Component-resolved diagnostics in pet allergy

Introduction

Furry mammals kept as pets are important allergen sources, and the prevalence of sensitization to dander from various animals appears to be increasing worldwide. Several mammalian allergens from diverse species and distinct protein families have been characterized, and some are available for component-resolved diagnostics (CRD). This review presents an overview of mammalian respiratory allergens with a focus on cat, dog, and horse. The potential of CRD in fine-tuning the diagnostic work-up following traditional methods based on whole extracts and before immunotherapy are discussed. Finally, the review highlights the clinical utility of CRD, particularly as a marker/predictor of increased asthma risk and disease severity.

Background

Domesticated furry animals are among the most common sources of respiratory allergens, causing development of sensitization and respiratory allergic diseases. Symptoms of pet allergy range in severity from the discomfort associated with rhinitis and conjunctivitis to potentially life-threatening asthmatic episodes[1].

An international survey of over 27,000 participants estimated that 57% of the population have at least one pet at home, most commonly dogs (33%) and cats (23%)[2] *(Figure 1)*. Allergy to cat and dog is considered a major risk factor for the development of asthma and rhinitis, and is associated with severe childhood asthma[3].

A study of almost 13,000 German children reported a sensitization rate of 12.6% to animal danders. The prevalence increased with age from 5.7% in 3–6 year olds to 11.5% in 7–10 year olds, and reached 17.2% in 14–17 year olds[4]. A Swedish birth cohort study of over 4,000 children reported a similar increase in sensitization rates to horse, cat, and dog from 4–16 years, respectively reaching 10.6%, 19.0%, and 22.6%[5, 6]. Increased prevalence of sensitization to common airborne allergens, including from cat and dog, has also been observed in adults[7], although prevalence rates are lower and monosensitization is more frequent[8]. In Brazil, sensitization to furry animals, especially to dog, increased dramatically among allergic children and adolescents between 2004 and 2016, seemingly reflecting a sedentary "indoor lifestyle"[9].

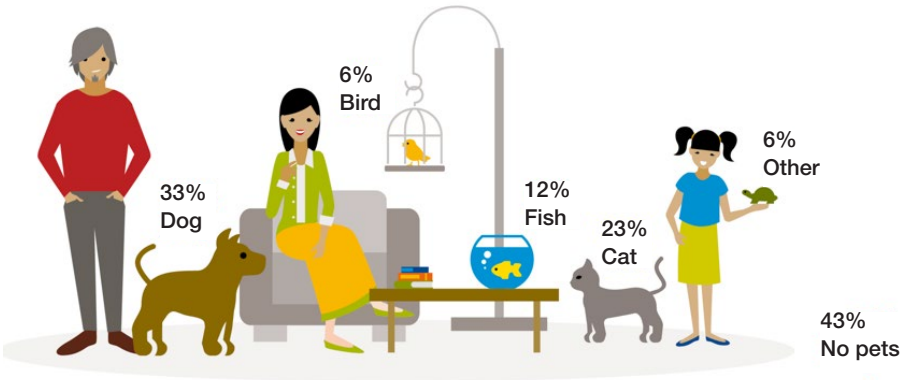

Figure 1.

Percentage of people living with pets (survey of >27,000 internet users in 22 countries)[2]

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This increase is worrying, because sensitization to cat and dog is a risk factor for the development of asthma and rhinitis, as reported in a study that showed that sensitization to key pet components was observed in young children before the development of respiratory symptoms[10].

Geographic variation in the prevalence of allergic sensitization to furry animals has been attributed to cultural differences, environmental factors, and rate of pet ownership[11, 12]. A large international multicenter study in adults reported 8.8% (range, 1.2%–22.4%) sensitization to cat as measured by skin tests[13] and the sensitization rate for dog was 20.4% among adult Korean subjects[14]. Approximately 26% of European adults coming to the clinic for suspected allergy to inhalant allergens are sensitized to cat and 27% to dog, according to another large patient-based study of skin prick testing (SPT) for aeroallergens (Global Asthma and Allergy European Network [GAL2EN])[12, 15].

A number of pet animal allergen components, mostly produced as recombinant proteins, are now available for component-resolved diagnostics (CRD), offering improved diagnostic work-up *(Figure 2)*, particularly in patients with polysensitization and/or severe asthma[16, 17].

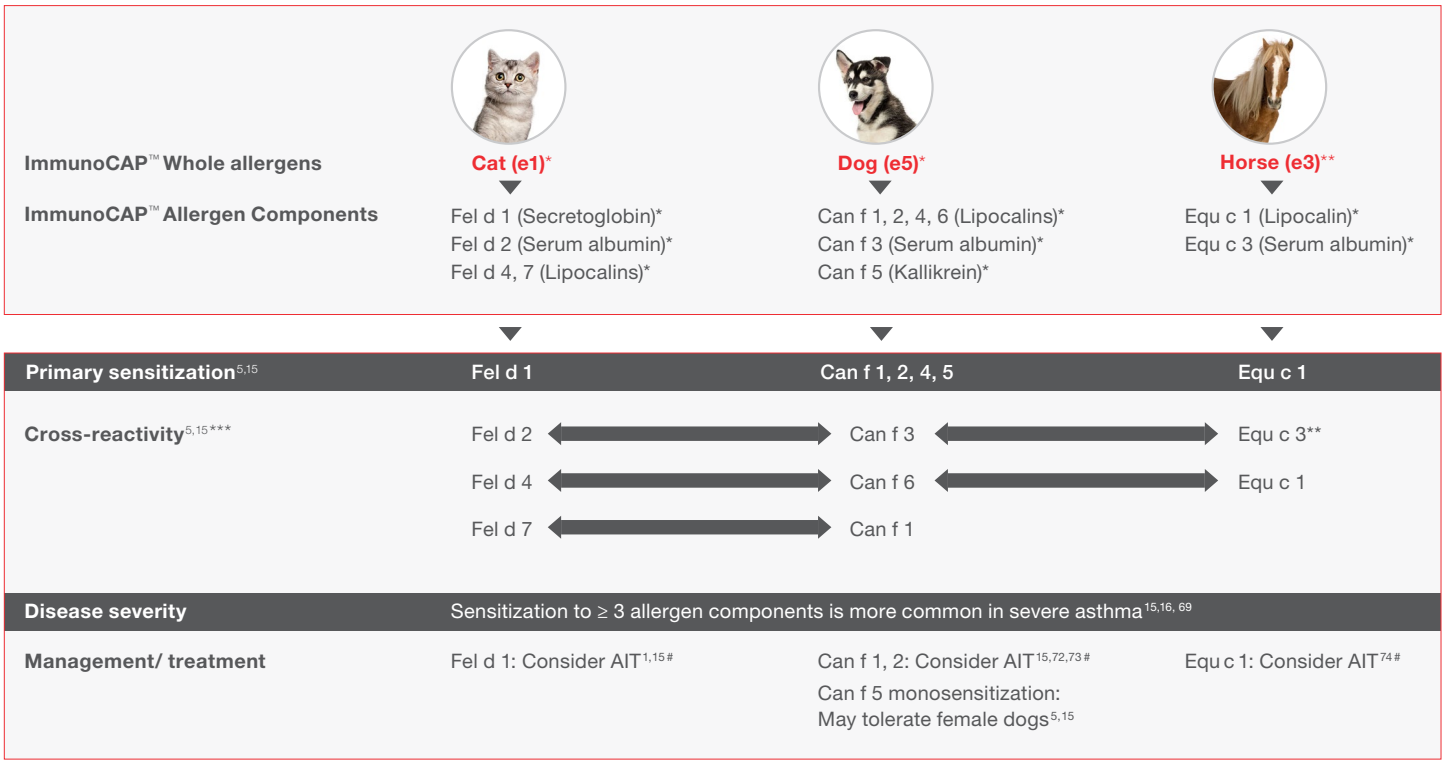
Allergens and sensitization phenotypes

Allergy diagnosis is supported by the detection of allergen-specific IgE (sIgE) antibodies using whole extracts from animal dander, or individual allergenic proteins. The use of CRD provides a tool to identify sIgE responses to specific molecular targets, which may represent species-specific or cross-reactive sensitizations[18, 19].

Allergens from pet animals are mainly present in their fur, saliva and urine and are spread into the environment through shedding of hair and dander[17]. A study reported that all of 831 sampled homes in the United States contained dog allergen and most contained cat allergen, even those that have never had an animal in the house[20].

The World Health Organization/International Union of Immunological Societies (WHO/IUIS, available from www.allergen.

Figure 2.
Suggested test profile: Suspicion of allergy to furry animals



*Full product names available on page 7.
**Available on ImmunoCAP™ ISAC sIgE 112 chip.
***When sIgE to two or more cross-reactive components from different species is detected, the highest sIgE level may indicate the primary sensitizer.
#Allergen Immunotherapy

org) lists 36 allergens from mammals (excluding milk allergens, Table 1). Major mammalian-derived respiratory allergens can be classified into the following protein families: lipocalins, secretoglobins, serum albumins, kallikreins, and latherins[17, 21].

Lipocalins

The most important group of mammalian respiratory allergens are lipocalins. These allergens are produced in secretory glands and are present in skin, urine, saliva, sweat and sebum. At least one allergen from the lipocalin protein family has been identified in each species, such as major allergens Can f 1 in dog and Equ c 1 in horse (Table 1)[22].

IgE reactivity to cat lipocalin Fel d 4 has been observed in up to 63% of cat-allergic subjects (23) and high levels of IgE antibodies to Fel d 4 have been associated with atopic dermatitis (AD) in children with cat allergy[24]. IgE reactivity to Fel d 7 has been reported in 38% of subjects with cat allergy[25]. Fel d 7 has 62% amino acid identity with Can f 1[22].

Among dog allergic patients, 50%–90% have antibodies to Can f 1, 20%–33% to Can f 2[3, 26, 27] and 35% to Can f 4[28]. Sera from 56%[18/32] of children with dog allergy reacted with Can f 6[29].

Two horse lipocalins, Equ c 1 and Equ c 2, have been identified. Up to 76% of patients with horse allergy react to Equ c 1[30]. Sensitization to Equ c 1 has been associated with severe childhood asthma[16]. In a population of 85 horse allergic individuals, 53 (62%) were sensitized to Equ c 2 with an IgE level ≥0.1 kU^A/L[31].

The lipocalins comprise a diverse protein family with specific patterns of cross-reactivity among certain of its members, for example between Can f 6, Fel d 4 and Equ c 1[32-34], Mus m 1[30] and Rat n 1[35], Equ c 1, and Fel d 7 and Can f 1[36]. Lipocalin allergens Equ c 1, Fel d 4, Can f 6, Cav p 6, Ory c 4, Rat n 1 and Mus m 1 show sequence identities between 47% and 69% *(Figure 3a)*[5].

Secretoglobins

Two mammalian allergens have been categorized as members of the secretoglobin protein family, namely Fel d 1 from cat and Ory c 3 from rabbit[32]. Sequence identity between both allergens is very low and no IgE cross-reactivity has been observed[37]. Fel d 1 is mainly produced in sebaceous and salivary glands and is transferred to fur by grooming[38].

Fel d 1 is the most important allergen in cat allergy, shown to react with IgE from 90% of cat-sensitized individuals, and to account for up to 90% of IgE reactivity to cat dander[1].

Serum albumins

According to the WHO/IUIS allergen nomenclature database, seven mammalian serum albumin allergens have been identified, including Bos d 6 (domestic cattle), Can f 3 (dog), Cav p 4 (guinea pig), Equ c 3 (domestic horse), Fel d 2 (cat), and Sus s 1 (domestic pig).

Serum albumins are highly abundant proteins, present in blood, dander, milk and other secretions, and are considered minor allergens. However, high levels of IgE antibodies to Fel d 2 have been associated with AD in children with cat allergy[24]. Serum albumins remain relevant, because they are responsible for species cross-reactivity due to high sequence identity (up to 82%)[21], for example between cat and pig *(Figure 3b)*. In a group of 39 highly sensitized cat-allergic patients, 23% had sIgE to Fel d 2 (cat) and more than half had sIgE to Sus s 1 (pig)[39].

Monosensitization to serum albumins seems rare. Sensitization to serum albumins is in the vast majority of cases observed in combination with sIgE directed against major allergens[32]. Serum albumins may play a significant role as cross-reacting allergens in individuals sensitized to dander of multiple animal species, in association with lipocalins[40]. Indeed, because of cross-reactivity, children with persistent milk allergy and bovine serum albumin sensitization show an increased risk of allergy to animal dander with symptoms of rhinoconjunctivitis and asthma[41].

Kallikrein

Can f 5 is so far the only identified allergen from the kallikrein protein family. The protein was isolated from the urine of a male dog, and was shown to be present in dog dander[42]. Among patients allergic to dogs, 31–70% showed IgE reactivity to Can f 5, and 19.5–42.2% of these patients were monosensitized to Can f 5[42-46]. Can f 5-sIgE also been reported as the most common dog component sensitization in a Swedish adult population[8].

The Can f 5 amino acid sequence shows no significant similarity to any known animal dander or urinary allergen[17]. Therefore, monosensitization to Can f 5 could be a highly specific marker for allergy to male dogs[47]. Of note, Can f 5 cross-reacts with prostate-specific antigen of human seminal plasma (HSP), as suggested by several reports worldwide[48-50]. A study in women sensitized to Can f 5 found that 8/27 patients reported allergic symptoms to HSP during intercourse[51].

Latherins

Two allergens belonging to the latherin protein family, namely Equ c 4 and Fel d 8, have been identified. Equ c 4 is an

Figure 3.
Amino acid sequence identity (adapted from[5]).

A. Lipocalins

	Bos d 23k	Bos d 2	Mus m 1	Equ c 2	Equ c 1	Fel d 7	Fel d 4	Can f 6	Can f 4	Can f 2	Can f 1
Can f 1	22	26	21	23	28	63	26	26	24	24	100
Can f 2	24	20	26	26	26	23	25	24	26	100	
Can f 4	37	32	28	35	29	23	27	26	100		
Can f 6	28	27	47	30	57	24	69	100			
Fel d 4	28	31	50	31	68	20	100				
Fel d 7	21	23	21	23	26	100					
Equ c 1	28	33	47	34	100						
Equ c 2	46	32	28	100							
Mus m 1	27	30	100								
Bos d 2	33	100									
Bos d 23k	100										

B. Serum albumins

	Sus s 1	HSA	Fel d 2	Equ c 3	Cav p 4	Can f 3	Bos d 6
Bos d 6	79	76	78	74	70	76	100
Can f 3	78	80	87	76	73	100	
Cav p 4	72	72	76	72	100		
Equ c 3	76	76	78	100			
Fel d 2	79	82	100				
HSA (human serum albumin)	75	100					
Sus s 1	100						

abundant protein constituent in sweat, saliva and dander of horse[52]. An IgE binding frequency to Equ c 4 of 77% (17/22 sera from horse-sensitized subjects) has been reported[53].

Fel d 8 has been characterized from the salivary glands of cats. However, with an IgE binding frequency of 19% among cat allergic individuals, it is not considered a major cat allergen[25].

Clinical utility of component-resolved diagnostics

Component-based IgE testing can distinguish a primary sensitization from a cross-sensitization to a higher extent than whole allergen extracts[5]. While the possibility of using single components to replace whole extracts in daily diagnostic practice has been widely debated[54, 55], a dominating view is that extracts are still needed as first line testing to ensure the detection of patients sensitized to components other than those that are available for CRD.

The availability of tests with allergen components has driven new epidemiological studies to analyze the prevalence and clinical relevance of sIgE directed against individual allergens. Thus, CRD may be useful in detecting atypical sensitization profiles that involve minor allergen components, and in describing patient-specific IgE profiles to establish predictive risk markers and to develop strategies for therapeutic intervention[1, 32]. When clinical history and investigations are inconclusive, molecular allergology can add valuable clinical information in the diagnostic work-up *(Figure 2)*[56].

Table 1.
Pet allergen components (www.allergen.org)

Animal	Component	Protein type
Domestic cattle	Bos d 2	Lipocalin
	Bos d 3	S100 calcium-binding protein A7
Dog	Can f 1*	Lipocalin
	Can f 2*	Lipocalin
	Can f 3	Serum albumin
	Can f 4*	Lipocalin
	Can f 5*	Arginine esterase, prostatic kallikrein
	Can f 6*	Lipocalin
	Can f 7	Epididymal Secretory Protein E1, or Niemann Pick type C2 protein
Guinea pig	Cav p 1	Lipocalin
	Cav p 2	Lipocalin
	Cav p 3	Lipocalin
	Cav p 4	Serum albumin
	Cav p 6	Lipocalin
Donkey	Equ a 6	Lysozyme
Domestic horse	Equ c 1*	Lipocalin
	Equ c 2	Lipocalin
	Equ c 3*	Serum albumin
	Equ c 4	Latherin
	Equ c 6	Lysozyme
Cat	Fel d 1*	Secretoglobin (Uteroglobin, chain 1)
	Fel d 2*	Serum albumin
	Fel d 3	Cystatin
	Fel d 4*	Lipocalin
	Fel d 5w	Immunoglobulin A
	Fel d 6w	Immunoglobulin M
	Fel d 7*	Lipocalin (Von Ebner gland protein)
	Fel d 8	Latherin-like protein
Golden hamster Syrian hamster	Mes a 1	Lipocalin
Mouse	Mus m 1*	Lipocalin/ urinary prealbumin
Rabbit	Ory c 1	Lipocalin
	Ory c 3	Secretoglobin (Lipophilin)
	Ory c 4	Lipocalin
Siberian hamster	Phod s 1	Lipocalin
Rat	Rat n 1	Alpha-2u-globulin/ Lipocalin
Domestic pig	Sus s 1	Serum albumin

**Mammalian allergens currently available for CRD.*

Improved clinical sensitivity and specificity

Component-resolved diagnostics may prove useful in terms of improved clinical sensitivity in cases where a relevant allergen is scarcely represented in the natural allergens extract, as is the case of PR-10 allergens in certain plant foods such as fruits and nuts. Superior clinical specificity of component IgE testing compared to whole extract testing has been demonstrated for allergies to several foods, including peanut[57], hazelnut[58, 59], and cashew nut[60, 61].

Markers of increased asthma risk and disease severity

Component-resolved diagnostics could provide markers of increased asthma risk, since asthmatic children with cat allergy have been shown to have higher Fel d 1-specific IgE levels than children with rhinitis only[62]. A study of 696 cat allergic Swedish children found that asthma symptoms upon contact with cat were significantly associated with sIgE to cat allergens Fel d 1 and Fel d 4 in cat-allergic children[45]. Among dog-sensitized children, the majority were sensitized to more than one dog component, and co-sensitization to Can f 5, Can f 1, and Can f 2 conferred the greatest risk for asthma[45]. The study further confirmed that asthma was associated with higher levels of component sensitization. Progression of allergic sensitization over time has been shown to involve IgE recognition of an increasing number of components from the sensitizing allergen source, forming the basis for the concept of molecular spreading, in which sensitization to a greater number of components from the same allergen source correlates with disease severity[63]. A recent study reported that asthmatic pediatric patients with IgE to Fel d 2 serum albumin, Fel d 4 and Fel d 7 lipocalins were more likely to have persistent type-2 inflammation[64]. A cross-sectional cohort study in 269 children found that asthma was significantly associated with sensitization to members of the lipocalin protein family[65].

The relationship between sensitization to specific allergen components and disease has been investigated by Simpson et al., who identified patterns of response to allergen component groups and investigated associations with asthma in children. Sensitization to a group that included 27 components of plant, animal, and fungal origin from 12 protein families was most strongly associated with asthma and decreased lung function (lower FEV1, $p < 0.001$)[66]. Similar results have been reported in several subsequent studies[67, 68]. In another study, multisensitization to three or more animal-derived components (lipocalins, kallikrein, and secretoglobin) was associated with severe asthma, increased bronchial inflammation, and a trend towards more courses of oral corticosteroid treatment[69]. Similar findings were recently reported in a study of dog sensitized children[56].

A small early study comparing children with severe asthma vs. controlled asthmatic children demonstrated that those with severe asthma had higher levels of IgE antibodies towards cat, dog, and horse components[16]. The use of allergen components compared with whole extracts as predictors of disease severity was evaluated by Asarnoj et al. in a large cross-sectional and longitudinal population-based pediatric study. Sensitization to Fel d 1 and Can f 1 at 4 years of age and molecular polysensitization to cat or dog components predicted allergy to cat and dog at 16 years of age significantly better than did IgE to whole cat or dog extracts[68].
In comparing allergen extract vs. component sensitization, Patelis et al. showed that adults sensitized to both cat extract and one or more of cat components Fel d 1, Fel d 2 and Fel d 4 had higher exhaled nitric oxide (FeNO) ($p=0.008$) and more bronchial responsiveness ($p=0.002$) than subjects sensitized the extract but not to any of the cat components tested[54]. Further, subjects that were sensitized to the tested cat components were more likely to develop asthma ($p=0.005$) and rhinitis ($p=0.007$) over a 12-year period than those that were not, highlighting the value of CRD in predicting disease severity[54]. Another adult study identified sensitization to furry animal allergen components, sensitization patterns and clusters as associated with a substantially increased risk of asthma, rhinitis, and concomitant asthma/rhinitis. Sensitization to Fel d 1, Can f 1, Can f 2, and Can f 3, polysensitization, and multisensitized cluster were further associated with increased FeNO and eosinophil levels. Thus, sensitization to furry animal allergen components appears to be a predictor of asthma outcome and an indicator of severity[70].

Taken together, available data indicate that measuring component-specific IgE offers insights into the progression and severity of allergy.

Allergen components in allergen immunotherapy

Treatment options for individuals who are allergic to furry animals include allergen avoidance, medications, and allergen immunotherapy (AIT)[5]. The ability of components to distinguish primary sensitization from cross-sensitization is important when immunotherapy is envisaged, in order to determine if immunotherapy is applicable and to choose the primary sensitizing allergen source for therapy. The availability of CRD has raised the possibility of better targeted AIT, the only treatment able to change the natural course of allergic disease.

Future perspectives

Generally speaking, CRD allows detailed analysis of the patient's sensitization profile, and may ultimately facilitate individualized treatments and patient management options.

Some questions remain to be answered on the clinical utility of CRD in patients with allergy to furry animals[3]. For the time being, CRD should be considered complementary to extract based testing rather than a replacement *(Figure 2)*. The role of allergenic molecules as markers and predictors of disease severity needs to be further explored, and whether CRD could identify patients who are most likely to respond to AIT. Component-resolved diagnostics may also have a role in monitoring treatment responses following immunotherapy.

Overall, CRD has a role in developing patient-tailored treatment that could reduce healthcare costs, save time for patients, reduce adverse effects, and improve patient quality of life.

Product list

ImmunoCAP Allergen e5, Dog dander, ImmunoCAP Allergen e101, Allergen component rCan f 1 Dog, ImmunoCAP Allergen e102, Allergen component rCan f 2 Dog, ImmunoCAP Allergen e221, Allergen component nCan f 3 Dog serum albumin, ImmunoCAP Allergen e229, Allergen component rCan f 4, Dog, ImmunoCAP Allergen e226, Allergen component rCan f 5, Dog, ImmunoCAP Allergen e230, Allergen component rCan f 6, Dog. ImmunoCAP Allergen e1, Cat dander, ImmunoCAP Allergen e94, Allergen component rFel d 1 Cat, ImmunoCAP Allergen e220, Allergen component rFel d 2 Cat serum albumin, ImmunoCAP Allergen e228, Allergen component rFel d 4, Cat, ImmunoCAP Allergen e231, Allergen component rFel d 7, Cat. ImmunoCAP Allergen e227, Allergen component rEqu c 1, Horse.

References

1. Bonnet B, Messaoudi K, Jacomet F, et al. *An update on molecular cat allergens: Fel d 1 and what else? Chapter 1: Fel d 1, the major cat allergen.* Allergy Asthma Clin Immunol 2018;14:14.
2. Global GfK survey: Pet ownership. *Available from: https://www.gfk.com/global-studies/global-studies-pet-ownership/* (accessed 25 June 2018).
3. Konradsen JR, Fujisawa T, van Hage M, et al. *Allergy to furry animals: New insights, diagnostic approaches, and challenges.* J Allergy Clin Immunol 2015;135:616-25.
4. Schmitz R, Ellert U, Kalcklosch M, et al. *Patterns of sensitization to inhalant and food allergens - findings from the German Health Interview and Examination Survey for Children and Adolescents.* Int Arch Allergy Immunol 2013;162:263-70.
5. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, et al. *EAACI Molecular Allergology User's Guide.* Pediatr Allergy Immunol 2016;27 Suppl 23:1-250.
6. Wickman M, Asarnoj A, Tillander H, et al. *Childhood-to-adolescence evolution of IgE antibodies to pollens and plant foods in the BAMSE cohort.* J Allergy Clin Immunol 2014;133:580-2.
7. Warm K, Lindberg A, Lundback B, et al. *Increase in sensitization to common airborne allergens among adults - two population-based studies 15 years apart.* Allergy Asthma Clin Immunol 2013;9:20.
8. Suzuki S, Nwaru BI, Ekerljung L, et al. *Characterization of sensitization to furry animal allergen components in an adult population.* Clin Exp Allergy submitted.
9. Aranda CS, Cocco RR, Pierotti FF, et al. *Increased sensitization to several allergens over a 12-year period in Brazilian children.* Pediatr Allergy Immunol 2018;29:321-4.
10. Nagao M, Borres MP, Sugimoto M, et al. *Sensitization to secretoglobin and lipocalins in a group of young children with risk of developing respiratory allergy.* Clin Mol Allergy 2017;15:4.
11. Liccardi G, Triggiani M, Piccolo A, et al. *Sensitization to Common and Uncommon Pets or Other Furry Animals: Which May Be Common Mechanisms?* Transl Med UniSa 2016;14:9-14.
12. 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;64:1498-506.
13. Bousquet PJ, Chinn S, Janson C, et al. *Geographical variation in the prevalence of positive skin tests to environmental aeroallergens in the European Community Respiratory Health Survey I.* Allergy 2007;62:301-9.
14. Park YB, Mo EK, Lee JY, et al. *Association between pet ownership and the sensitization to pet allergens in adults with various allergic diseases.* Allergy Asthma Immunol Res 2013;5:295-300.
15. Davila I, Dominguez-Ortega J, Navarro-Pulido A, et al. *Consensus document on dog and cat allergy.* Allergy 2018;73:1206-22.
16. Konradsen JR, Nordlund B, Onell A, et al. *Severe childhood asthma and allergy to furry animals: refined assessment using molecular-based allergy diagnostics.* Pediatr Allergy Immunol 2014;25:187-92.
17. Zahradnik E, Raulf M. *Respiratory Allergens from Furred Mammals: Environmental and Occupational Exposure.* Vet Sci 2017;4.
18. Valenta R, Lidholm J, Niederberger V, et al. *The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT).* Clin Exp Allergy 1999;29:896-904.
19. Liccardi G, Bilo 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;47:163-7.
20. Arbes SJ, Jr., Cohn RD, Yin M, et al. *Dog allergen (Can f 1) and cat allergen (Fel d 1) in US homes: results from the National Survey of Lead and Allergens in Housing.* J Allergy Clin Immunol 2004;114:111-7.
21. Chruszcz M, Mikolajczak K, Mank N, et al. *Serum albumins-unusual allergens.* Biochim Biophys Acta 2013;1830:5375-81.
22. Hilger C, Kuehn A, Hentges F. *Animal lipocalin allergens.* Curr Allergy Asthma Rep 2012;12:438-47.
23. Smith W, Butler AJ, Hazell LA, et al. *Fel d 4, a cat lipocalin allergen.* Clin Exp Allergy 2004;34:1732-8.
24. Wisniewski JA, Agrawal R, Minnicozzi S, et al. *Sensitization to food and inhalant allergens in relation to age and wheeze among children with atopic dermatitis.* Clin Exp Allergy 2013;43:1160-70.
25. Smith W, O'Neil SE, Hales BJ, et al. *Two newly identified cat allergens: the von Ebner gland protein Fel d 7 and the latherin-like protein Fel d 8.* Int Arch Allergy Immunol 2011;156:159-70.
26. Konieczny A, Morgenstern JP, Bizinkauskas CB, et al. *The major dog allergens, Can f 1 and Can f 2, are salivary lipocalin proteins: cloning and immunological characterization of the recombinant forms.* Immunology 1997;92:577-86.
27. Saarelainen S, Taivainen A, Rytkonen-Nissinen M, et al. *Assessment of recombinant dog allergens Can f 1 and Can f 2 for the diagnosis of dog allergy.* Clin Exp Allergy 2004;34:1576-82.
28. Mattsson L, Lundgren T, Olsson P, et al. *Molecular and immunological characterization of Can f 4: a dog dander allergen cross-reactive with a 23 kDa odorant-binding protein in cow dander.* Clin Exp Allergy 2010;40:1276-87.
29. Wang YJ, Li L, Song WJ, et al. *Canis familiaris allergen Can f 6: expression, purification and analysis of B-cell epitopes in Chinese dog allergic children.* Oncotarget 2017;8:90796-807.
30. Saarelainen S, Rytkonen-Nissinen M, Rouvinen J, et al. *Animal-derived lipocalin allergens exhibit immunoglobulin E cross-reactivity.* Clin Exp Allergy 2008;38:374-81.
31. Lidholm J, Lundgren T, Larsson H, et al. *Complete sequence and recombinant production of horse dander allergen Equ c 2.* Allergy 2017;72:341 (abstract 505).
32. Hilger C, van Hage M, Kuehn A. *Diagnosis of Allergy to Mammals and Fish: Cross-Reactive vs. Specific Markers.* Curr Allergy Asthma Rep 2017;17:64.
33. Hilger C, Swiontek K, Arumugam K, et al. *Identification of a new major dog allergen highly cross-reactive with Fel d 4 in a population of cat- and dog-sensitized patients.* J Allergy Clin Immunol 2012;129:1149-51.
34. Nilsson OB, Binnmyr J, Zoltowska A, et al. *Characterization of the dog lipocalin allergen Can f 6: the role in cross-reactivity with cat and horse.* Allergy 2012;67:751-7.
35. Jeal H, Harris J, Draper A, et al. *Dual sensitization to rat and mouse urinary allergens reflects cross-reactive molecules rather than atopy.* Allergy 2009;64:855-61.

36. Apostolovic D, Sanchez-Vidaurre S, Waden K, et al. *The cat lipocalin Fel d 7 and its cross-reactivity with the dog lipocalin Can f 1.* Allergy 2016;71:1490-5.
37. Hilger C, Kler S, Arumugam K, et al. *Identification and isolation of a Fel d 1-like molecule as a major rabbit allergen.* J Allergy Clin Immunol 2014;133:759-66.
38. Pomes A, Chapman MD, Wunschmann S. *Indoor Allergens and Allergic Respiratory Disease.* Curr Allergy Asthma Rep 2016;16:43.
39. Hilger C, Kohnen M, Grigioni F, et al. *Allergic cross-reactions between cat and pig serum albumin. Study at the protein and DNA levels.* Allergy 1997;52:179-87.
40. Liccardi G, Asero R, D'Amato M, et al. *Role of sensitization to mammalian serum albumin in allergic disease.* Curr Allergy Asthma Rep 2011;11:421-6.
41. Vicente-Serrano J, Caballero ML, Rodriguez-Perez R, et al. *Sensitization to serum albumins in children allergic to cow's milk and epithelia.* Pediatr Allergy Immunol 2007;18:503-7.
42. Mattsson L, Lundgren T, Everberg H, et al. *Prostatic kallikrein: a new major dog allergen.* J Allergy Clin Immunol 2009;123:362-8.
43. Uriarte SA, Sastre J. *Clinical relevance of molecular diagnosis in pet allergy.* Allergy 2016;71:1066-8.
44. Ukleja-Sokolowska N, Gawronska-Ukleja E, Zbikowska-Gotz M, et al. *Analysis of feline and canine allergen components in patients sensitized to pets.* Allergy Asthma Clin Immunol 2016;12:61.
45. Bjerg A, Winberg A, Berthold M, et al. *A population-based study of animal component sensitization, asthma, and rhinitis in schoolchildren.* Pediatr Allergy Immunol 2015;26:557-63.
46. Basagana M, Luengo O, Labrador M, et al. *Component-Resolved Diagnosis of Dog Allergy.* J Investig Allergol Clin Immunol 2017;27:185-7.
47. Schoos AM, Bonnelykke K, Chawes BL, et al. *Precision allergy: Separate allergies to male and female dogs.* J Allergy Clin Immunol Pract 2017;5:1754-6.
48. Tanaka M, Nakagawa Y, Kotobuki Y, et al. *A case of human seminal plasma allergy sensitized with dog prostatic kallikrein, Can f 5.* Allergol Int 2018.
49. Basagana M, Bartolome B, Pastor-Vargas C, et al. *Involvement of Can f 5 in a case of human seminal plasma allergy.* Int Arch Allergy Immunol 2012;159:143-6.
50. Kofler L, Kofler H, Mattsson L, et al. *A case of dog-related human seminal plasma allergy.* Eur Ann Allergy Clin Immunol 2012;44:89-92.
51. Gonzalez-de-Olano D, Gandolfo-Cano M, de-Calzada-Bustingorri MP, et al. *Prevalence of allergy to human seminal fluid among women with allergy to male dog and sensitization to Can f 5.* Clin Exp Allergy 2018 Oct;48(10):1368-1370.
52. McDonald RE, Fleming RI, Beeley JG, et al. *Latherin: a surfactant protein of horse sweat and saliva.* PLoS One 2009;4:e5726.
53. Goubran Botros H, Poncet P, Rabillon J, et al. *Biochemical characterization and surfactant properties of horse allergens.* Eur J Biochem 2001;268:3126-36.
54. Patelis A, Gunnbjornsdottir M, Alving K, et al. *Allergen extract vs. component sensitization and airway inflammation, responsiveness and new-onset respiratory disease.* Clin Exp Allergy 2016;46:730-40.
55. Eder K, Becker S, San Nicolo M, et al. *Usefulness of component resolved analysis of cat allergy in routine clinical practice.* Allergy Asthma Clin Immunol 2016;12:58.
56. Kack U, Asarnoj A, Gronlund H, et al. *Molecular allergy diagnostics refine characterization of children sensitized to dog dander.* J Allergy Clin Immunol 2018 Oct;142(4):1113-1120
57. Klemans RJ, Otte D, Knol M, et al. *The diagnostic value of specific IgE to Ara h 2 to predict peanut allergy in children is comparable to a validated and updated diagnostic prediction model.* J Allergy Clin Immunol 2013;131:157-63.
58. Masthoff LJ, Mattsson L, Zuidmeer-Jongejan L, et al. *Sensitization to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults.* J Allergy Clin Immunol 2013;132:393-9.
59. Eller E, Mortz CG, Bindslev-Jensen C. *Cor a 14 is the superior serological marker for hazelnut allergy in children, independent of concomitant peanut allergy.* Allergy 2016;71:556-62.
60. Savvatianos S, Konstantinopoulos AP, Borga A, et al. *Sensitization to cashew nut 2S albumin, Ana o 3, is highly predictive of cashew and pistachio allergy in Greek children.* J Allergy Clin Immunol 2015;136:192-4.
61. Lange L, Lasota L, Finger A, et al. *Ana o 3-specific IgE is a good predictor for clinically* relevant cashew allergy in children. Allergy 2017;72:598-603.
62. Gronlund H, Saarne T, Gafvelin G, et al. *The major cat allergen, Fel d 1, in diagnosis and therapy.* Int Arch Allergy Immunol 2010;151:265-74.
63. Hatzler L, Panetta V, Lau S, et al. *Molecular spreading and predictive value of preclinical IgE response to Phleum pratense in children with hay fever.* J Allergy Clin Immunol 2012;130:894-901.e5.
64. 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 Sep;48(9):1186-1194.
65. Schoos AM, Kattan JD, Gimenez G, et al. *Sensitization phenotypes based on protein groups and associations to allergic diseases in children.* J Allergy Clin Immunol 2016;137:1277-80.
66. Simpson A, Lazic N, Belgrave DC, et al. *Patterns of IgE responses to multiple allergen components and clinical symptoms at age 11 years.* J Allergy Clin Immunol 2015;136:1224-31.
67. Perzanowski MS, Ronmark E, James HR, et al. *Relevance of specific IgE antibody titer to the prevalence, severity, and persistence of asthma among 19-year-olds in northern Sweden.* J Allergy Clin Immunol 2016;138:1582-90.
68. 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;137:813-21.e7.
69. Nordlund B, Konradsen JR, Kull I, et al. *IgE antibodies to animal-derived lipocalin, kallikrein and secretoglobin are markers of bronchial inflammation in severe childhood asthma.* Allergy 2012;67:661-9.
70. Nwaru BI, Suzuki S, Ekerljung L, et al. *Furry animal allergen component sensitization and clinical outcomes in adult asthma and rhinitis.* J Allergy Clin Immunol Pract accepted.
71. Gerth van Wijk R. *Diagnosis of dog allergy: beware of the dog.* J Allergy Clin Immunol 2018 Oct;142(4):1058-1059.
72. Liccardi G, Calzetta L, Milanese M, Lombardi C, Savi E, Passalacqua G, Rogliani P. *Critical aspects in dog allergen immunotherapy (DAI). May Component Resolved Diagnosis (CRD) play a role in predicting the efficacy?* Hum Vaccin Immunother. 2018 Jun 3;14(6):1438-1441.
73. Curin M, Reininger R, Swoboda I, Focke M, Valenta R, Spitzauer S. *Skin prick test extracts for dog allergy diagnosis show considerable variations regarding the content of major and minor dog allergens.* Int Arch Allergy Immunol. 2011;154:258-263.
74. Fernández-Távora L, Rico P, Martín S. *Clinical experience with specific immunotherapy to horse dander.* J Invest Allergol Clin Immunol 2002,12 (1):29-33.

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