

ORIGINAL ARTICLE

Epidemiology of Allergic Disease

Characterization of sensitization to furry animal allergen components in an adult population

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Summary

Background: There are paucity of data on sensitization to furry animal allergen components in adults. Furry animals are major sensitizers and contributors to asthma burden in northern Europe and North America.

Objective: To characterize sensitization patterns to furry animal allergen components in Swedish adults.

Methods: Based on the West Sweden Asthma Study, a random population ($n = 1103$) and an asthma sample ($n = 769$) were tested for allergen sensitization using Phadiatop®. Those with $\text{IgE} \geq 0.35 \text{ kU}_A/\text{L}$ were tested for cat (Fel d 1, 2, and 4), dog (Can f 1, 2, 3, and 5), and horse (Equ c 1) allergen component sensitization. We defined allergen component poly-sensitization patterns, identified data-driven sensitization clusters, described component sensitization overlaps, and assessed determinants of sensitization patterns.

Results: The prevalence of allergen component sensitization ranged from 0.8% for Fel d 2 and Can f 3 to 8.9% for Fel d 1. The most common dog component was Can f 5 (3.6%); 2.1% were sensitized to Equ c 1. Those sensitized to Fel d 2 and Fel d 4 were commonly sensitized to Fel d 1. The most common dog component overlap was between Can f 1/Can f 2 and Can f 5. Mono-sensitization was 5.6%, double sensitization 1.5% and poly-sensitization 2.1%. Sensitization was always higher in the asthma than in the random sample. Three sensitization clusters were derived, namely non-sensitized (90% in random vs 66% in asthma sample); Fel d 1-driven sensitized (7% vs 19%); and multi-sensitized (3% vs 15%). Key determinants of sensitization were gender, age, raised on a farm, family history of allergy or asthma, smoking, and occupational exposure to dust or fumes.

Conclusions & Clinical Relevance: Fel d 1 and Can f 5 are the most common cat and dog components sensitization in this adult Swedish population. Mono-sensitization is more common than poly-sensitization. This detailed characterization highlights the current distribution of furry animal allergen components in Swedish adults, and their impact on clinical outcomes of asthma will be further explored.

1 | INTRODUCTION

Allergic sensitization is a major risk factor for asthma and other allergic conditions both in children and adults.^{1,2} The patterns of sensitization to different allergens within a geographic area are innumerable and vary widely across regions of the world.³ In northern Europe and North America, sensitization patterns to furry animals are major sensitizers and important contributors to the burden of asthma and allergic rhinitis.⁴⁻⁷ Factors that influence sensitization include climate, environmental and occupational exposures, and lifestyle, but it is also important to note that prevalence is changing over time, with noticeable increase in prevalence over the last decades.⁸⁻¹¹

Detection of allergen sensitization has conventionally been undertaken using skin prick tests (SPT) and/or immunoassays of serum-specific IgE (sIgE) levels, but these approaches are hampered by the fact that cross-reactive molecules, such as serum albumin, are present in the extracts used, which can lead to an overestimation of sensitization rates to a particular allergen.¹² Over the last decade, the use of molecular-based component-resolved diagnostics has increased, offering the opportunity to characterize individual allergenic molecules, specific phenotypes of allergens, as well as a possibility of detecting potential cross-reactivity to allergens.¹³⁻¹⁵ While increasing number of studies have described the patterns of allergen components in childhood populations, only very few component-resolved studies have been conducted in adult populations. Furthermore, characterization of sensitization patterns to furry animals and comparison of potential patterns and their determinants between the general population and asthmatic patients has not been previously undertaken in adults.

The aim of this study was to determine the patterns of sensitization to furry animal allergen components in an unselected adult population enriched with asthmatics. We studied the frequency of sensitization to specific allergen components, their overlaps, and their poly-sensitization patterns. Furthermore, we applied data-driven cluster analysis to uncover underlying sensitization clusters of the allergen components. The frequency of allergen component sensitization, sensitization patterns, and sensitization clusters were compared between a random and an asthma sample. Finally, we examined the demographic and environmental determinants of the sensitization patterns, also doing so separately for the random and asthma samples.

2 | MATERIAL AND METHODS

2.1 | Study population and questionnaire

The West Sweden Asthma Study (WSAS) has been described in detail previously.¹⁶ WSAS is a population-based study established to measure the trends in asthma, allergy, and respiratory conditions in adults living in Western Sweden; determine risk and prognostic factors of these conditions; and catalyse clinical and mechanistic studies to understand the underlying causes of these conditions. In 2008, a postal questionnaire was mailed to 30 000

randomly selected subjects aged 16-75 years living in Western Sweden in the region of Västra Götaland, which includes the city of Gothenburg. The questionnaire has been described elsewhere.^{16,17} We received 18 087 completed questionnaires, giving a response rate of 62 % after correction for untraced and diseased subjects. A non-response study showed no differences in the prevalence of asthma, rhinitis, and respiratory symptoms between responders and non-responders.¹⁷ Of the responders to the postal questionnaire, 2000 were randomly selected and invited to clinical examinations and detailed structured interviews; 1172 subjects participated (among them 130 with doctor's diagnosed asthma). In addition, all other questionnaire responders who had reported that they had doctor-diagnosed asthma or reported ever having had asthma and used asthma medication or reported symptoms common in asthma during the previous year were also invited to the clinical examinations ($n = 1524$); 834 of the subjects participated. In total, 2006 subjects participated in the clinical examinations and detailed structured interviews; 964 of the participants had asthma (ie total of 130 with asthma from the random sample and 834 originally identified to have asthma; Figure 1). Of the 2006 who participated in the clinical examinations, 1872 (93%, of which 698 had asthma) gave blood samples for Phadiatop measurements of IgE levels to a mix of aeroallergens (Figure 1). The reasons for not undergoing Phadiatop measurements were inability to find the veins for serum collection or refusal to give blood samples. The study was approved by the regional Ethics Committee at the University of Gothenburg (approval number: 593-08).

2.2 | Allergen-specific IgE measurements

At the clinical examinations, blood samples were collected and sera stored at -20°C before analysis of IgE levels against a mix of aeroallergens (Phadiatop). Subjects with $\text{IgE} \geq 0.35 \text{ kU/L}$ against the aeroallergen mix were further characterized regarding IgE antibody levels against each individual allergen of the panel: cat, dog, horse, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Cladosporium herbarum*, birch, timothy grass, and mugwort. All measurements of IgE performed used ImmunoCAP system (Immunodiagnosics; Thermo Fisher Scientific, Uppsala, Sweden). The results were presented in kU/L, and values of $\text{IgE} \geq 0.35 \text{ kU/L}$ for an individual allergen were taken as positive for allergic sensitization. Subjects who were sensitized to either cat, dog, or horse were further analysed for IgE to the following specific allergen components: Fel d 1, Fel d 2, and Fel d 4 for cat; Can f 1, Can f 2, Can f 3, and Can f 5 for dog; and Equ c 1 for horse.

2.3 | Definition of patterns of allergic sensitization

We were interested in describing the patterns of allergic sensitization to each of the animal allergen components measured, as well as the patterns of sensitization to the lipocalins (Fel d 4, Can f 1, Can f 2, and Equ c 1), mono-, double-, and poly-sensitization. Mono-

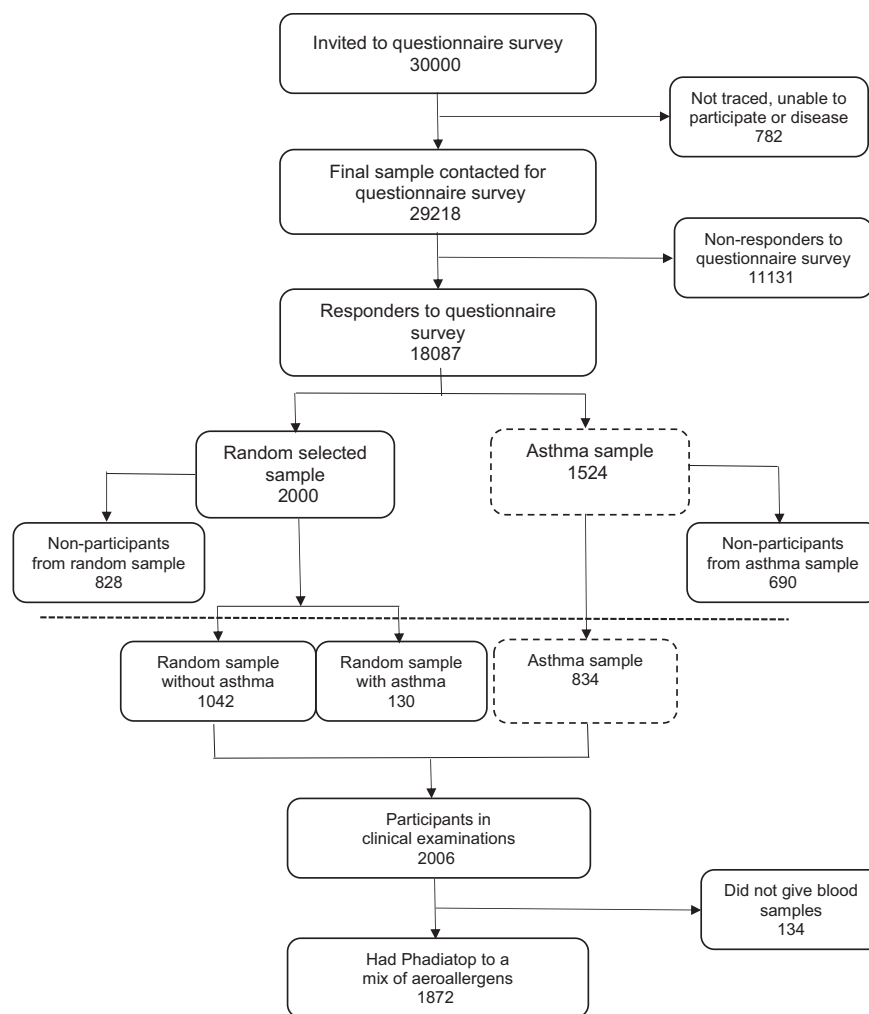


FIGURE 1 Flowchart of the study population. The study was based on a questionnaire survey from which a random sample and asthma sample were selected

sensitization was defined as positivity to only one of the animal allergen components; double sensitization was defined as positivity to only two components; and poly-sensitization was defined as positivity to more than two components. It is of particular interest to investigate the overlap of the lipocalins, as they display a well-known overall similarity in their fold in spite of the relatively low degree of amino acid sequence identity.

2.4 | Cluster analysis of allergen components

Cluster analysis is one of the assortment of data-driven multivariate statistical procedures used to define or group individuals on the basis of measured variables, so that individuals with similar characteristics are grouped together in clusters.¹⁸ Once constructed, there is a strong association between members of a specific cluster, but weak association between members of different clusters.¹⁸ In addition to the component patterns described above, we implemented a data-driven definition of sensitization patterns by performing a cluster analysis of the data, including the measured allergen components (Fel d 1, Fel d 2, Fel d 4, Can f 1, Can f 2, Can f 3, Can f 5, and Equ c 1) in the model. We used k-means clustering approach to derived

the clusters, with the Jaccard similarity coefficient (which is relevant for binary data) as a measure for forming the clusters, so that individuals with similar sensitization patterns can be clustered together. Based on preliminary assessment of the data, we determined that a three-cluster model was the most robust to describe the data.

2.5 | Demographic and environmental factors

Information on several demographic factors and environmental exposures were collected from the survey questionnaire. In the current analysis, we examined the role of the following factors as determinants of sensitization patterns: age, smoking, body mass index (BMI), degree of urbanization, exposure to dust/fumes at the place of work, whether or not participants were raised on a farm, educational attainment, and family history of allergy or asthma. The categorization of these variables for analysis is given in Table 1.

2.6 | Statistical analyses

All analyses were done separately for the random and asthma samples. The Pearson chi-square test was used to evaluate the differences

TABLE 1 Background characteristics of participants by Phadiatop vs no Phadiatop measurement

Background characteristics	N = 2006 n (%)	No Phadiatop measurement, n = 134 n (%)	Had Phadiatop measurement, n = 1872 n (%)	P-value
Gender				
Males	888 (44.3)	38 (28.4)	850 (45.4)	<0.001
Females	1118 (55.7)	96 (71.6)	1022 (54.6)	
Age, years				
≤30	289 (14.4)	17 (12.7)	272 (14.5)	0.794
31-45	546 (27.2)	38 (28.3)	508 (27.1)	
46-60	586 (29.2)	43 (32.1)	543 (29.0)	
61-75	585 (29.2)	36 (26.9)	549 (29.3)	
Smoking status				
Non-smokers	1112 (55.7)	75 (56.0)	1037 (55.7)	0.987
Ex-smokers	548 (27.44)	36 (26.9)	512 (27.5)	
Current smokers	337 (16.9)	23 (17.1)	314 (16.8)	
Body mass index, kg/m ²				
<25	719 (35.9)	44 (32.8)	675 (36.1)	0.014
25-29.9	869 (43.3)	49 (36.6)	820 (43.8)	
≥30	417 (20.8)	41 (30.6)	376 (20.1)	
Degree of urbanization				
>10 000 inhabitants	1486 (74.1)	90 (67.2)	1396 (74.6)	0.059
≤10 000 inhabitants	520 (25.9)	44 (32.8)	476 (25.4)	
Exposure to dust/fumes at work				
No	1458 (72.7)	102 (76.7)	1356 (73.4)	0.402
Yes	523 (26.1)	31 (23.3)	492 (26.6)	
Missing information	25 (1.2)			
Raised on a farm				
No	1742 (86.8)	110 (82.7)	1632 (87.9)	0.078
Yes	247 (12.3)	23 (17.3)	224 (12.1)	
Missing information	17 (0.8)			
Education				
Less than high school	380 (18.9)	21 (15.8)	359 (19.3)	0.399
High school	745 (37.2)	47 (35.3)	698 (37.5)	
Tertiary education	871 (43.4)	65 (48.9)	806 (43.3)	
Missing	10 (0.5)			
Family history of allergy or asthma				
No	1087 (54.2)	65 (48.5)	1022 (54.6)	0.172
Yes	919 (45.8)	69 (51.5)	850 (45.4)	

between categorical variables. We used Venn diagrams to describe the overlaps in sensitization between the allergen components. Logistic regression was used to study the relation of the demographic and environmental factors to sensitization patterns (mono-, double-, and poly-sensitization) and each derived cluster (all the factors were concurrently adjusted in these models). The odds ratios (OR) derived from the logistic regression analyses were accompanied by their respective 95% confidence intervals (95% CI). All analyses were carried out using Stata statistical package version 15 (Stata-Corp, Texas, USA).

3 | RESULTS

3.1 | Characteristics of participants

Of the 2006 participants who were clinically examined, 1872 (93%) had Phadiatop measurement (Figure 1). Of those with Phadiatop measurement, positivity was detected in 41% (n = 775). Those with Phadiatop measurement and those without were similar regarding their demographic characteristics and environmental exposures, except for differences in gender and BMI (Table 1).

3.2 | Prevalence of sensitization to allergen components

In Table 2, we present the frequency of sensitization to allergen components separately for the random and asthma samples. As would be expected, the frequencies of sensitization to each of the allergen components, as well as mono-, double-, and poly-sensitization were substantially higher in the asthma sample than in the random sample. By using the random sample, we estimated the population-level prevalence of furry animal allergen component sensitization as follows: Fel d 1 was the most commonly sensitized component (8.9%) while Fel d 2 (0.8%) and Can f 3 (0.8%) were equally the least sensitized components. The most commonly sensitized dog allergen component was Can f 5 (3.3%); Equ c 1 was present in 2%; while sensitization to any lipocalin was present in 4.3%. The prevalence of mono-sensitization was 5.6%, double sensitization 1.5%, and poly-sensitization 2.1%. Except for Fel d 1, majority of those sensitized to each allergen component were more likely to be poly-sensitized, both in the random and asthma samples (Figure 2).

3.3 | Allergen component clusters

We derived three distinct clusters when k-means clustering was implemented in our data (Table 3). The table shows similar allergen component clusters for the random and asthma samples. The first cluster ($n = 990$ in the random sample and $n = 509$ in the asthma sample), which we named “non-sensitized cluster”, comprised of those not sensitized to any allergen component. The second cluster ($n = 79$ in the random sample and $n = 148$ in the asthma sample), named “Fel d 1-driven sensitized cluster”, was the most common cluster when compared to cluster three, and comprised of those who were primarily mono-sensitized to Fel d 1. The third cluster ($n = 34$ in the random sample and $n = 112$ in the asthma sample), named “multi-component sensitized cluster”, comprised of those

who had greater propensity to be simultaneously sensitized to multiple allergen components than to single allergen component; while most people in this cluster were highly multi-sensitized to all other components, they were less likely to be sensitized to Table 3. In relating the derived sensitization clusters to poly-sensitization patterns, among those who were sensitized to allergen components, the results showed that both in the random and asthma samples, majority of those in cluster two were mono-sensitized, while majority of those in cluster three were poly-sensitized (Figure 3).

3.4 | Overlap of allergen component sensitization

3.4.1 | Sensitization to any cat, dog, and horse allergen components

Figure 4 (upper panels) shows the overlap of sensitization to any specific component within each furry animal, separately for the random and asthma samples. Sensitization to Equ c 1 was present in 2% vs 12% in the random and asthma samples, respectively. In the random sample, no one was sensitized to only Equ c 1, while it was only two people in the asthma sample. No one was simultaneously sensitized to Equ c 1 and any dog component in the random sample, but only one person was sensitized in the asthma sample. In the random sample, 16 people (1.4%) were simultaneously sensitized to any cat, dog, and horse allergen components, while it was 66 people (9%) in the asthma sample.

3.4.2 | Sensitization to specific cat allergen components

Figure 4 (lower panels) shows the overlap of sensitization among the cat allergen components, separately for the random population and asthma samples. In both samples, fewer individuals were sensitized to Fel d 2 or Fel d 4 without being sensitized to Fel d 1. Only five

TABLE 2 Frequency of sensitization to furry animal allergen components in the random and asthma samples among those with Phadiatop measurement

Allergen component	All, N = 1872 Number (%) sensitized	Random sample, n = 1103 Number (%) sensitized	Asthma sample, n = 769 Number (%) sensitized	P-value*
Fel d 1	311 (16.6)	98 (8.9)	213 (28.7)	<0.001
Fel d 2	44 (2.3)	9 (0.8)	35 (4.5)	<0.001
Fel d 4	117 (6.2)	23 (2.1)	94 (12.2)	<0.001
Equ c 1	113 (6.0)	22 (2.0)	91 (11.8)	<0.001
Can f 1	138 (7.4)	33 (3.0)	105 (13.6)	<0.001
Can f 2	45 (2.4)	15 (1.4)	30 (3.9)	<0.001
Can f 3	51 (2.7)	9 (0.8)	42 (5.5)	<0.001
Can f 5	104 (5.6)	37 (3.3)	67 (8.7)	<0.001
Lipocalins	198 (10.6)	48 (4.3)	150 (19.5)	<0.001
Mono-sensitization	164 (8.8)	62 (5.6)	102 (13.3)	<0.001
Double sensitization	72 (3.8)	17 (1.5)	55 (7.1)	<0.001
Poly-sensitization	138 (7.4)	34 (2.1)	104 (13.5)	<0.001

*P-value for comparison of prevalence between the random and asthma samples.

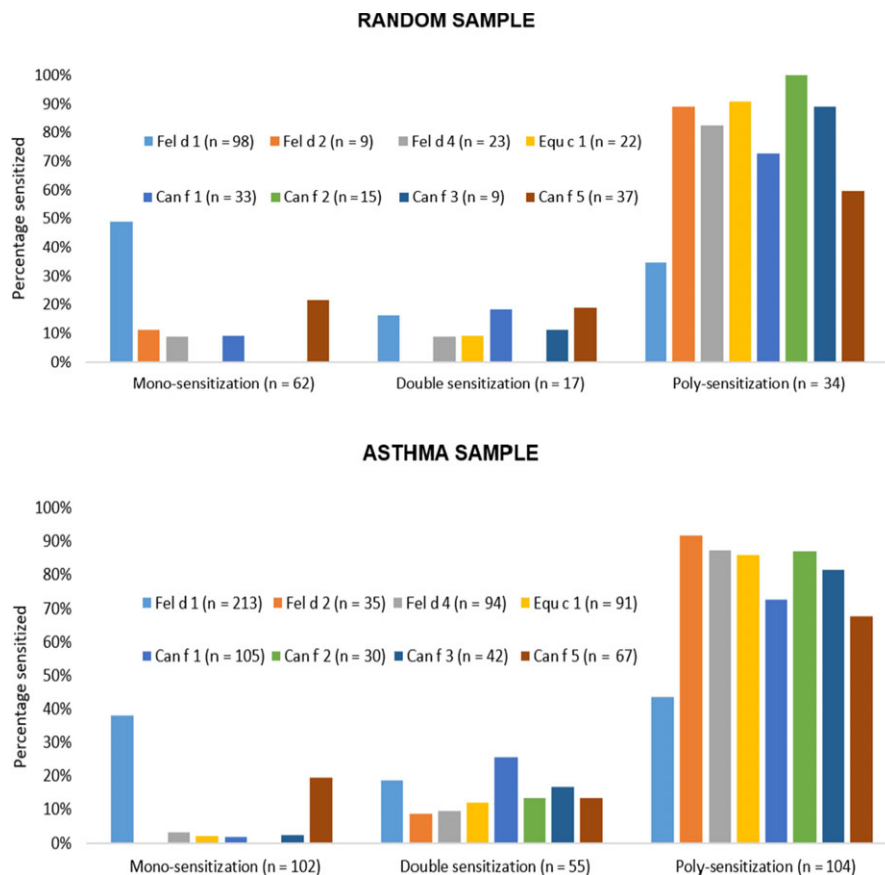


FIGURE 2 Proportion of mono-, double-, and poly-sensitization in those sensitized to specific furry animal allergen components. % = Percentage of those sensitized to respective allergen component within each sample (random = 1103, asthma = 769)

(0.4%) and 20 (2.6%) people were sensitized to all three cat allergen components in the random and asthma samples, respectively.

3.4.3 | Sensitization to specific dog allergen components

Figure 5 (upper panels) shows the overlap of sensitization among the dog allergen components (Can f 1 and Can f 2 were combined given their similarity in structure), separately for the random and asthma samples. In both samples, the most common overlap was between Can f 5 and Can f 1/Can f 2 than with Can f 3. Only 6 (0.5%) and 14 (2%) people were simultaneously sensitized to all dog components in the random and asthma samples, respectively.

3.4.4 | Sensitization to the lipocalins

Figure 5 (lower panels) shows the overlap of sensitization among the lipocalins (Fel d 4, Can f 1/Can f 2, and Equ c 1), separately for the random and asthma samples. In both samples, the most common overlap was between Equ c 1 and Fel d 4. In the random sample, 8 (1%) people and 55 (7%) in the asthma sample were simultaneously sensitized to all lipocalin components.

3.5 | Determinants of sensitization patterns and membership in the allergen component clusters

In Table S1, we present the ORs for the associations between the demographic and environmental exposures and patterns of

sensitization to animal allergen components, separately for the random and asthma samples. In the random sample, being a female compared to a male was associated with a decreased risk of double sensitization, while family history of allergy or asthma increased the risk of all sensitization patterns (Table S1). In the asthma sample, older age compared to younger age was associated with a decreased risk of mono- and poly-sensitization; being raised on a farm was associated with a decreased risk of mono-sensitization, while a family history of allergy or asthma increased the risk of mono-sensitization (Table S1).

Table S2 shows the ORs for the associations between demographic and environmental exposures and allergen component sensitization clusters, separately for the random and asthma samples. In the random sample, female sex and old age were associated with an increased likelihood of belonging to cluster one (non-sensitized), while a family history of allergy or asthma decreased the likelihood of belonging to that cluster. While female sex was associated with a decreased risk, family history of allergy or asthma increased the likelihood of belonging to cluster two. In the asthma sample, female sex, older age, and being raised on the farm increased the likelihood of belonging to cluster one, while family history of allergy or asthma decreased the likelihood of membership of that cluster. Old age decreased the likelihood of belonging to cluster two, while family history of allergy or asthma increased the likelihood of belonging to that cluster. Older age decreased the likelihood of belonging to cluster three, while residence in less urban setting increased the likelihood.

TABLE 3 Furry animal allergen component clusters and proportion of those sensitized to specific components belonging to each cluster in the random and asthma samples

Random sample				
	n	Cluster 1 "Non-sensitized cluster" n = 990% ^a	Cluster 2 "Fel d 1-driven sensitized cluster" n = 79% ^a	Cluster 3 "Multi-component sensitized cluster" n = 34% ^a
Fel d 1	98	0.0	80.6	19.4
Fel d 2	9	0.0	11.1	88.9
Fel d 4	23	0.0	26.1	73.9
Equ c 1	22	0.0	45.4	54.5
Can f 1	33	0.0	39.4	60.6
Can f 2	15	0.0	13.3	86.7
Can f 3	9	0.0	22.2	77.8
Can f 5	37	0.0	32.4	67.6
Asthma sample				
	n	Cluster 1 "non-sensitized group" n = 509% ^a	Cluster 2 "Fel d 1-driven sensitized group" n = 148% ^a	Cluster 3 "Multi-component sensitized group" n = 112% ^a
Fel d 1	213	0.0	69.5	30.5
Fel d 2	35	0.0	5.7	94.3
Fel d 4	94	0.0	20.2	79.8
Equ c 1	91	0.0	24.2	75.8
Can f 1	105	0.0	31.4	68.6
Can f 2	30	0.0	6.7	93.3
Can f 3	42	0.0	7.1	92.9
Can f 5	67	0.0	19.4	80.6

Yellow: <25% sensitized; Brown: 25%-50% sensitized; Green: ≥50% sensitized.

^aPercentage of those sensitized to respective allergen components that fall into each sensitization cluster.

4 | DISCUSSION

In this representative random population sample enriched with asthmatics, we report that the prevalence of sensitization to furry animal allergen components in adults ranged from 0.8% for Fel d 2 and Can f 3 to 9% for Fel d 1. The most commonly sensitized dog component was Can f 5 (3.6%), whereas 2.1% were sensitized to Equ c 1. Mono-sensitization was more common than double or poly-sensitization. The frequency of sensitization was much higher in the asthma sample than in the random population sample. We found varying levels of overlaps among the components: those sensitized to Fel d 2 and Fel d 4 were more likely to be sensitized to Fel d 1, but not vice versa. Among the dog components, the most common overlap was between Can f 1/Can f 2 and Can f 5. The most common overlap among the lipocalins was between Equ c 1 and Fel d 4. Using cluster analysis, we derived three distinct clusters of allergen

component sensitization, comprising of those not sensitized to any allergen component; those primarily mono-sensitized to Fel d 1; and those simultaneously sensitized to multiple components. Key determinants of sensitization patterns and membership of the component clusters were gender, age, being raised on a farm, family history of allergy or asthma, smoking habits, and exposure to dust or fumes at work place.

All allergen component analyses were performed with singleplex testing (ImmunoCAP). The majority of previous animal component studies¹⁹⁻²⁴ have been analysed with multiplex testing using microarray technology (ISAC), which does not have the same degree of resolution as singleplex testing.^{25,26} Only few clinical animal allergy studies have used singleplex testing.^{27,28} The chip-based technology is less quantitative, which makes the singleplex IgE antibody assays those of choice for routine diagnostic allergy testing.²⁹ Thus, the sensitivity and specificity of different platforms differ, and this should be taken into consideration when reporting and comparing scientific results.²⁹ A growing spectrum of molecules representing single allergens of clinical relevance have been identified, characterized and produced for commercial in vitro assays and component-resolved diagnosis thus allowing a detailed molecular profiling of the polyclonal IgE repertoire of the allergic patient. Measuring serum IgE to a whole allergen extract is insufficiently precise to reflect on how the sensitization influences the clinical impact of allergy on clinical outcomes, such as asthma, especially for dog allergy diagnosis.^{21,30} The whole extracted allergens from furry animals consist primarily of major allergen components such as Fel d 1 and Can f 1. EAACI Molecular Allergology User's Guide states that IgE to Can f 1 is the most important prognostic marker of dog allergy and superior to dog allergen extract and that Fel d 1 is equally good as IgE to cat extract in predicting cat allergy.²⁶ We defined the poly-sensitization patterns based on sensitization across components of the three animals because cat, dog, and horse are close related. The lipocalins are major allergens in all three animals and a previous study found that children with severe asthma were sensitized to more than three animal components.²² The lipocalin sequence identity can to a certain extent predict IgE cross-reactivity, but even lipocalins with low sequence identity may cross-react. Although epitope spreading has been previously defined as multiple sensitization within the same species, for example, grass,³¹ in real life, it is still rare to have people who are exclusively poly-sensitized from only one species, for example, from only one animal. A common phenomenon is that people are poly-sensitized to components from different animals, in real life. We included horse into the analysis because, although horse allergy is a rare phenomenon, horse sensitization is common. Patelis et al²¹ found horse sensitization in 40% of young asthmatics and very few of them reported horse allergy. Equ c 1 (horse) and Fel d 4 (cat) have a 68% sequence identity.³² Our present data throw further light into this intriguing interrelationship among cat, dog, and horse allergen component sensitization.

This study focused on a population in West Sweden; thus, our results may not be applicable to other parts of the world where other airborne allergens are dominating, for example house dust mite



FIGURE 3 Distribution of sensitization patterns within each allergen component cluster. % = Percentage of those sensitized to respective allergen component within each sample (random = 1103, asthma = 769)

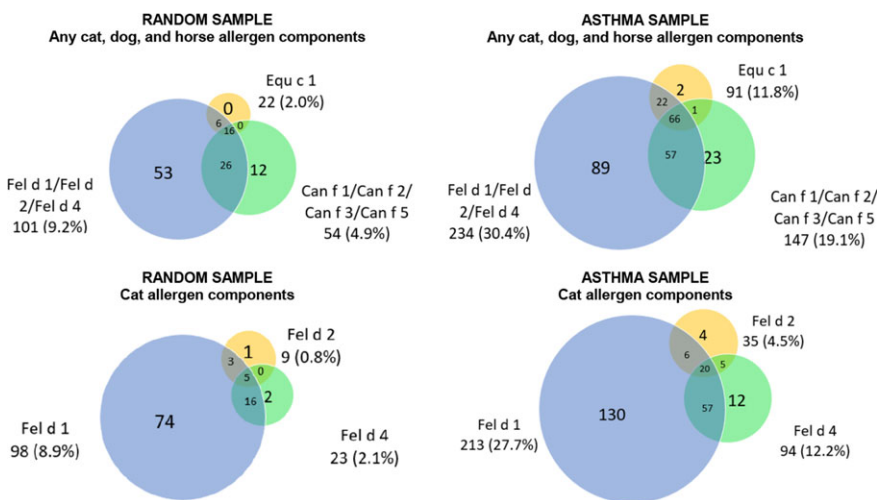
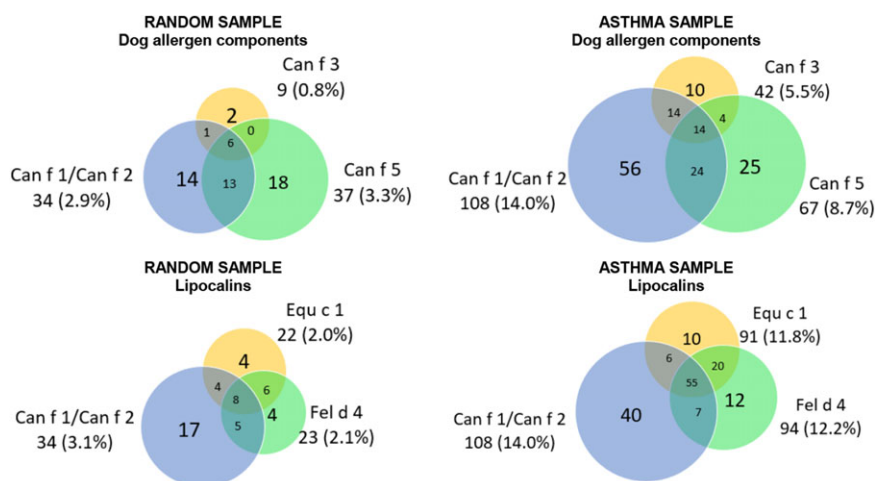


FIGURE 4 Overlap among cat (Fel d 1, Fel d 2, and Fel d 4) allergen components in the random (left figure) and asthma (right figure) samples % = Percentage of those sensitized to respective allergen component within each sample (random = 1103, asthma = 769)

in warmer and more humid climates. Nevertheless, furry animals and pollen are the most important airborne sensitizers in large geographic areas in the northern hemisphere,³³⁻³⁸ as such, this detailed characterization of the patterns of sensitization to furry animal allergen components complements an important knowledge gap on the current distribution of furry animal sensitization in adults of these populations at the molecular level. A further limitation of our study is the cross-sectional design, so that we are unable to establish any temporality in the associations between the demographic and environmental determinants and patterns of allergen component sensitization, as well as in the pairwise associations among the specific allergen components and between specific allergen components and mono-, double-, or poly-sensitization. For more definitive understanding of the nature and degree of the temporality of these

associations, a prospective cohort study design will be required. We aim to investigate these perspectives in future aspects of this study in which a cohort design is currently being implemented. Additional limitation of this analysis is that we did not study the role of furry pet ownership, furry pet-related occupation or histories of direct or indirect contact with furry pets as part of the determinants of patterns of allergic sensitization; factors which are known to be important for sensitization to furry pet allergens. We were only able to analyse the included specific allergen components of cat, dog, and one component of horse, because at the time of this study, these molecules were the only ones commercially available from the manufacturer. Therefore, it cannot be excluded that other animal allergen components are important in our study population. For instance, sensitization to Fel d 7, Can f 4, and Can f 6 has been developed



and has been suggested to be of clinical importance, but these are not currently available for commercial use; there is need to investigate them in future studies.²⁶

The current study represents the first detailed characterization of patterns of sensitization to furry animal allergen components in an unselected adult population using ImmunoCAP, doing so separately for both a random and an asthma sample. In a previous study, Uriarte and Sastre reported the results of similar allergen components as we included in this study, except for the exclusion of Fel d 2 and addition of Fel d 3 and Equ c 3.³⁹ However, that study combined both children and adults with an age range of 3–60 years, contrary to an adult-only population of our study (16–75 years). That previous study also mixed measurements obtained using both simplex (ImmunoCAP) and multiplex (ISAC) testing and the study population constituted symptomatic asthma and rhinitis patients recruited from an outpatient clinic. By relying on the population-representative random sample of our study, we have estimated that Fel d 1 (9%) was the most commonly animal allergen component in our study population; the corresponding frequency among the asthma sample was 28%. The most commonly sensitized dog allergen component was Can f 5. Similar findings have been reported in two different cohorts from northern (11–12 years of age)²³ and southwestern (4–16 years of age)²⁴ Sweden in which it was shown that Fel d 1 and Can f 5 were the most commonly sensitized cat and dog allergen components, respectively. Those sensitized to Fel d 2 and Fel d 4 were more likely to be sensitized to Fel d 1, but not vice versa, a finding which was also reported among children.²⁷ Our results further showed that mono-sensitization was the norm, and then followed by poly-sensitization. This contrasts the findings reported among children in which the proportion of children poly-sensitized to furry animal components was double that of mono-sensitization.²³ By applying clustering analysis techniques, we were able to derive three distinct allergen component clusters; primary mono-sensitization Fel d 1 was more common than simultaneous multi-sensitization to several allergen components. There have been no previous studies investigating the utility of clustering techniques to derive clinically important clusters of allergen component sensitization in an adult population.

Other studies done among children have clearly shown that such data-driven approaches can be potentially useful to derive clinically useful clusters that may highlight sensitization phenotypes. Further studies are required in this aspect.^{19,24}

5 | CONCLUSIONS

By characterizing the patterns of sensitization to furry animal allergen component sensitization in adults in random and asthma samples, we have shown that Fel d 1 and Can f 5, respectively, are the most common cat and dog components and that mono-sensitization was the norm rather than poly-sensitization as seen in children. By applying statistical clustering, we have also shown that more of the mono-sensitized individuals in our population have mainly Fel d 1-driven sensitization. Further studies, particularly prospectively designed cohort studies, are required to confirm the temporality of the associations between the investigated demographic and environmental factors and patterns of allergic sensitization to the furry animal allergen components. Furthermore, we will subsequently investigate whether the characterized sensitization patterns and derived clusters differentially predict clinical outcomes of asthma and indicators of asthma severity in this population.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTION

BL, JL, LE, and MPB designed the study. LE was responsible for the study database. RM, BL and JL participated in data collection. SSu and SSj planned and executed the IgE analyses. SSj was also responsible for a precision of the analysis of serum IgE. SSu and BN planned and executed the statistical analyses and drafted the manuscript. All authors participated in data interpretation, writing of the manuscript and review the data presented. All authors approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Table S1. Relation of demographic and environmental factors to mono-, double-, and poly-sensitization in participants selected based on random sample (left figure) and in participants with asthma (right figure). Odds ratio (OR), 95% confidence interval (95% CI). All variables were concurrently adjusted.

	RANDOM SAMPLE			ASTHMA SAMPLE		
	Mono-sensitization OR (95% CI)	Double sensitization OR (95% CI)	Poly-sensitization OR (95% CI)	Mono-sensitization OR (95% CI)	Double sensitization OR (95% CI)	Poly-sensitization OR (95% CI)
Gender						
Males	1	1	1	1	1	1
Females	0.62 (0.36-1.08)	0.29 (0.10-0.88)	0.96 (0.46-2.01)	0.73 (0.46-1.14)	1.00 (0.54-1.84)	0.74 (0.47-1.17)
Age, years						
≤30	1	1	1	1	1	1
31-45	0.56 (0.26-1.21)	0.85 (0.22-3.21)	0.92 (0.32-2.69)	0.57 (0.31-1.04)	1.58 (0.69-3.64)	0.87 (0.47-1.60)
46-60	0.84 (0.39-1.82)	0.32 (0.06-1.64)	1.39 (0.49-3.96)	0.58 (0.30-1.12)	1.13 (0.44-2.87)	0.80 (0.41-1.55)
61-75	0.51 (0.21-1.26)	0.33 (0.06-1.90)	0.32 (0.07-1.47)	0.41 (0.19-0.88)	0.54 (0.16-1.81)	0.16 (0.06-0.43)
Smoking status						
Non-smokers	1	1	1	1	1	1
Ex-smokers	0.70 (0.35-1.39)	1.67 (0.50-5.58)	0.47 (0.17-1.27)	0.74 (0.43-1.29)	0.68 (0.31-1.47)	1.65 (0.99-2.76)
Current smokers	0.63 (0.27-1.47)	1.44 (0.35-5.86)	0.72 (0.26-2.01)	0.68 (0.35-1.31)	1.38 (0.64-2.97)	0.63 (0.31-1.26)
Body mass index, kg/m ²						
<25	1	1	1	1	1	1
25-29.9	0.79 (0.43-1.44)	1.71 (0.51-5.72)	1.59 (0.69-3.64)	1.18 (0.70-1.97)	1.01 (0.52-1.99)	1.18 (0.72-1.95)
≥30	0.75 (0.32-1.75)	2.23 (0.53-9.40)	1.96 (0.70-5.45)	1.35 (0.74-2.45)	1.19 (0.54-2.61)	0.70 (0.37-1.33)
Degree of urbanization						
>10 000 inhabitants	1	1	1	1	1	1
≤10 000 inhabitants	0.49 (0.21-1.11)	1.49 (0.46-4.86)	1.00 (0.42-2.41)	0.91 (0.55-1.52)	1.19 (0.62-2.89)	1.38 (0.86-2.23)
Exposure to dust/fumes at work	0.77 (0.38-1.56)	0.24 (0.05-1.16)	0.65 (0.25-1.69)	1.08 (0.65-1.79)	0.64 (0.30-1.35)	0.69 (0.41-1.17)
Raised on a farm	0.61 (0.21-1.76)	-----**	0.47 (0.11-2.08)	0.25 (0.08-0.84)	0.82 (0.28-2.41)	0.77 (0.33-1.80)
Education						
Less than high school	1	1	1	1	1	1
High school	1.05 (0.38-2.90)	2.61 (0.29-23.45)	2.09 (0.43-10.02)	1.05 (0.52-2.14)	1.29 (0.45-3.72)	1.25 (0.62-2.50)
Tertiary education	1.73 (0.67-4.45)	1.88 (0.21-16.93)	2.19 (0.47-10.27)	1.51 (0.74-3.05)	2.04 (0.72-5.81)	0.82 (0.39-1.71)
Missing						
Family history of allergy or asthma	2.11 (1.23-3.62)	3.49 (1.16-10.45)	2.45 (1.19-5.05)	1.62 (1.00-2.62)	1.73 (0.91-3.31)	1.08 (0.68-1.71)

** Too small numbers, inestimable

Table S2. Relation of demographic and environmental factors to specific clusters of allergen component sensitization in participants selected based on random sample (left figure) and in participants with asthma (right figure). Odds ratio (OR), 95% confidence interval (95% CI). All variables were concurrently adjusted.

	RANDOM SAMPLE			ASTHMA SAMPLE		
	Cluster 1 "Non-Sensitized Group" OR (95% CI)	Cluster 2 "Fel d 1-Driven Sensitized Group" OR (95% CI)	Cluster 3 "Multi-Component Sensitized Group" OR (95% CI)	Cluster 1 "Non-Sensitized Group" OR (95% CI)	Cluster 2 "Fel d 1-Driven Sensitized Group" OR (95% CI)	Cluster 3 "Multi-Component Sensitized Group" OR (95% CI)
Gender						
Males	1	1	1	1	1	1
Females	1.65 (1.08-2.52)	0.51 (0.31-0.83)	0.99 (0.47-2.08)	1.41 (1.01-1.98)	0.78 (0.53-1.16)	0.76 (0.49-1.17)
Age, years						
≤30	1	1	1	1	1	1
31-45	1.48 (0.81-2.69)	0.66 (0.33-1.32)	0.76 (0.27-2.15)	1.29 (0.81-2.05)	1.17 (0.68-1.98)	0.53 (0.29-0.96)
46-60	1.18 (0.64-2.17)	0.63 (0.30-1.30)	1.53 (0.57-4.14)	1.50 (0.91-2.47)	1.01 (0.56-1.81)	0.50 (0.26-0.95)
61-75	2.45 (1.18-5.07)	0.47 (0.20-1.06)	0.23 (0.04-1.23)	4.34 (2.37-7.97)	0.42 (0.20-0.85)	0.18 (0.08-0.42)
Smoking status						
Non-smokers	1	1	1	1	1	1
Ex-smokers	1.44 (0.85-2.44)	0.86 (0.48-1.56)	0.40 (0.13-1.19)	0.97 (0.65-1.43)	0.71 (0.44-1.14)	1.60 (0.97-2.65)
Current smokers	1.33 (0.72-2.44)	0.70 (0.33-1.50)	0.89 (0.34-2.31)	1.36 (0.85-2.18)	0.82 (0.47-1.41)	0.74 (0.38-1.44)
Body mass index, kg/m ²						
<25	1	1	1	1	1	1
25-29.9	0.91 (0.57-1.46)	1.27 (0.73-2.21)	0.75 (0.33-1.70)	0.82 (0.56-1.19)	1.12 (0.72-1.72)	1.25 (0.76-2.09)
≥30	0.79 (0.43-1.45)	1.33 (0.64-2.76)	1.07 (0.38-2.96)	0.95 (0.61-1.48)	0.85 (0.50-1.45)	1.33 (0.75-2.39)
Degree of urbanization						
>10 000 inhabitants	1	1	1	1	1	1
≤10 000 inhabitants	1.35 (0.78-2.35)	0.61 (0.31-1.21)	1.10 (0.46-2.66)	0.82 (0.57-1.17)	0.89 (0.58-1.39)	1.62 (1.01-2.57)
Exposure to dust/fumes at work	1.70 (0.98-2.95)	0.65 (0.34-1.23)	0.55 (0.20-1.51)	1.33 (0.91-1.94)	0.83 (0.52-1.30)	0.76 (0.46-1.27)
Raised on a farm	2.21 (0.93-5.27)	0.44 (0.15-1.26)	0.51 (0.12-2.23)	2.10 (1.18-3.96)	0.53 (0.24-1.16)	0.55 (0.22-1.34)
Education						
Less than high school	1	1	1	1	1	1
High school	0.66 (0.30-1.48)	1.02 (0.42-2.48)	4.81 (0.60-38.44)	0.82 (0.49-1.36)	1.16 (0.61-2.19)	1.22 (0.63-2.36)
Tertiary education	0.51 (0.24-1.12)	1.63 (0.70-3.79)	3.89 (0.49-30.92)	0.74 (0.44-1.24)	1.58 (0.84-2.99)	0.97 (0.48-1.94)
Family history of allergy or asthma	0.39 (0.25-0.58)	3.17 (1.92-5.22)	1.42 (0.70-2.87)	0.63 (0.45-0.89)	1.62 (1.08-2.45)	1.21 (0.77-1.90)