d 1	43.24 (16/37)	52.56 (41/78)	**76.19 (16/21)**	68.94 (91/132)	**12.790**	**0.005**	a	ab	ab	b
Fel d 2	2.70 (1/37)	3.85 (3/78)	4.76 (1/21)	18.94 (25/132)	**15.760**	**0.002**	ab	a	ab	b
Chicken	0.00 (0/37)	2.56 (2/78)	4.76 (1/21)	4.55 (6/132)	1.920	0.577	a	a	a	a
Sheep	2.70 (1/37)	2.56 (2/78)	14.29 (3/21)	16.67 (22/132)	**13.867**	**0.002**	ab	a	ab	b
Rat	0.00 (0/37)	5.13 (4/78)	14.29 (3/21)	16.67 (22/132)	**12.896**	**0.003**	a	ab	ab	b
Mouse	0.00 (0/37)	1.28 (1/78)	4.76 (1/21)	15.15 (20/132)	**16.817**	**<0.001**	ab	a	ab	b
Cow	5.41 (2/37)	6.41 (5/78)	14.29 (3/21)	18.94 (25/132)	**8.766**	**0.027**	a	a	a	a
Horse	0.00 (0/37)	3.85 (3/78)	4.76 (1/21)	15.15 (20/132)	**12.201**	**0.004**	a	a	a	a

Chi-square test was used to compare the positive rate between the groups. If the difference between groups is statistically significant, use bold fonts. Pairwise comparison of the chi-square test, if the same letter is included between 2 groups, indicate no signicant difference, and vice versa. AR, allergic rhinitis; MI, mild intermittent; M/SI, moderate-to-severe intermittent; MP, mild persistent; M/SP, moderate-to-severe persistent; Can f 1–5, components of dog extract; Fel d 1–2, components of cat extract.

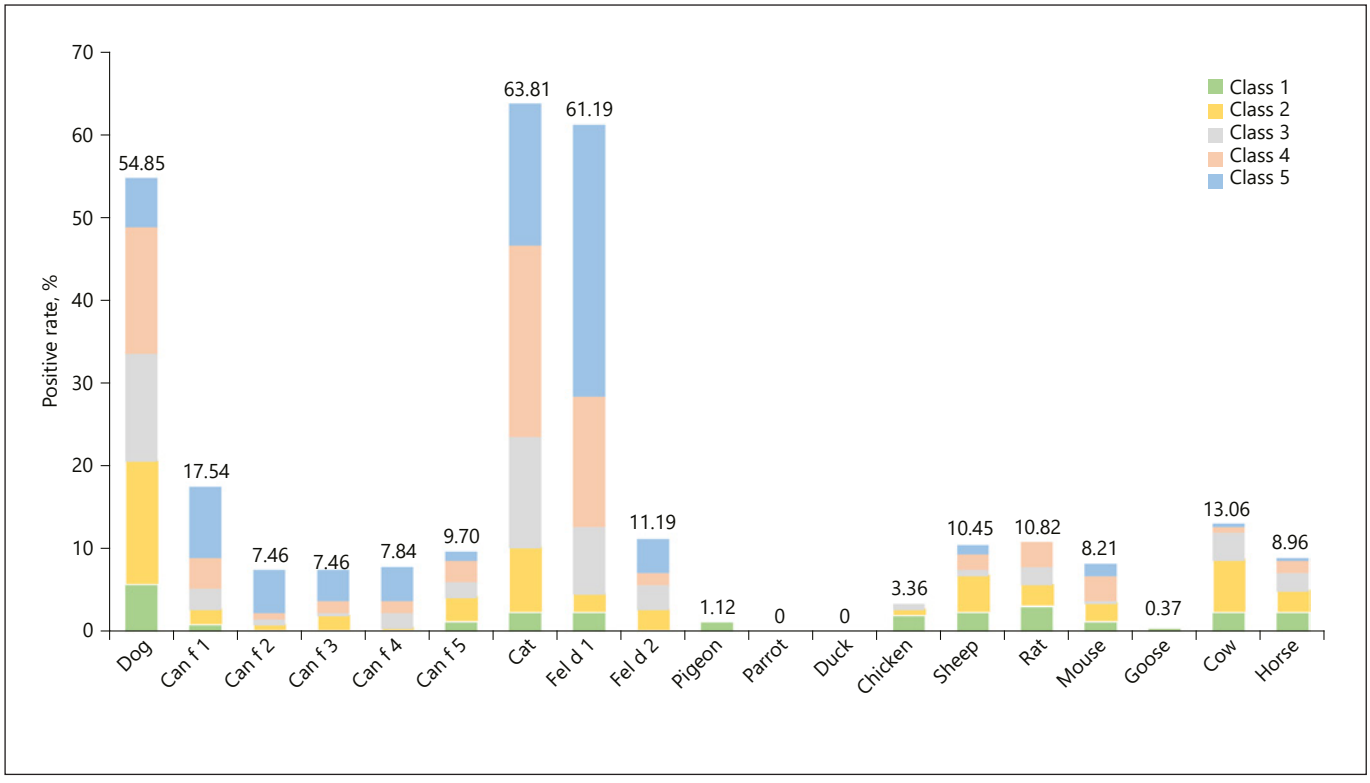

Fig. 1. Prevalence of sensitization and the class of sIgE of furry animals. Positive rate (%), ratios of positive sIgE for furry animal allergens to the total patients (n = 268); Can f 1–5, components of dog extract; Fel d 1–2, components of cat extract.

Table 3. Positive rate (percentage) of the 12 crude extracts and 7 components in different types of cat and dog sensitization group

	e1N-e2N, % (*n*)	e1P-e2N, % (*n*)	e1N-e2P, % (*n*)	e1P-e2P, % (*n*)	Chi-square value	*p* value	e1N-e2N	e1P-e2N	e1N-e2P	e1P-e2P
Can f	0.00 (0/39)	0.00 (0/82)	100 (58/58)	100 (89/89)	**268.000**	**<0.001**	a	a	b	b
Can f 1	5.13 (2/39)	10.98 (9/82)	17.24 (10/58)	29.21 (26/89)	**14.988**	**0.002**	a	a	ab	b
Can f 2	2.56 (1/39)	0.00 (0/82)	8.620 (5/58)	15.73 (14/89)	**17.846**	**<0.001**	abc	b	c	ac
Can f 3	0.00 (0/39)	2.44 (2/82)	1.72 (1/58)	19.10 (17/89)	**26.365**	**<0.001**	a	a	a	b
Can f 4	0.00 (0/39)	1.22 (1/82)	**13.79 (8/58)**	13.48 (12/89)	**15.067**	**0.002**	abc	b	c	ac
Can f 5	0.00 (0/39)	2.44 (2/82)	**17.24 (10/58)**	15.73 (14/89)	**17.750**	**<0.001**	ab	b	c	ac
Fel d	0.00 (0/39)	100 (82/82)	0.00 (0/58)	100 (89/89)	**268.000**	**<0.001**	a	b	a	b
Fel d 1	7.69 (3/39)	**97.56 (80/82)**	8.62 (5/58)	85.39 (76/89)	**182.134**	**<0.001**	a	b	a	c
Fel d 2	2.56 (1/39)	3.66 (3/82)	1.72 (1/58)	28.08 (25/89)	**33.961**	**<0.001**	a	a	a	b
Chicken	2.564 (1/39)	1.219 (1/82)	1.724 (1/58)	6.741 (6/89)	3.882	0.242	a	a	a	a
Sheep	2.56 (1/39)	3.66 (3/82)	3.45 (2/58)	24.71 (22/89)	**25.138**	**<0.001**	a	a	a	b
Rat	2.56 (1/39)	4.88 (4/82)	6.90 (4/58)	22.47 (20/89)	**16.847**	**<0.001**	a	a	ab	b
Mouse	0.00 (0/39)	2.44 (2/82)	1.72 (1/58)	21.34 (19/89)	**26.419**	**<0.001**	a	a	a	b
Cow	7.69 (3/39)	4.88 (4/82)	6.90 (4/58)	26.96 (24/89)	**22.924**	**<0.001**	ab	a	b	b
Horse	2.56 (1/39)	6.10 (5/82)	3.45 (2/58)	17.97 (16/89)	**11.838**	**0.006**	a	a	a	a

Chi-square test was used to compare the positive rate between the groups. If the difference between groups is statistically significant, use bold fonts. Pairwise comparison of the chi-square test, if the same letter is included between 2 groups, indicate no signicant difference, and vice versa. e1, dog extract; e2, cat extract; N, negative; P, positive; e1N, dog allergen reactive negetive; e1P, dog allergen reactive positive; e2N, cat allergen reactive negetive; e2P, cat allergen reactive positive; Can f 1–5, components of dog extract; Fel d 1–2, components of cat extract.

Fel d 1–2, were also significantly higher among M/SP-AR patients than among those in the MP-AR, M/SI-AR, and MI-AR groups ($p < 0.01$). Positivity rates for other furry animals, including e81, e73, e71, e4, and e3, were also significantly higher in M/SP-AR patients than in patients in the MP-AR, M/SI-AR, and MI-AR groups ($p < 0.05$; Table 2).

The positivity rates for dog components Can f 1–3; cat components Fel d 2; and uncommon furry animals e81, e73, e71, e4, and e3 were significantly higher in e1P-e2P patients than in e1P-e2N, e1N-e2P, and e1N-e2N patients, indicating that these components may be cross-sensitized or co-sensitized with e81, e73, e71, e4, and e3. Dog components Can f 4 and Can f 5 had the highest positivity rates among the e1P-e2N patients, but the difference was not statistically significant compared to the e1P-e2P patients. However, Fel d 1 had the highest positive rate in e1N-e2P patients and was significantly higher in e1N-e2P patients than in e1P-e2P patients, indicating that Fel d 1 is the main component of cat allergens and has no cross-sensitization or co-sensitization with dog allergens (Table 3).

SIgE Levels for Furry Animal Allergens and VAS Scores in Different Groups

VAS scores in adults were significantly lower than those in minors (3.11 ± 0.90 vs. 3.62 ± 2.05, $p < 0.001$). AR and AS patients had higher VAS scores than the single AR patients (4.10 ± 0.92 vs. 3.42 ± 1.16, $p < 0.0001$). VAS scores for M/SP-AR patients were significantly higher than those for MP-AR patients (4.61 ± 0.62 vs. 4.18 ± 0.62, $p < 0.01$). VAS scores for M/SI-AR patients were significantly higher than those for MI-AR patients (3.08 ± 0.65 vs. 2.53 ± 0.82, $p < 0.001$). Finally, VAS scores for e1P-e2P patients were higher than those for e1N-e2P, e1P-e2N, and e1N-e2N patients (4.39 ± 0.78 vs. 3.58 ± 1.02 vs. 3.90 ± 0.98 vs. 2.89 ± 1.10, respectively, $p < 0.01$; Fig. 2).

Clinical Relevance of Furry Animal Allergens

AR and AS patients did not have an IgE response to more combined furry animal allergens than patients with single AR; however, M/SP-AR patients had an IgE response to more furry animal allergens than patients with MP-AR, M/SI-AR, and MI-AR. The e1P-e2P patients had an IgE response to more combined furry animal allergens compared to those in the e1P-e2N, e1N-e2P, and e1N-e2N groups (Fig. 3). A significant correlation was observed between the number of combined allergens and VAS scores, with increasing combined furry animal allergens resulting in a higher VAS score ($r = 0.495$, $p < 0.01$; Fig. 4a). SIgE levels for Can f 1–4 and Fel d 1–2 were also significantly correlated with VAS scores, with higher levels resulting in higher VAS scores ($p < 0.05$). No correlation was observed between Can f 5 and VAS score (Fig. 4b).

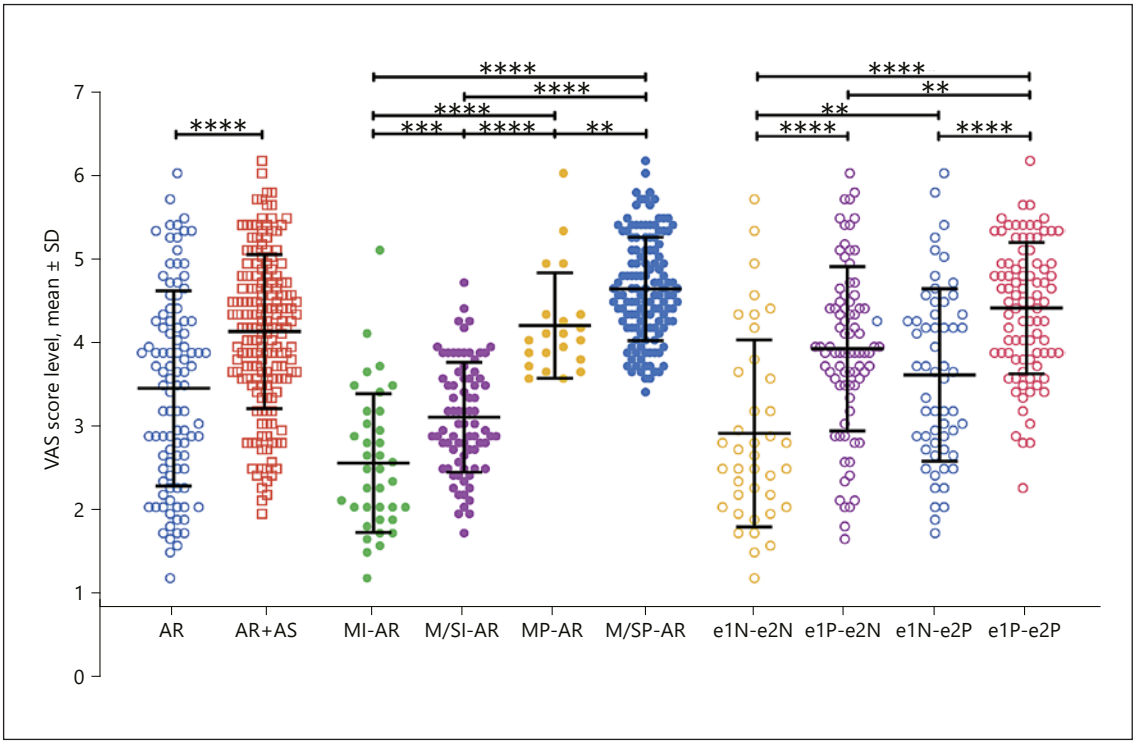

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Fig. 2. SIgE levels of furry animal allergens and VAS scores in different groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. VAS, visual analog scale; AR, allergic rhinitis; AR and AS, allergic rhinitis and asthma; MI, mild intermittent; M/SI, moderate-to-severe intermittent; MP, mild persistent; M/SP, moderate-to-severe persistent; e1, dog extract; e2, cat extract; N, negative; P, positive.

Table 4. Risk factors of M/S AR patients (VAS ≥5)

Variable	OR	95% CI	*p* value
Age, years	0.909	0.868–0.951	0.000
Combined with asthma	2.630	1.128–6.129	0.025
Can f 4 sensitization	4.789	1.222–18.773	0.025
Fel d 1 sensitization	3.058	1.273–7.345	0.012
Mouse sensitization	17.837	5.096–62.430	0.000
Constant	0.096		0.000

Binary logistic regression analysis of risk factors for HDM-induced AR with VAS ≥5.

Can f 4, the component of dog extract; Fel d 2, the component of cat extract. M/S, moderate-to-severe; AR, allergic rhinitis; VAS, visual analog scale; HDM, house dust mite.

Risk Factors for M/S AR

Binary logistic regression analysis showed that AR combined with AS (OR 2.630) or sensitization to Can f 4 (OR 4.789), Fel d 1 (OR 3.058), or mouse extracts (OR 17.837) were significant risk factors for HDM-induced AR with a VAS score ≥5 ($p < 0.05$; Table 4).

Discussion/Conclusion

Single sensitization to furry animals occurs mostly in the United States and European countries [13–15] and rarely in China; however, sensitization to HDM combined with furry animals is common, especially in Guangzhou [1]. Studies have shown that patients with severe atopic dermatitis have a significantly higher frequency of IgE reactivity to allergens like cats and HDMs [16]. Allergen-specific IgE titers of cockroach or HDM allergen components and sensitization profiles were associated with asthma and rhinitis [17, 18], and sensitization to Per a 2 (cockroach allergen component) correlates with more severe airway allergies and elevated proinflammatory chemokines [19]. However, there are no relevant data on the effects of furry animal sensitivity on the symptoms, duration, severity, and quality of life for patients with HDM-induced AR.

The target population in this study was patients with HDM-induced AR, with positive SPT results for cat and/or dog allergens. Positive sIgE rates for dog and cat extracts were approximately 60%. We previously reported that both

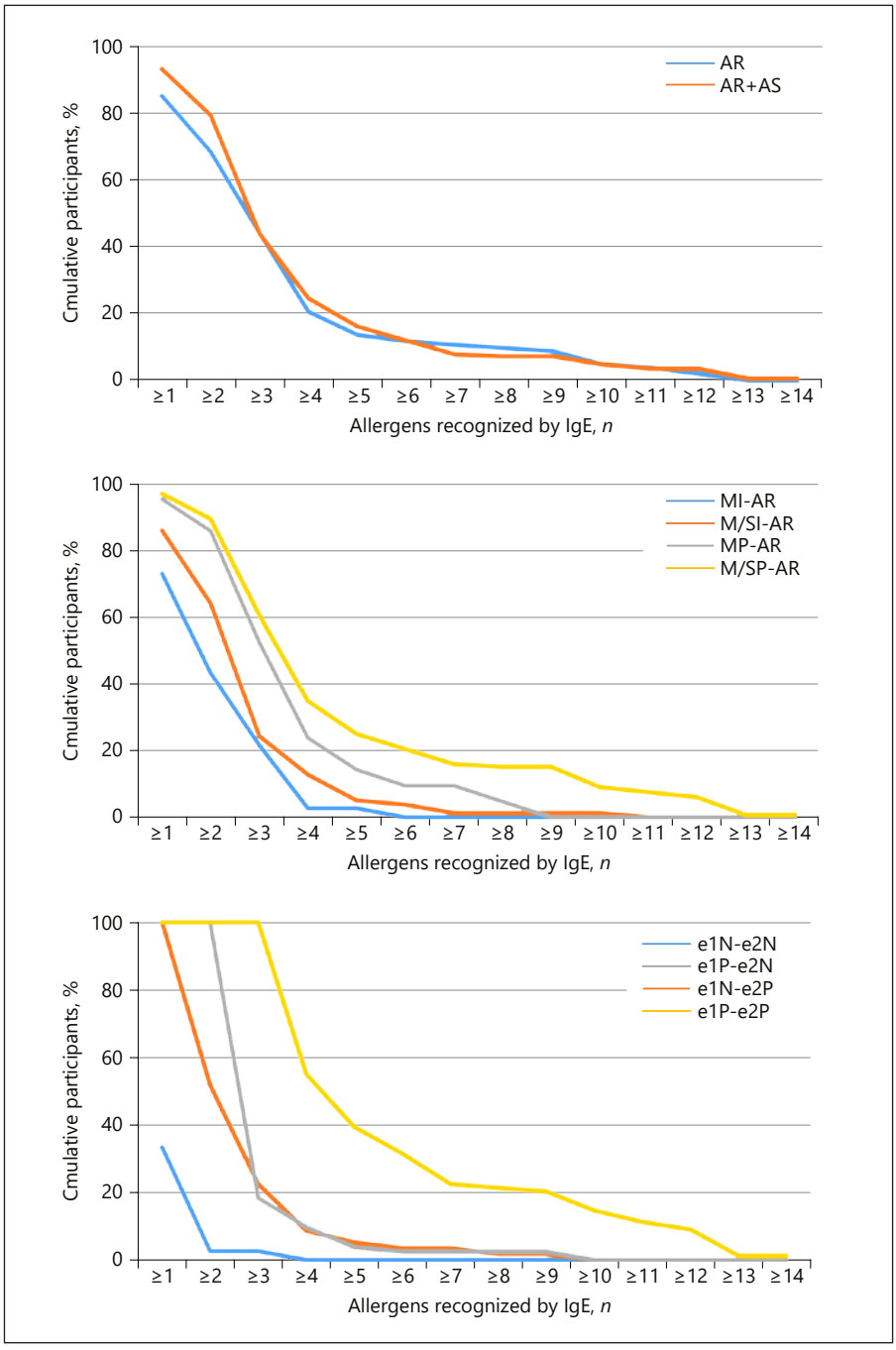

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Fig. 3. The number of furry animal allergens and cumulative of participants in different groups. AR, allergic rhinitis; AR and AS, allergic rhinitis and asthma; MI, mild intermittent; M/SI, moderate-to-severe intermittent; MP, mild persistent; M/SP, moderate-to-severe persistent; e1, dog extract; e2, cat extract; N, negative; P, positive.

cat and dog sIgE levels were mostly in classes 1–2 [8], but in the present study, sIgE levels for dog extracts were mainly in classes 2–4 and cat extracts were in classes 4–5. Instead of focusing on the epidemiological investigation of all patients with allergies, the present study focused on those with HDM-induced AR, which may be the explanation for the differences from the previous study; alternatively, sIgE levels for cat and dog extracts may increase gradually.

Can f 1 and Can f 5 are the main components of dog extracts. In this study, positive rates for Can f 1 and Can f 5 were 17.54 and 9.70%, respectively, and were significantly higher in adults than in minors. Bjerg et al. [20] and Mattsson et al. [21] reported that positivity rates for Can f 1 and f 5 were 39 and 46% in children and 50 and 70% in adults, respectively, which are consistent with our conclusion that positivity rates for Can f 1 and f 5 were high-

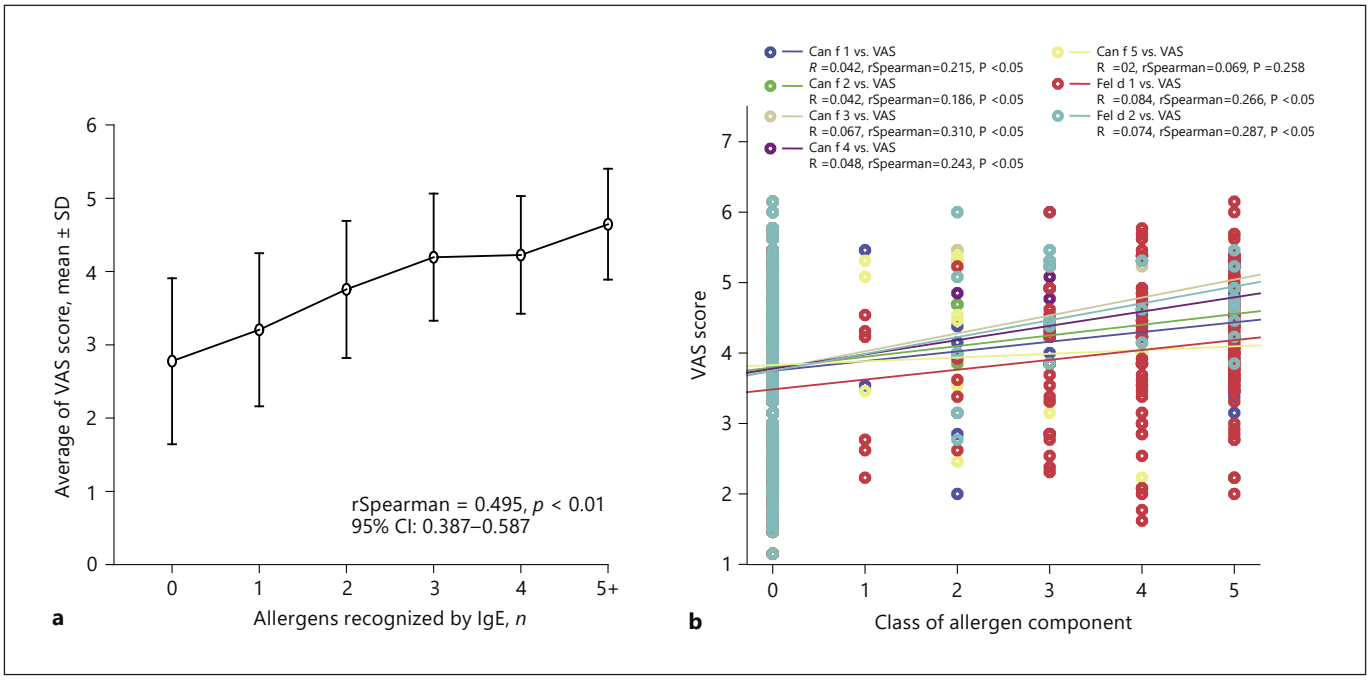

Fig. 4. a Correlation between the number of furry animal allergens and VAS scores. **b** Correlation between components of dog and/or cat and VAS scores. VAS, visual analog scale; Can f 1–5, components of dog extract; Feld 1–2, components of cat extract.

er in adults than in minors; however, we obtained lower positive rates than those reported, which may due to differences in cultures and environments between countries and regions. In addition, the positivity rate for horse extracts was significantly higher in adults than in minors, which may be due to adults riding horses more, and is consistent with the results obtained by Zahradnik and Raulf [22]. Can f 5 has a structure like human prostate-specific antigen (PSA); thus, patients sensitized to Can f 5 have an increased risk of allergic reactions to semen [21]. In fact, 24% of patient allergies to dog extracts with sIgE for e1 can bind to PSA in human semen [23]. In our study, the positivity rate for Can f 5 in women was significantly higher than that in men. Cross-reactivity of Can f 5 with PSA may explain this phenomenon, and this patient population may also have a semen allergy.

Fel d 1, the most important component of cat allergens, is associated with asthma symptoms [20]. However positive sIgE responses to Fel d 1 alone cannot predict the risk of asthma in children unless the level is above class 3 [24]. Fel d 2 is associated with the severity of rhinitis and asthma [25]. Our study did not find significantly higher positivity rates for Fel d 1 and Fel d 2 in patients with AR and AS compared to patients with single AR; however, a correlation analysis showed that Fel d 1 and Fel d 2 sIgE levels were positively correlated with VAS scores, indicating that the higher the sIgE level for Fel d 1 and Fel d 2, the worse the symptoms and quality of life in HDM-induced AR patients. We also observed the same correlation for dog components Can f 1–4. Likewise, Soderstrom also reported more obvious symptoms and increased severity in AR patients with higher sIgE levels of furry animal allergens [26].

In this study, patients in the M/SP AR group had an IgE response to more combined allergens than the MP-AR, M/SI-AR, and MI-AR groups. Furthermore, VAS scores climbed with increasing amounts of poly-allergens. It has been reported that, among children who are allergic to dogs, the higher the number of combined allergens, the more severe the AR and AS symptoms [25]. We also observed higher VAS scores in patients sensitized to both dogs and cats. Nordlund showed that children sensitized to 3 or more furry animals were prone to severe uncontrolled AS [10]. Corren et al. [27] showed that patients with AR and AS who were sensitized to multiple animals tended to have more severe symptoms and longer duration of disease when exposed to animals. In our study, we observed no difference in the number of positive allergens between AR and AR and AS patients; however, we conclude that the greater the number of furry

animal allergens as measured by IgE, the more severe the symptoms, the longer the duration, and the worse the quality of life.

The main cat allergen Fel d 4 and dog allergen Can f 1 are lipocalins, which explains the cross-reactivity between the 2 extracts [28]. Fel d 1-like antigens are found in dog dander extracts; cross-inhibition experiments showed that 25% of patients with sIgE positivity to Fel d 1 could inhibit 50% of dog allergens [29]. Can f 6, as a dog component, shows a high sequence similarity and cross-reactivity with Fel d 4 and Equ c 1, major cat and horse allergens [30]. Fel d 7 and Can f 1 showed high similarities in protein structure and common epitopes were found by using cross-reactive antisera [31]. These results suggest wide cross-reactions between cat and dog allergens. In this study, 33.1% of patients were sensitized to both dog and cat extracts; these patients also had higher positivity rates to dog components (Can f 1–3); cat components (Fel d 2); and sheep, rat, mouse, cow, and horse extracts compared to those with negative responses to one or more dog and cat extracts. This indicates cross-sensitization and co-sensitization between these components and extracts. Both Can f 3 and Fel d 2 are serum albumins with wide cross-reactivity with other mammalian proteins [32]. Can f 1, Can f 2, and Equ c 1 (the main component of horse extract), Rat n 1 (the main component of rat extract), and Mus m 1 (the main component of mouse extract) are lipocalins [3], which may explain the extensive cross- sensitization or co-sensitization between these furry animals. Patients who are sensitized to cats and dogs have a 14-fold increased risk of developing sensitization to other furry animals (cows, horses, mice, hamsters, and rabbits), suggesting that sensitization to cats and dogs tends to cause sensitization to a variety of furry animals [33]. Cross-reactive lipocalins may play a very important role in individuals with multiple sensitizations to furry animals without direct contact [34]. Asarnoj et al. [35] showed that the common IgE tests with crude allergen extracts might be enough for cat allergies but is not likely to be enough for dog allergies. In our study, sensitization to Fel d 1, the main component of cat extract, was observed in 91.28% of patients, higher than that for cat and dog sensitization combined, while positivity rates for Can f 1–5 was <25% in patients sensitized to dog extracts. This is consistent with Asarnoj's findings.

The risk assessment identified Can f 4, Fel d 1, and sensitization to mouse extracts as risk factors for M/S AR (VAS ≥5). Can f 4, a lipocalin, is a minor component in dog extracts [36]; data are scarce regarding the relationship between Can f 4 and clinical symptoms/severity of allergic airway diseases. Sensitization to Fel d 1 (predominantly uteroglobin) can predict childhood asthma [24]. Mus m 1, the main component of the mouse extracts, is predominantly a lipocalin and found in urinary prealbumin [3]. Uriarte reported that sensitization to albumins rather than lipocalins or uteroglobins was a risk factor for severe respiratory symptoms [25], a finding inconsistent with ours, which may require further study.

This study is the first to describe the effects of sensitization to furry animals on HDM-induced AR in China. Moreover, our findings provide practical suggestions for prevention and treatment by clinicians. However, this study also has limitations. First, as >80% of AR cases in Guangzhou are caused by HDM, there were few patients positive for cat and dog allergens and negative for HDM. Therefore, we included patients with HDM-induced AR and combined cat and/or dog sensitization. It is not clear whether there is an interaction between HDM and cat and/or dog allergens, which might be have affected the results. Second, in this study, patients with positive sIgE responses for the main dog components Can f 1 and Can f 5 comprised <50% of the crude-extract positive patients, indicating that there is heterogeneity between the crude extract and the main components, which may have led to a bias in the results. Because the reagents lacked components for uncommon furry animals, we performed sIgE detection using only crude extracts for sheep, horses, cows, rats, and mice. We hope to address these limitations in future research.

In conclusion, component-resolved diagnosis is clinically significant in patients with HDM-induced AR when combined with sensitization to furry animals. Dog components Can f 1–3; cat component Fel d 2; and rat, sheep, mouse, cow, and horse extracts had extensive cross-sensitization and co-sensitization. The higher the sIgE level of dog components Can f 1–4, cat components Fel d 1–2, or the more furry animal allergens combined that are recognized by IgE, the worse the symptoms and quality of life in patients with HDM-induced AR. Combined with AS, sensitization to Can f 4, Fel d 1, or mouse extracts were risk factors for M/S HDM-induced AR.

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Statement of Ethics

The study was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (GYYY-2016-73). Written consent was obtained from all adult patients or parents of children.

Disclosure Statement

The authors declare that they have no conflicts of interest.

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Author Contributions

B.S. and H.C.: conceived and designed the experiments. Z.H. and W. Luo: performed the experiments. Z.H. and W. Li: analyzed the data. H.C.: wrote and revised the manuscript. P.Z. and H.H.: mainly performed the clinical tests and collected the data.

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