

DR. WOLFGANG HEMMER (Orcid ID : 0000-0001-8613-0399)

DR. STEFAN WÖHRL (Orcid ID : 0000-0002-6324-0007)

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Molecular sensitization patterns in animal allergy: relationship with clinical relevance and pet ownership

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Wolfgang HEMMER, PhD (ORCID0000-0001-8613-0399)

Gabriele SESTAK-GREINECKER, MD

Tobias BRAUNSTEINER

Felix WANTKE, MD

Stefan WÖHRL, MSc, MD (ORCID 0000-0002-6324-0007)

Floridsdorf Allergy Center (FAZ), Vienna, Austria

Corresponding author:

Wolfgang Hemmer, PhD

Floridsdorf Allergy Center (FAZ)

Pius-Parsch-Platz 1/3

A-1210 Vienna, Austria

Phone: +43 1 270253065

E-mail: hemmer@faz.at

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Abstract

Background: In-vitro diagnosis using single molecules is increasingly complementing conventional extract-based diagnosis. We explored in routine patients with animal allergy to what extent molecules can explain polysensitization and identify primary sensitizers and how individual IgE patterns correlate with previous pet ownership and clinical relevance.

Methods: Serum samples from 294 children and adults with suspect allergic rhino-conjunctivitis or asthma and a positive skin prick test to cat, dog and/or horse were tested by ImmunoCAP for IgE antibodies against eleven different allergens from cat (Fel d 1,2,4,7), dog (Can f 1,2,3,4,5,6) and horse (Equ c 1).

Results: Patients monosensitized to cat (40.8%) or dog (6.1%) showed simple IgE patterns dominated by Fel d 1 (93%) and Can f 5 (67%), respectively. Double-sensitization to cat+dog (25.9%), cat+horse (5.4%) and polysensitization (20.7%) was associated with an increasing prevalence of the cross-reactive lipocalins Fel d 4/Can f 6/Equ c 1 and Fel d 7/Can f 1. While these lipocalins were not reliable markers for genuine sensitization per se, comparison of sIgE levels may give a clue on the primary sensitizer. Sensitizations to dog appeared to result from cross-reactivity with cat in 48%, with half of these sensitizations lacking clinical relevance. Individual sensitization patterns strongly mirrored current or previous pet ownership with the exception of Fel d 1 which regularly caused sensitization also in non-owners.

Conclusions: Allergen components can reasonably illuminate the molecular basis of animal (poly)sensitization in the majority of patients and are helpful in distinguishing between primary sensitization and sometimes less relevant cross-reactivity.

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67 **Key words:** animal allergens, furry animals, indoor allergens, molecular allergy diagnosis, pet
68 allergy

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74 **Abbreviations:**

75 PSA prostate-specific antigen

76 SA serum albumin

77 SI sequence identity

78 SPT skin prick test

79

80 Introduction

81 Furry animals are a major source of indoor allergens in Westernized countries and a well-known
82 risk factor for the development of allergic rhinitis and asthma.^{1,2} Up to 50% of European
83 households keep a pet, but significant allergen exposure may also occur through allergens spread by
84 pet owners to schools, day-care centers, undergrounds, and elsewhere in the public.²⁻⁴ In Austria,
85 40% of households harbor a pet, most often cats (63%) followed by dogs (42%) and small animals
86 such as rabbits and Guinea pigs (13%).⁵ Accordingly, sensitization to animal dander is frequent
87 within the local atopic population (>40%) with the majority of patients suffering from cat allergy,
88 and with around half of patients showing simultaneous sensitization to two or more animal species
89 with often obscure significance.⁶

90 Routine diagnosis of allergy to furry animals commonly relies on the use of dander and hair
91 extracts offered for a broad range of species. Extracts are sensitive but cannot further specify the
92 molecular basis of sensitization and explain the origin of polysensitization. Recently, a growing
93 number of recombinant or purified natural allergens from cat, dog and horse has become available
94 for routine assessment of specific serum IgE. The implementation of allergen-resolved diagnosis
95 into daily practice has proven extremely helpful in e.g. pollen and venom allergy where targeted
96 testing for species-specific marker allergens has substantially improved diagnosis and selection of
97 the proper immunotherapy vaccine.^{7,8} Molecular studies in allergy to furry animals have thus far
98 focused on selected cohorts of high-risk children and adults with asthma.⁹⁻¹⁴ These
99 studies consistently reported that complex sensitization patterns involving minor allergens correlate
100 with disease severity and that certain allergens might represent clinically useful risk markers for
101 severe rhinitis and

102 The benefit of molecular diagnostics for better understanding and managing pet allergy in a
103 random outpatient population is not yet well documented. Though immunotherapy is less often
104 carried out with animal allergens due to efficacy and safety concerns, knowledge of individual
105 sensitization patterns and identification of the putative primary sensitizer in polysensitized
106 subjects seems crucial for selection of the proper vaccine. Understanding the molecular
107 background of (poly)sensitization might also be helpful in estimating clinical relevance, especially
108 in those with unexpected sensitization to many animals. In our population, this involves above all
109 cat-allergic subjects who wish to switch to a dog instead but remarkably often show unexpected co-

110 sensitization also to dog during routine allergy screening. Finally, there is still limited knowledge
111 about how much individual IgE profiles are determined by current or previous pet ownership.
112 The present study investigates in a large routine population the usefulness of currently available
113 animal allergens for illuminating the molecular origin of (poly)sensitization and for discriminating
114 between genuine sensitization and cross-sensitization, and how individual IgE profiles correlate
115 with pet ownership and clinical relevance.

116

117 **Material& Methods**

118 *Patients*

119 Specific IgE was analyzed in 294 children and adults with sensitization to furry animals (mean age
120 32±17 yrs, range 2-80 yrs, median 30 yrs, 116m/178f). Study participants were selected from
121 patients attending our outpatient clinic for suspect respiratory allergy and undergoing routine skin
122 prick testing (SPT) with a panel of inhalant allergens including cat, dog and horse dander (ALK
123 Soluprick, Hørsholm, Denmark). Patients were included into the study upon a positive SPT ≥3
124 mm to at least one of the three tested animals. All participants gave informed written consent. The
125 study was a side project in cause of TB's medical diploma thesis at the Medical University of
126 Vienna (ethics committee vote 2213/2018).

127

128 *Assessment of allergic symptoms and animal exposure*

129 Allergic symptoms upon contact with cat, dog and horserelied on patients' self-assessment and
130 were roughly graded as 1) moderate/severe (rhino-conjunctivitis, breathing difficulties/asthma), 2)
131 mild (contact skin reactions, sneezing, and 3) no symptoms, as specified in **Suppl. Table 1**. Some
132 participants reported symptoms also after contact with small animals such as rabbits, Guinea pigs
133 and rats.

134 Concerning cat/dog exposure, patients were arbitrarily classified as "owners" or "non-owners".

135 Accordingly, pet ownership refers to subjects currently keeping a cat/dog in their homeor having
136 kept a cat/dog in the past or otherwise having regular close contact with these animalse.g. in the
137 home of their partner, parents or grandparents."Horse riders" refers to subjects currently practicing
138 horse riding or having done so in the past or otherwise having regular contact with horses (e.g.
139 parents of horse-riding children). When evaluating the clinical relevance of sensitization to a
140 particular animal species, only subjects confirming contact with that species were considered.

141

142 *Assessment of specific IgE to animal allergens*

143 Sera were analyzed by Phadia ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) for IgE
144 antibodies against the following recombinant or purified components:rFel d 1 (cat uteroglobulin);
145 nFel d 2 (cat/dogserum albumin),rFel d 4 (cat lipocalin), rFel d 7 (cat lipocalin), rCan f 1 (dog

146 lipocalin), rCan f 2 (dog lipocalin), nCan f 3 (dog serum albumin), rCan f 4 (dog lipocalin), rCan f
147 5 (dog arginine esterase = dog PSA), rCan f 6 (dog lipocalin), and rEqu c 1 (horse lipocalin).
148 ImmunoCAP results >0.10 kUa/L were considered positive.

149 The seven tested lipocalins belong to four different similarity clusters within the large lipocalin
150 allergen family showing only low sequence identity among each other as illustrated in
151 **Suppl. Table 2:** 1) Can f 1/Fel d 7, 2) Can f 2, 3) Can f 4, 4) Can f 6/Fel d 4/Equ c 1. In case of
152 simultaneous sensitization to cross-reactive lipocalins or serum albumins, the allergen yielding the
153 highest IgE score was arbitrarily considered as the putative primary sensitizer.

154

155 *Statistical analysis*

156 Pearson's chi-squared test was used to evaluate if sensitization to certain allergens is significantly
157 associated with pet ownership or clinical relevance. Linear correlation between antibody binding
158 to cross-reactive lipocalins was calculated by Pearson correlation coefficient using MedCalc
159 Statistical Software version 19.2.

160

Results

1. Skin prick test results with animal dander extracts

Of the 294 patients included, 273 (92.9%) were positive to cat, 155 (52.7%) to dog, and 80 (27.2%) to horse. Half of the patients (53.0%) reacted with two or all three animals as specified in **Figure 1**. Monovalent sensitization was common for cat (44.0%), but uncommon for dog (11.6%) and horse (3.8%). 73% of patients were also sensitized to other inhalant allergens, mostly to pollens (39%), house dust mite (12%), or both (18%).

2. Molecular sensitization profiles

2.1. Molecular sensitization profiles in cat allergy

Patients with monovalent cat sensitization (120/273) mostly reacted with Fel d 1 alone (**Table 1, Figure 2a**). Reactivity with allergens other than Fel d 1 was nearly always below 1 kUa/L. Out of 9 Fel d 1-negative sera, none was positive for Fel d 2 or 4, one was weakly positive for Fel d 7 (0.37 kUa/l), and four were positive for cat extract indicating sensitization to allergens not covered by the tested molecule panel.

Cat+dog double-positive patients (n=76) more often had IgE against Fel d 4 and Fel d 7, but Fel d 1 was still positive in 96% showing that cat allergy rarely resulted from cross-reactivity with dog. Cat+horse double-positivity (n=16) was associated with a unique binding pattern characterized by a strikingly high prevalence of Fel d 4 (81.3%) and Equ c 1 (93.8%). All except two patients reacted with both Fel d 4 and Equ c 1 suggesting that cross-reactivity between them is a major cause of cat-horse double-positivity.

2.2. Molecular sensitization profiles in dog allergy

In dog monosensitized patients, Can f 5 was the by far most prevalent allergen (66.7%) followed by the lipocalins Can f 1 (33.3%) and Can f 4 (22.2%), all three together identifying 94% of patients (**Table 1, Figure 2b**). Can f 2 and 6 did not contribute to sensitivity.

In contrast, in dog+cat double-positive patients (n=76) Can f 1 was the dominant dog allergen (35.5%) followed by Can f 5 (23.7%) and Can f 6 (17.1%) (**Table 1, Figure 2b**). This is obviously

189 due to the fact that dog sensitization in cat-dog double-positive patients often involves cross-
190 reactivity with the cat lipocalins Fel d 4 and Fel d 7. Comparing IgE levels to the lipocalin pairs
191 Can f 1/Fel d 7 and Can f 6/Fel d 4 suggests that sensitization to Can f 1 and Can f 6 results from
192 cross-reactivity with Fel d 7 and Fel d 4, respectively, in more than half of patients. The very same
193 was observed in polysensitized patients. **Suppl. Figure 1** summarizes IgE binding patterns to dog
194 allergens after stratification for putative primary sensitization and cross-sensitization for all 155
195 dog-sensitized patients.

196 Of importance, as much as 43% of cat+dog skin test double-positive patients (33/76) did not react
197 with any of the six tested dog allergens, but 79% of them (26/33) were positive to dog extract.
198 Reactivity with dog extract strongly correlated with Fel d 1 ($r=0.88$, data not shown).

199

200 *2.3. Molecular sensitization profiles in horse allergy*

201 Sensitization to horse was largely confined to patients with cat+horse double-positivity and
202 polysensitization (**Table 1, Figure 2**). Equ c 1 represented a major horse allergen in all subgroups
203 altogether identifying 77% of patients. When comparing IgE levels to the lipocalins Equ c 1/Fel d
204 4/Can f 6, each of them may be highest suggesting that all three lipocalins can act as the primary
205 sensitizer depending on individual animal exposure. Serum albumin (SA) may explain another
206 10% of cases, especially in polysensitized patients.

207

208 *2.4. Molecular sensitization profiles in polysensitized patients*

209 Expectedly, patients sensitized to cat+dog+horse ($n=61$) showed the most complex binding
210 patterns primarily due to a high prevalence of the cross-reactive lipocalins Fel d 4/Can f 6/Equ c 1
211 (~80-85%) and Can f 1/Fel d 7 (~60%) (**Table 1, Figure 2**). Although also SA was a
212 frequent allergen in this group (37.7%), only 5 out of 61 cases (8.2%) were primarily based on SA
213 cross-reactivity.

214

215 *3. Cross-reactivity between lipocalins*

216 Fel d 4/Can f 6/Equ c 1: There was considerable overlap in IgE-binding to these lipocalins with
217 87% of sera (92/106) reacting to two or all three molecules (**Figure 3a**). Single-positive sera were

218 predominantly seen with Fel d 4 and most had levels below 1 kUa/L. Correlation between the
219 three lipocalins was poor when looking at the whole population ($r=0.33-0.38$). When stratifying
220 patients for putative primary sensitization to Fel d 4 (62/106), Can f 6 (25/106) and Equ c 1
221 (19/106), correlation was strong ranging from $r=0.61$ for Fel d 4/Can f 6 to $r=0.85$ for Can f 6/Equ c
222 1) (**Suppl. Figure 2a**).

223 Fel d 7/Can f 1: 79% of sera (65/82) reacted with both allergens (**Figure 3b**). Single-positive
224 sera were more often seen with Can f 1 and mostly showed IgE values below 1 kUa/L, but single
225 samples showed levels as high as 15 kUa/L and virtually no IgE reactivity with Fel d 7. Correlation
226 between Can f 1 and Fel d 7 was only moderate when looking at the whole population ($r=0.47$) but
227 was very strong after stratification for putative primary sensitizers ($r=0.77-0.89$; **Suppl. Figure 2b**).

228

229 **4. Relationship between molecular sensitization profiles and pet ownership**

230 **4.1. Cat owners vs. non-owners**

231 Sensitization to Fel d 1 was not significantly associated with cat ownership indicating that
232 sensitization is common also without possessing a cat. In contrast, sensitization to minor cat
233 allergens was significantly associated with cat ownership with putative primary sensitization to Fel
234 d 2, 4 and 7 being strikingly confined to cat owners (**Table 2**).

235

236 **4.2. Dog owners vs. non-owners**

237 Reactivity with the dog-specific allergens Can f 2, 4 and 5 was strongly confined to dog owners
238 (85%). This was also true for the cross-reactive lipocalins Can f 1 and Can f 6 where putative
239 primary sensitization was almost exclusively found in dog owners (>90%) (**Table 3**).

240 In addition, dog gender influenced sensitization to the male-specific allergen Can f 5. Among 75
241 dog-owners where dog gender was identified, Can f 5 was positive in 36/55 patients keeping a
242 male dog but in none only 2/20 patients keeping a female dog ($p<0.0001$) (**Suppl. Table 3**). Can f
243 5 sensitization was equally common among male and female patients. Also Can f 2 sensitization
244 was associated with keeping a male dog ($p<0.05$).

245

246 **4.3. Horse riders vs. non-riders**

247 Sensitization rates to Equ c 1, Feld 4 and Can f 6 did not differ between riders and non-riders.
248 However, higher IgE levels for Equ c 1 than for Feld 4/Can f 6 were found in 59% of horse riders
249 but in only 20% of non-riders ($p=0.005$) suggesting that regular contact with horses commonly
250 causes genuine sensitization to Equ c 1 and subsequent cross-sensitization to Fel d 4 and Can f
251 6 (Suppl. Table 4).

253 5. Clinical relevance of dog sensitization

254 We were particularly interested in this study in understanding the origin and clinical relevance of
255 dog sensitization in cat-allergic subjects also showing a positive skin test to dog in the routine
256 allergy test. When dissecting IgE profiles of all 155 patients with dog sensitization, 52% (80/155)
257 presumably had genuine dog sensitization, 25% (38/155) likely were cross-sensitized, and 24%
258 (37/155) did not react with any of the tested dog allergens at all. The clinical relevance of dog
259 sensitization varied between these groups:

260 1) Dog-sensitized patients negative for all tested dog allergens rarely reported symptoms upon dog
261 exposure (3/27, 11.1%), even if confining analysis to dog owners within this group (15.4%; 2/13).
262 In contrast, 76% (81/107) of patients positive for at least one dog allergen complained about
263 symptoms ($p<0.0001$) (**Figure 4, upper panel**).

264 2) Symptoms were more severe (but not more common) in patients with putative primary dog
265 sensitization as compared to those cross-sensitized to dog via Can f 1, 3 or 6 ($p<0.05$) (**Figure 4,**
266 **bottom panel**).

267 3) Clinical relevance increased with the number of recognized dog allergens: all components
268 negative ($n=27$): 11%; 1 positive component ($n=41$): 63%, 2 positive components ($n=30$): 80%;
269 ≥ 3 positive components ($n=36$): 86%.

271 Discussion

272 Previous molecular studies in animal allergy were mainly performed in children or adults from
273 high-risk asthma cohorts,^{9-11,14} whereas the benefit from molecular diagnostics in the context of
274 routine allergy screening in unselected patient collectives is uncertain. The present study shows
275 that molecules can reliably explain also the often complex molecular background in polysensitized
276 patients and prove helpful in discriminating between genuine sensitization and cross-reactivity.

277 When discussing the diagnostic sensitivity and importance of single molecules, results will not
278 only depend on the specific assay platforms and cut-off levels used but also strongly on patient
279 populations. Systematically stratifying patients for mono-, di- and polysensitization as performed
280 in the present work has not been done before, but appears to be crucial since the significance of a
281 certain allergen strongly rests on concomitant sensitization to other animal species and can be
282 distorted by selection bias. For example, the prevalence of Fel d 4 sensitization in cat allergy has
283 been reported as 31-63% (see Table 2). Our data show that Fel d 4 is a negligible allergen in cat-
284 monosensitized patients but extremely common in cat-horse double-positive and in polysensitized
285 patients ($\geq 80\%$). Such discrepancies were seen for most other allergens, too. In agreement with
286 previous studies,²²⁻²⁴ Can f 5 was the most prevalent dog allergen in our study, but only in dog
287 monosensitized subjects. Overall, Can f 1 and Can f 6 were more common, obviously often
288 resulting from cross-reactivity with Fel d 4 and Fel d 7 in our population which is characterized by
289 a high prevalence of cat allergy. This underlines that the relative role of single allergens may differ
290 geographically and also depend on which other pets are commonly kept in the community.

291 Altogether, the overall prevalence of the tested allergens in our routine population-based study is
292 low compared to published data, pointing towards some selection bias in many studies. Actually,
293 only Fel d 1 and Equ c 1 fulfilled the criterion for a major allergen. Dog harbors a broad range of
294 relevant allergens with none clearly predominating and each potentially causing
295 monosensitization. Molecular diagnosis in dog allergy therefore requires simultaneous testing for
296 all components, perhaps excluding Can f 2 where monovalent sensitization is rare^{11,16} and which
297 never contributed to sensitivity in our study.

298 Molecular diagnosis in animal allergies has become complex and sometimes confusing essentially
299 due to the increasing number of allergens identified from the large lipocalin family. In light of the
300 extreme heterogeneity within this protein family, labeling allergens simply as “lipocalins” is little

301 helpful. As an example, Can f 1, 2, 4 and 6 all represent lipocalins but sequence identities between
302 them are very low (<30%) and actually each of them represents a completely independent
303 allergen. A closer examination of currently identified mammalian respiratory lipocalin allergens
304 reveals that they allocate to at least eight different subfamilies. To better get across among
305 allergists this tremendous heterogeneity, a new nomenclature might be
306 welcomed denominating allergens after the corresponding subgroup within the family, like van
307 Ebner gland proteins (VEGP), major urinary proteins (MUP) or odorant-binding proteins (OBP).

308 The present study provides interesting data for two new lipocalin allergens, Fel d 7 from cat and
309 Can f 6 from dog, which have become available in the ImmunoCAP platform only recently. Both
310 allergens turned out extremely helpful by clarifying the relationship with their cross-reactive
311 counterparts Can f 1 and Fel d 4, respectively, both of which have been available already well
312 before. In consistency with previous papers^{9,11,17,18} we observed substantial cross-reactivity
313 between Fel d 4/Can f 6/Equ c 1 and between Can f 1/Fel d 7 thereby reasonably explaining many
314 cases of double- and polysensitization. Horse allergy was nearly always associated with cat allergy
315 in our patients due to the strong cross-reactivity Equ c 1/Fel d 4, though we may have missed
316 sensitization to other relevant horse allergens not yet available for molecular diagnosis, such as
317 Equ c 4, a latherin potentially cross-reactive with cat Fel d 8.

318 The data obtained with Fel d 7 are provocative with regard to the role of Can f 1 which currently is
319 considered a marker allergen for genuine dog allergy.^{20,25} Our findings do not support this
320 view since almost half of patients with Can f 1 sensitization show much higher IgE levels to Fel d 7
321 and therefore likely are just cross-sensitized to Can f 1. In fact, nearly all of the Can f 1/Fel d 7
322 double-positive patients showing higher values for Fel d 7 than for Can f 1 were cat owners, while
323 those with higher values for Can f 1 were mostly dog owners. Hence, cats commonly cause dog
324 allergy, and vice versa. The very same can be seen for the Fel d 4-like lipocalins Fel d 4/Can f
325 6/Equ c 1.

326 Accordingly, neither of these lipocalins can be considered per se a reliable marker for genuine
327 sensitization. Anyhow, we believe that comparison of individual IgE scores gives a reasonable
328 clue on the primary sensitizer in many patients. This may be helpful especially in dog allergy and
329 when immunotherapy is considered, since many dog-allergic patients are not sensitized to dog-
330 specific marker allergens like Can f 2, 4 or 5. We are aware that defining the primary sensitizer on
331 the basis of IgE levels is speculative and that reliable identification requires laborious inhibition

332 experiments. In insect venom allergy, discrimination between primary *Vespula* and *Polistes*
333 allergy by comparing IgE levels to Ves v 5 and Pol d 5 has been shown to correlate pretty well
334 with the results from inhibition experiments.²⁶ Likewise, in Fagales pollen allergy, where birch
335 pollen Bet v 1 usually represents the primary sensitizer, antibody-binding to homologous PR-10
336 proteins from other Fagales pollens and from foods is always clearly weaker than to Bet v
337 1.^{27,28} What also supports our approach is that the obtained classification into primary and
338 secondary sensitization fits remarkably well together with reported pet ownership.

339 A novel finding of the present study is the strong correlation between individual sensitization
340 profiles and previous animal exposure. An obvious exception is Fel d 1 where sensitization
341 was unrelated to cat ownership, presumably due to its ubiquitous occurrence in the public together
342 with its high allergenicity. Sensitization to the minor cat allergens Fel d 4 and 7 and to dog Can f
343 1, 2, 4, 5 and 6, however, was significantly linked to cat and dog ownership, respectively. The
344 male-specific dog allergen Can f 5 further supports this observation as in agreement with another
345 recent study²⁹ sensitization was almost exclusively found in subjects keeping a male
346 dog. Undoubtedly, sensitization to all these allergens does occur also in subjects without close
347 animal contacts, but this appears to be infrequent and might be primarily found in highly atopic
348 subjects. Complex sensitization profiles involving minor allergens have been repeatedly described
349 as a risk factor for asthma, especially in children.⁹⁻¹⁴ Our findings suggest that complex
350 sensitization patterns may alternatively be considered as a surrogate marker for pet ownership and
351 high allergen burden finally promoting asthma.

352 We were particularly interested in this study in the significance of cat+dog double-positivity
353 because this is a common, though often unexpected finding encountered in many cat-allergic
354 patients during routine allergy testing. As many as 43% of cat-dog double-positive patients did not
355 have detectable IgE against any of the six tested dog allergens indicating that some dog
356 components are lacking. Interestingly, the vast majority of these IgE-negative patients denied any
357 symptoms upon dog exposure even if actively keeping a dog, while cat sensitization was very well
358 relevant in 83% of them. We speculate that the responsible missing dog allergen is an
359 uteroglobulin-like dog protein (UniProt E2QUN9) sharing 65% sequence identity with Fel d 1.
360 This is supported by our observation that IgE-binding to dog extracts in these patients strongly
361 correlated with Fel d 1 ($r=0.88$, data not shown). In vitro cross-reactivity between Fel d 1 and the
362 dog homologue has been shown previously but its clinical role remained obscure.³⁰ Overall, as

363 much as 48% of dog sensitizations found in our population appear to result from cross-reactivity
364 with cat allergens and more than half of these sensitizations seem to have low relevance.
365 Identifying those patients with non-relevant sensitization by their molecular IgE patterns would be
366 extremely helpful in daily practice and greatly improve patient management. However, a
367 limitation of our study is that both animal exposure and clinical relevance entirely relied on
368 patients' self-assessment and that interference with other respiratory allergies could not always be
369 excluded. Therefore the postulated limited clinical significance of Fel d 1-mediated cat-dog cross-
370 reactivity needs to be confirmed in future studies.

371 With regard to other dog allergens, sensitization to Can f 1 and 6 seemed to have high clinical
372 relevance throughout, even if resulting from cross-reactivity with cat. Can f 4 and particularly Can
373 f 5, however, often seemed to have limited importance, as also observed by others.²⁷ One reason
374 for this may be that Can f 5 is an urinary protein³¹ less likely found in high concentrations indoors.
375 On the other hand, Can f 5 can be cited as a tangible example illustrating the advantages of
376 molecules over extracts as it identifies subjects with an allergy to male dogs alone. The
377 expectation that these patients may safely keep a female dog has been recently proven
378 experimentally.³² In the present study, 28% of dog-allergic patients were monosensitized to Can f
379 5, which is in fair agreement with most previous papers reporting 20-58%.^{11,12,24,33} Accordingly,
380 switching to a female dog represents a reasonable option for a significant proportion of dog-
381 allergic individuals.

382 In conclusion, in-vitro diagnosis with currently available animal allergens can effectively
383 illuminate the molecular background and origin of (poly)sensitization to cat, dog and horse in the
384 majority of patients, and often allows for discrimination between genuine sensitization and cross-
385 sensitization. It is also a promising tool that might prove helpful in estimating clinical
386 relevance though but this requires more studies in larger collectives and some yet lacking
387 molecules to allow for more complete IgE binding profiles.

388

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488 **Legends:**

490 **Figure 1:** Sensitization patterns to cat, dog and horse dander as revealed by skin prick testing in
491 294 patients with animal sensitization.

494 **Figure 2:** IgE binding patterns with cat and dog allergens in relation to mono-, double- and
495 polysensitization. **a)** IgE-binding to cat allergens in patients with a positive skin prick test to cat
496 (n=273); **b)** IgE-binding to dog allergens in patients with a positive skin prick test to dog (n=155);

499 **Figure 3:** Co-reactivity patterns with animal lipocalins. **a)** Overlap of IgE-binding to the lipocalins
500 Fel d 4, Can f 6 and Equ c 1 (64-71% SI) in patients with IgE to at least one of the three lipocalins
501 (n=106). **b)** Overlap of IgE-binding to the lipocalins Fel d 7 and Can f 1 (69% SI) in patients with
502 IgE to at least one of the two lipocalins (n=82).

505 **Figure 4:** Relationship between IgE binding patterns and clinical relevance of dog sensitization
506 (n=134). Subjects with a positive skin prick test to dog dander but negative IgE test results to all
507 tested dog components (n=27) rarely reported symptoms upon dog exposure, whereas IgE to at
508 least one of the tested dog components (n=107) was highly predictive of clinical dog allergy, also
509 when sensitization was due to cross-reactivity with cat.

512 **Suppl. Figure 1:** Primary sensitization *versus* cross-sensitization to dog allergens in dog-
513 sensitized patients (n=155). IgE binding values for Can f 1, 3 and 6 were compared with those for
514 the homolog cat allergens Fel d 7, 2 and 4, and the molecule showing the higher score arbitrarily
515 considered as the primary sensitizer. Accordingly, Can f 1 and Can f 6 are the dog allergens most
516 often recognized, but reactivity often reflects cross-reactivity with cat. Can f 5 is the dog allergen
517 most often causing primary sensitization.

519

520 **Suppl. Figure 2:** Correlation between cat and dog lipocalins. **a)** Correlation between Fel d 4 and
521 Can f 6 in all subjects with IgE to either of the two lipocalins (n=106) after stratification for
522 putative primary sensitization to Fel d 4 (blue circles), Can f 6 (orange triangles) and Equ c 1
523 (green squares), respectively. **b)** Correlation between Can f 1 and Fel d 7 in all subjects with IgE
524 to either of the two lipocalins (n=82) after stratification for putative primary sensitization to Can f
525 1 (orange triangles) and Fel d 7 (blue circles), respectively.

526

Table 1: IgE binding frequency to cat, dog and horse allergens in 294 patients in relation to animal mono-, double- and polysensitization. Reported prevalence data in the bottom line are from references [10-12,17-23] and refer to patients with sensitization to cat, dog and horse, respectively. All values represent percent.

Positive skin test to	n	sIgE ≥ 0.1 kUa/L (%)									
		Fel d 1	Fel d 4	Fel d 7	Can f 1	Can f 2	Can f 4	Can f 5	Can f 6	Equ c 1	Fel d 2/ Can f 3
		uteroglobulin	lipocalin	lipocalin	lipocalin	lipocalin	lipocalin	PSA-like	lipocalin	lipocalin	serum albumin
Cat only	120	92.5	9.2	3.8	3.1	0.0	0.0	0.0	0.0	3.1	2.3
Dog only	18	0.0	5.6	11.1	33.3	0.0	22.2	66.7	5.6	11.1	5.6
Horse only	3	0.0	66.7	0.0	0.0	0.0	0.0	0.0	33.3	66.6	0.0
Cat + dog	76	96.1	27.6	29.3	35.5	5.3	11.8	23.7	17.1	17.1	13.2
Cat + horse	16	87.5	81.3	12.5	12.5	0.0	0.0	0.0	37.5	93.8	12.5
Cat + dog + horse	61	90.2	85.2	59.0	63.9	18.0	41.0	36.1	78.7	86.9	41.0
Cat all	273	92.7	35.9	24.2	26.4	5.5	24.1	14.7	43.6	31.1	14.7
Dog all	155	82.6	47.7	39.0	47.1	9.7	25.2	33.5	40.6	44.5	23.2

Horse all	80	86.3	83.8	47.5	51.3	13.8	39.7	27.5	71.3	87.5	33.8
All	294	86.1	34.4	22.9	26.9	5.1	24.4	17.7	40.2	30.6	13.9
Reported prevalence		84-100	31-63	38	29-76	12-40	35-59	32-71	23-61	27-100	4-59

Table 2: IgE binding patterns with cat allergens in relation to current or previous cat ownership in patients with a positive skin prick test to cat (n=273). Sensitization to Fel d 2, 4 and 7 was further separated into putative primary sensitization and cross-sensitization based on the comparison with the IgE scores obtained with the homologous dog allergens Can f 3, Can f 6 and Can f 1, respectively.

		All sensitized n=273	Cat owners n=187	No cat n=86	Chi ²
Fel d 1	positive ≥ 0.1 kU _a /L	253 (92.7%)	172 (92.0%)	81 (91.9%)	<i>n.s.</i>
Fel d 2	positive ≥ 0.1 kU _a /L	38 (15.0%)	31 (16.6%)	9 (10.5%)	<i>n.s.</i>
	primary sensitization to Fel d 2	27	26	3	<i>p<0.01</i>
	cross-reaction with Can f 3	11	5	6	<i>n.s.</i>
Fel d 4	positive ≥ 0.1 kU _a /L	98 (38.7%)	74 (39.6%)	24 (27.9%)	<i>p=0.06</i>
	primary sensitization Fel d 4	62	55	8	<i>p<0.0001</i>

	cross-reaction with Can f 6	36	19	16	<i>n.s.</i>
Fel d 7	positive ≥ 0.1 kU _a /L	66 (26.1%)	54 (31.4%)	12 (13.9%)	<i>p</i> <0.01
	primary sensitization to Fel d 7	43	40	3	<i>p</i> =0.0001
	cross-reaction with Can f 1	23	14	9	<i>n.s.</i>

Table 3: IgE binding patterns with dog allergens in relation to dog ownership in patients with a positive skin prick test to dog (n=155). Sensitization to the cross-reactive allergens Can f 1, 3 and 6 was further separated into putative primary sensitization and cross-sensitization based on the comparison with the IgE scores obtained with the homologous cat allergens Fel d 7, Fel d 2 and Fel d 4, respectively.

Allergen		All sensitized n=155	Dog owners n=98	No dog n=57	Chi ²
Can f 1	positive ≥ 0.1 kU _a /L	73 (47.1%)	58 (59.2%)	15 (26.3%)	$p < 0.0001$
	primary sensitization to Can f 1	40	36	4	$p < 0.0001$
	cross-reaction with Fel d 7	33	22	11	<i>n.s.</i>
Can f 2	positive ≥ 0.1 kU _a /L	15 (9.7%)	15 (15.3%)	0 (0.0%)	$p < 0.005$
Can f 3	positive ≥ 0.1 kU _a /L	36 (23.2%)	23 (23.5%)	13 (22.8%)	<i>n.s.</i>
	primary sensitization to Can f 3	14	12	2	$p = 0.06$
	cross-reaction with Fel d 2	22	11	11	<i>n.s.</i>
Can f 4	positive ≥ 0.1 kU _a /L	39 (25.2%)	33 (33.7%)	6 (10.5%)	$p = 0.001$

Can f 5	positive ≥ 0.1 kU _a /L	52 (33.5%)	45 (45.9%)	7 (12.3%)	$p < 0.0001$
Can f 6	positive ≥ 0.1 kU _a /L	63 (35.5%)	49 (50.0%)	14 (24.6%)	$p < 0.005$
	primary sensitization to Can f 6	21	21	0	$p < 0.001$
	cross-reaction with Fel d 4	42	28	14	$n.s.$

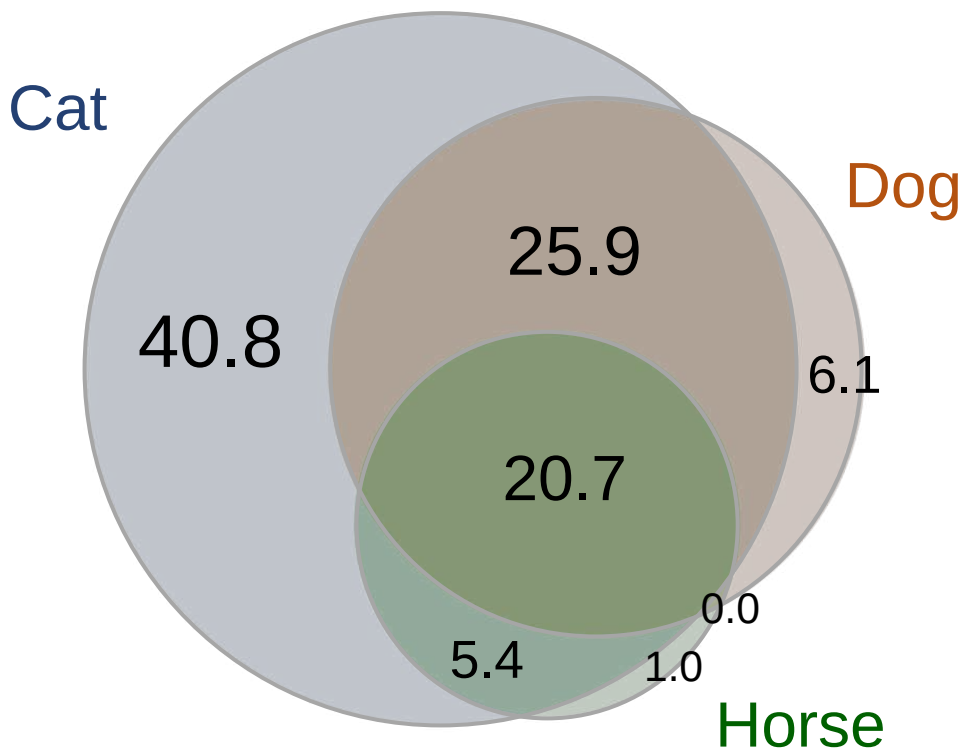


Figure 1: Sensitization patterns to cat, dog and horse dander as revealed by skin prick testing in 294 patients with animal sensitization. Figures represent percent.

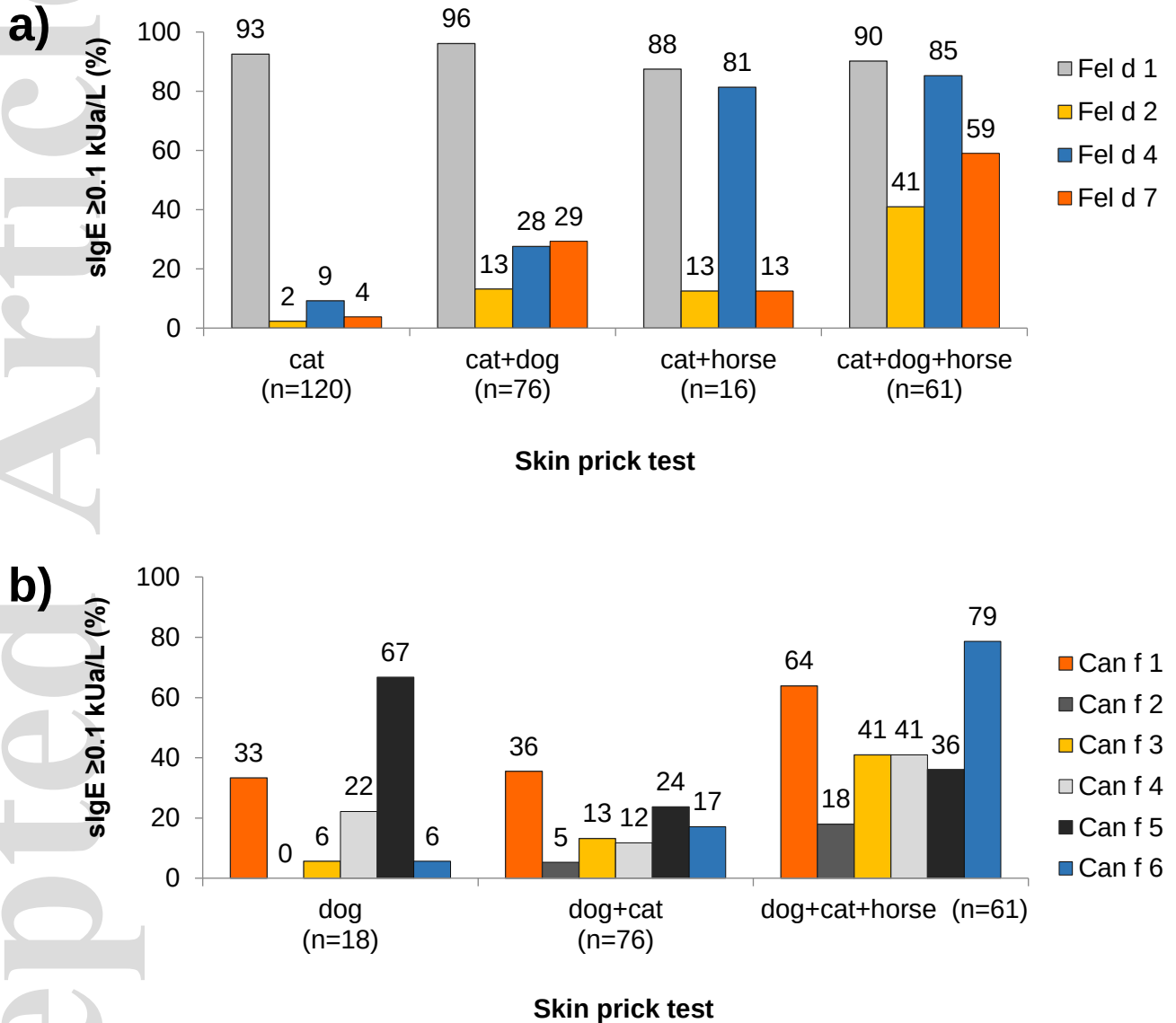


Figure 2: IgE binding patterns with cat and dog allergens in relation to mono-, oligo- and polysensitization. **a)** IgE-binding to cat allergens in patients with a positive skin prick test to cat (n=273); **b)** IgE-binding to dog allergens in patients with a positive skin prick test to dog (n=155);

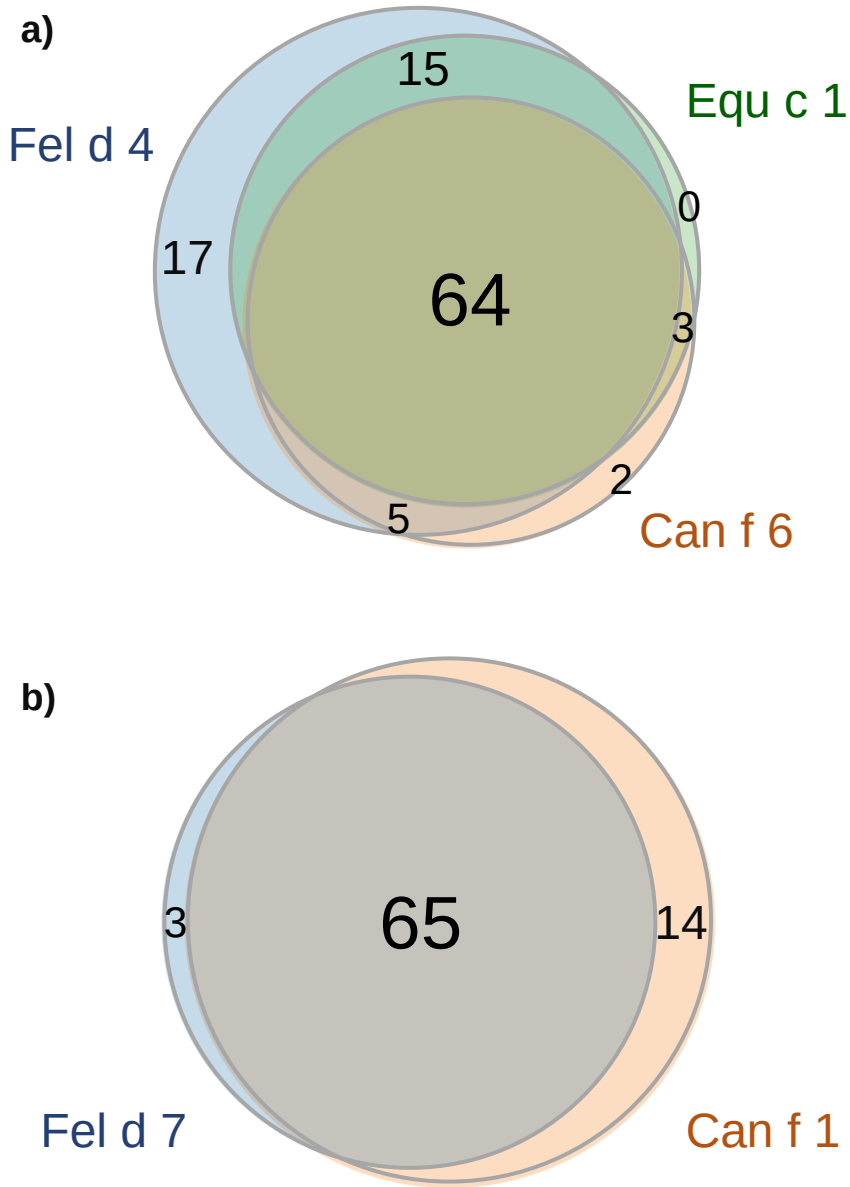


Figure 3: Co-reactivity patterns with animal lipocalins. **a)** Overlap of IgE-binding to the lipocalins Fel d 4, Can f 6 and Equ c 1 (64-71% SI) in patients with IgE to at least one of the three lipocalins (n=106). **b)** Overlap of IgE-binding to the lipocalins Fel d 7 and Can f 1 (69% SI) in patients with IgE to at least one of the two lipocalins (n=82).

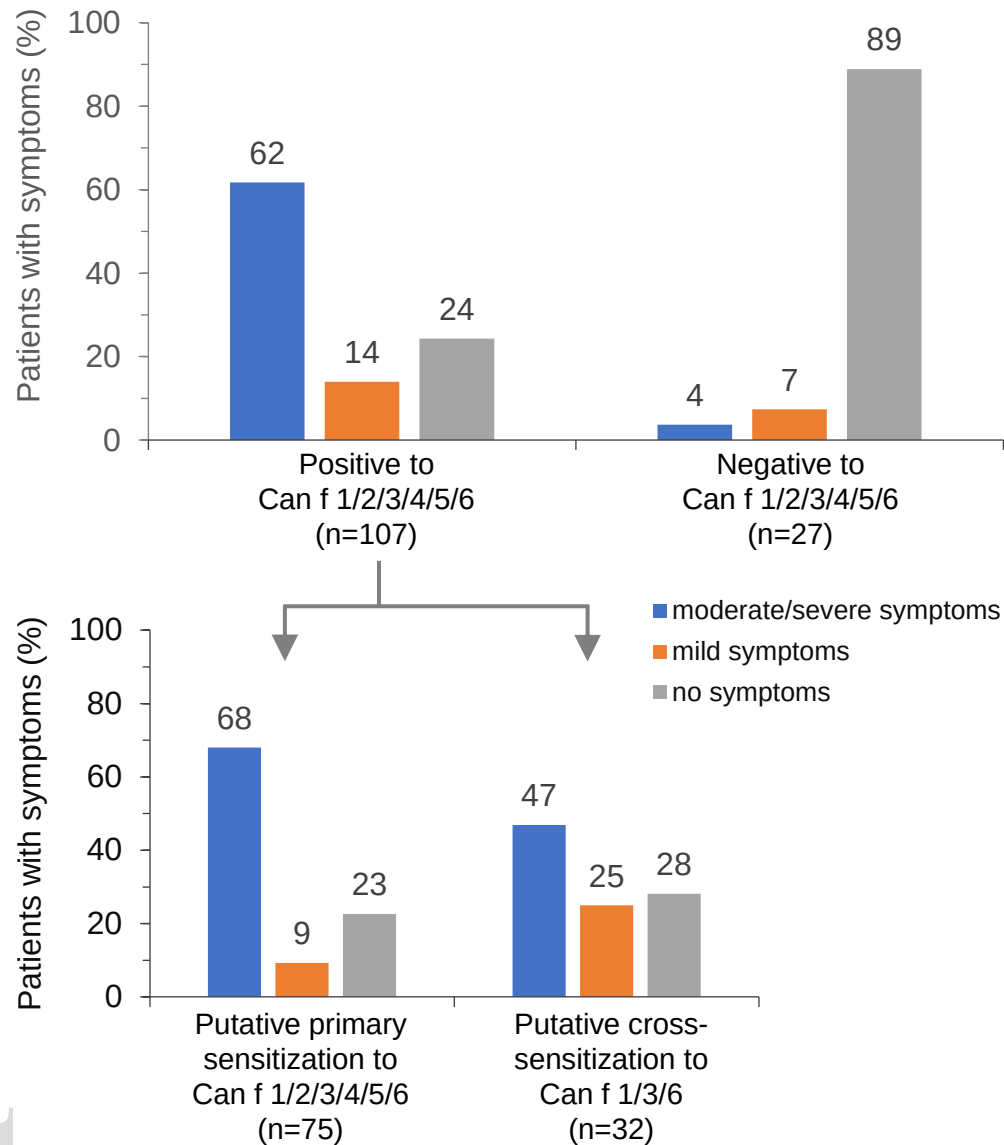
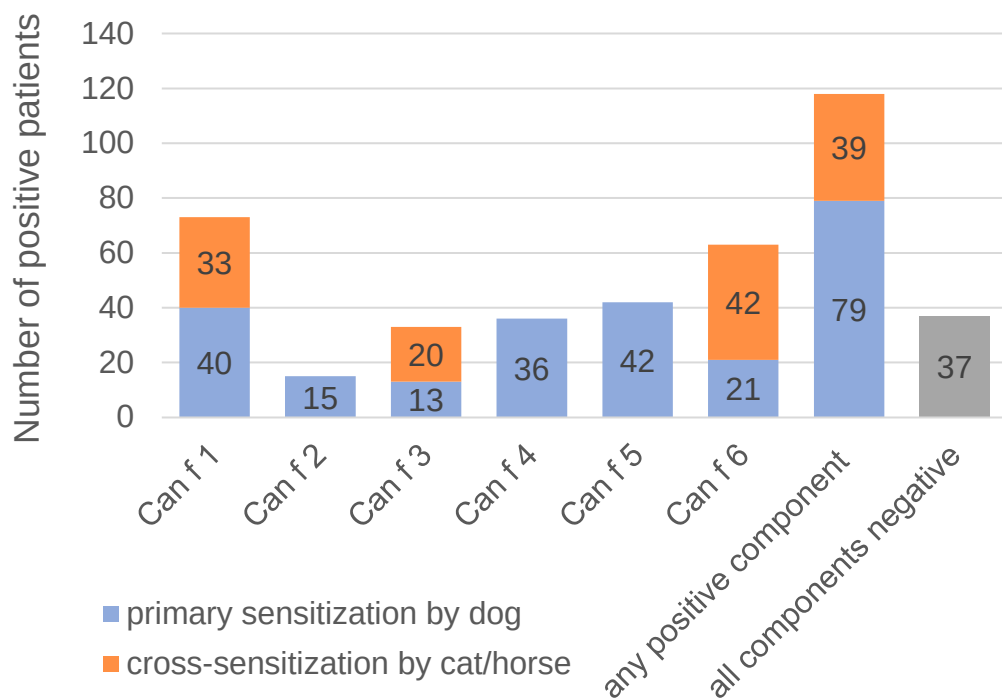
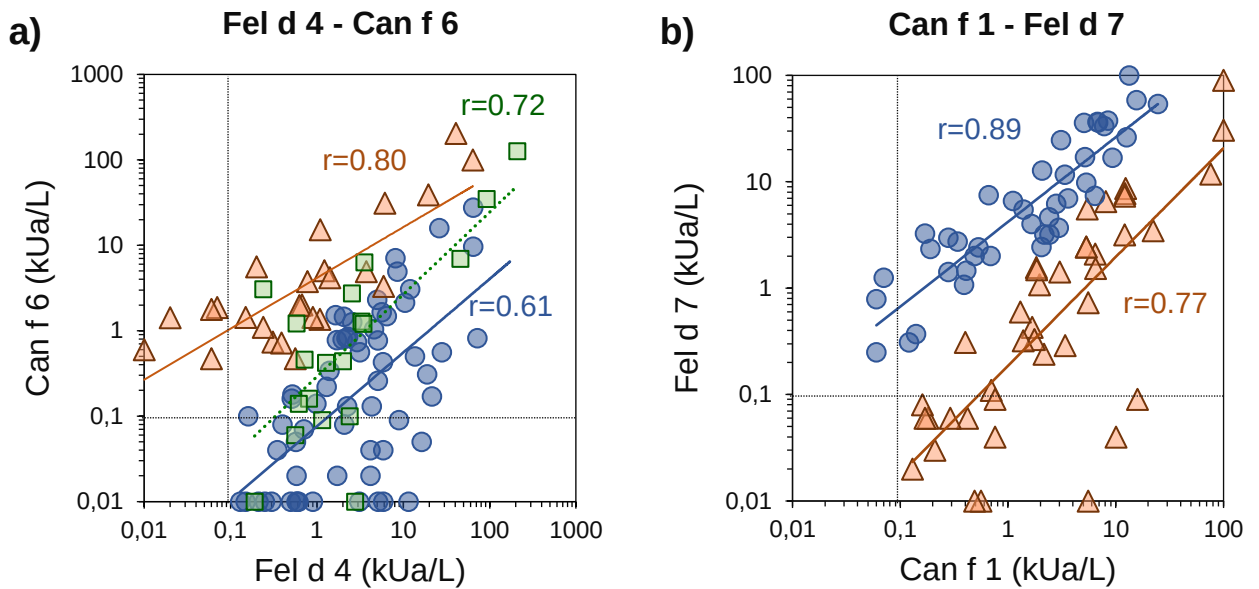


Figure 4: Relationship between IgE binding patterns and clinical relevance of dog sensitization. Subjects with a positive skin prick test to dog dander but negative IgE test results to all tested dog components rarely reported symptoms upon dog exposure, whereas the presence of IgE to at least one of the tested dog components was highly predictive of clinical dog allergy, also when sensitization was presumably due to cross-reactivity with cat.



Suppl. Figure 1: Primary sensitization versus cross-sensitization to dog allergens in dog-sensitized patients (n=155). IgE binding scores for Can f 1, 3 and 6 were compared with those for the homologous cat allergens Fel d 7, 2 and 4, and the molecule showing the higher binding score arbitrarily considered as the primary sensitizer. Accordingly, Can f 1 and Can f 6 are the most often recognized dog allergens, but reactivity often reflects cross-reactivity with cat. Can f 5 is the dog allergen most often causing primary sensitization.



Suppl. Figure 2: Correlation between cat and dog lipocalins. **a)** Correlation between Fel d 4 and Can f 6 in all subjects with IgE >0.1 kUa/L to either of the two lipocalins (n=106) after stratification for putative primary sensitization to Fel d 4 (blue circles), Can f 6 (orange triangles) and Equ c 1 (green squares), respectively. **b)** Correlation between Can f 1 and Fel d 7 in all subjects with IgE >0.1 kUa/L to either of the two lipocalins (n=82) after stratification for putative primary sensitization to Can f 1 (orange triangles) and Fel d 7 (blue circles), respectively.

Suppl. Table 1: Classification of severity grade based on self-reported symptoms after contact with animals.

Severity grade	Clinical symptoms upon animal exposure
1. Moderate/severe	Rhinorrhea, conjunctivitis, blocked nose for at least 1 hour, acute breathing difficulties, asthma exacerbation requiring use of beta-agonists
2. Mild	Sneezing without rhinitis, local skin reactions (itching, reddening, contact urticaria) after touching animals or after contact with saliva
3. Asymptomatic	none

Suppl. Table 2: Sequence identity between allergenic lipocalins from cat, dog and horse available on the ImmunoCAP platform. The seven allergens belong to four different similarity clusters within the lipocalin family.

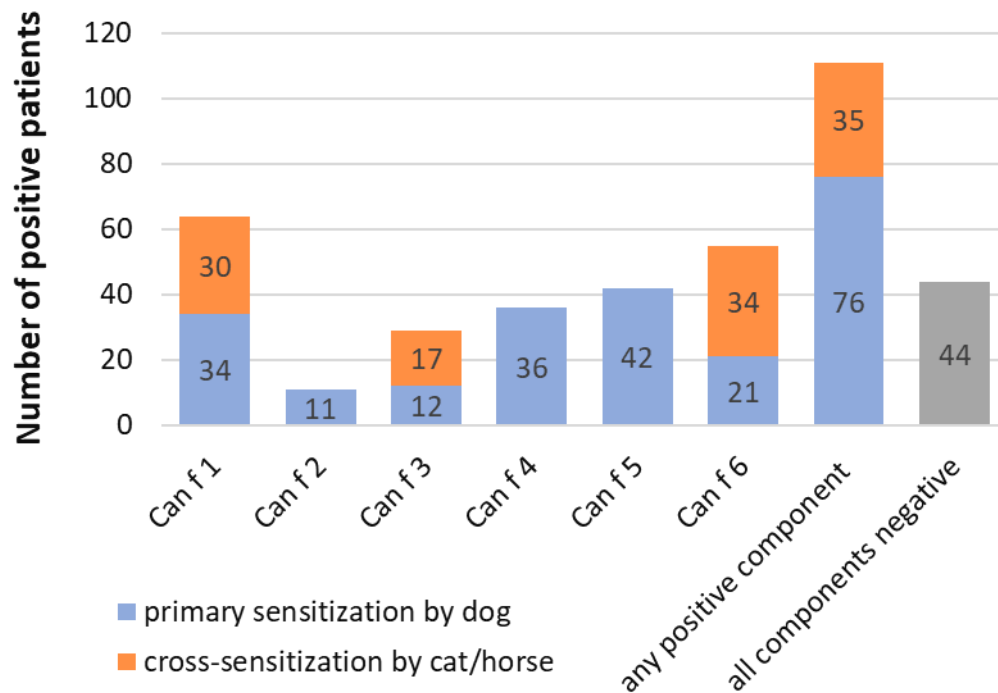
Animal species	IUIS	<i>Can f 1-like</i>				<i>Fel d 4-like</i>		
		Can f 4	Fel d 7	Can f 1	Can f 2	Fel d 4	Can f 6	Equ c 1
Dog	Can f 4	100	21	19	25	28	29	29
Cat	Fel d 7	21	100	63	26	23	25	26
Dog	Can f 1	19	63	100	26	26	24	27
Dog	Can f 2	25	26	26	100	28	26	27
Cat	Fel d 4	28	23	26	28	100	71	70
Dog	Can f 6	29	25	24	26	71	100	59
Horse	Equ c 1	29	26	27	27	70	59	100

Suppl. Table 3: IgE binding patterns with dog allergens in relation to dog ownership and dog gender in patients with dog sensitization (n=155).

Dog ownership	n	Can f 1	Can f 2	Can f 3	Can f 4	Can f 5	Can f 6
Yes	98	58 (59.2%)	15 (15.3%)	23 (23.5%)	33 (33.7%)	45 (45.9%)	49 (50.0%)
No	57	15 (26.3%)	0 (0.0%)	13 (22.8%)	6 (10.5%)	7 (12.3%)	14 (24.6%)
<i>Chi²</i>		<i>p<0.0001</i>	<i>p<0.005</i>	<i>n.s.</i>	<i>p<0.01</i>	<i>p<0.0001</i>	<i>p<0.005</i>
Male dog	55	33 (60.0%)	12 (21.8%)	14 (25.5%)	20 (36.4%)	36 (65.5%)	25 (45.5%)
Female dog	20	15 (75.0%)	0 (0.0%)	5 (25.0%)	7 (35.0%)	2 (10.0%)	11 (55.0%)
<i>Chi²</i>		<i>n.s.</i>	<i>p<0.05</i>	<i>n.s.</i>	<i>n.s.</i>	<i>p<0.0001</i>	<i>n.s.</i>

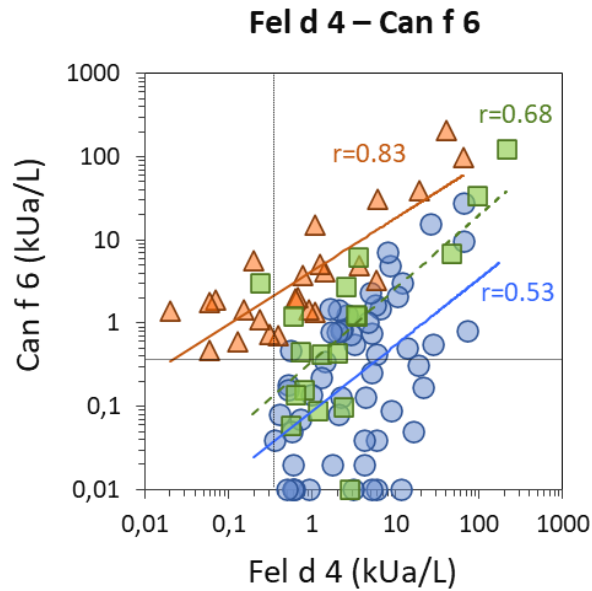
Suppl. Table 4: IgE reactivity with recombinant horse, cat and dog allergens in relation to horse exposure in patients with horse sensitization (n=80).

Regular exposure to horses	n	Fel d 1	Fel d 4	Equ c 1	Can f 6	Equ c 1 > Fel d 4/Can f 6
Yes	31	26 (83.9%)	23 (74.2%)	22 (71.0%)	19 (61.3%)	13/22 (59.1%)
No	49	43 (87.8%)	41 (83.7%)	44 (89.8%)	38 (77.6%)	9/44 (20.5%)
<i>Chi²</i>		<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>p=0.005</i>

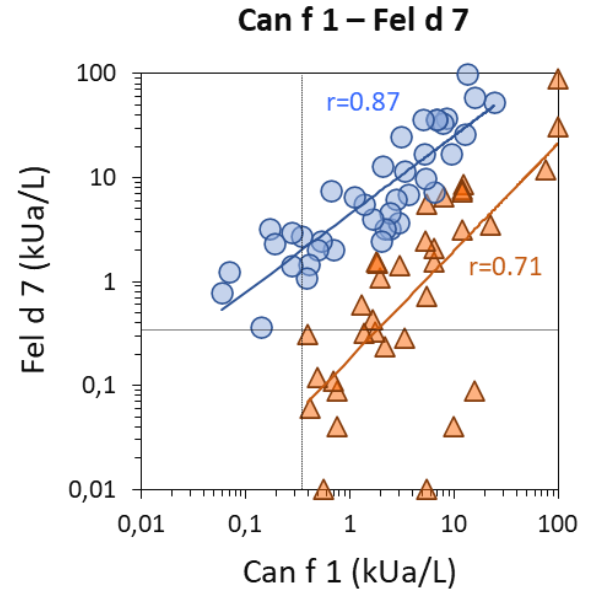


Suppl. Figure 1: Primary sensitization *versus* cross-sensitization to dog allergens in dog-sensitized patients (n=155). IgE binding values for Can f 1, 3 and 6 were compared with those for the homolog cat allergens Fel d 7, 2 and 4, and the molecule showing the higher score arbitrarily considered as the primary sensitizer. Accordingly, Can f 1 and Can f 6 are the dog allergens most often recognized, but reactivity often reflects cross-reactivity with cat. Can f 5 is the dog allergen most often causing primary sensitization.

a)



b)



Suppl. Figure 2: Correlation between cat and dog lipocalins. **a)** Correlation between Fel d 4 and Can f 6 in all subjects with IgE to either of the two lipocalins (n=99) after stratification for putative primary sensitization to Fel d 4 (blue circles), Can f 6 (orange triangles) and Equ c 1 (green squares), respectively. **b)** Correlation between Can f 1 and Fel d 7 in all subjects with IgE to either of the two lipocalins (n=74) after stratification for putative primary sensitization to Can f 1 (orange triangles) and Fel d 7 (blue circles), respectively.