

Component-resolved microarray analysis of IgE sensitization profiles to *Felis catus* major allergen molecules in Russian cat-allergic patients

Anna Sergeevna Dolgova, Anna Evgenevna Sudina, Anna Sergeevna Cherkashina & Olga Alekseevna Stukolova

To cite this article: Anna Sergeevna Dolgova, Anna Evgenevna Sudina, Anna Sergeevna Cherkashina & Olga Alekseevna Stukolova (2017): Component-resolved microarray analysis of IgE sensitization profiles to *Felis catus* major allergen molecules in Russian cat-allergic patients, Scandinavian Journal of Clinical and Laboratory Investigation, DOI: [10.1080/00365513.2017.1414955](https://doi.org/10.1080/00365513.2017.1414955)

To link to this article: <https://doi.org/10.1080/00365513.2017.1414955>



Published online: 11 Dec 2017.



Submit your article to this journal [↗](#)



Article views: 15



View related articles [↗](#)



View Crossmark data [↗](#)

ORIGINAL ARTICLE



Component-resolved microarray analysis of IgE sensitization profiles to *Felis catus* major allergen molecules in Russian cat-allergic patients

Anna Sergeevna Dolgova^a, Anna Evgenevna Sudina^a, Anna Sergeevna Cherkashina^b and Olga Alekseevna Stukolova^{a,b}

^aMolecular Diagnostics and Epidemiology Department, Central Research Institute of Epidemiology, Moscow, Russia; ^bResearch Institute of Occupational Health, Moscow, Russia

ABSTRACT

We aimed to determine the profile of IgE reactivity to three major cat allergens, Fel d 1, Fel d 2 and Fel d 4, in cat-allergic patients in the Moscow region in Russia. sIgE levels to recombinant proteins expressed in *Escherichia coli* (Fel d 1 and Fel d 4) and to Fel d 2 protein purified from cat serum were measured using a microarray method developed in our laboratory. Sera from 174 anonymous subjects with a positive reaction (≥ 0.35 IU/mL) to cat dander extract (e1, ImmunoCAP) and 56 negative controls were used for IgE testing. Fel d 1 was recognized by 92.5%, Fel d 2 by 29.9% and Fel d 4 by 39.1% of the tested patient sera. The sensitivity to these three proteins was approximately 98% compared to cat dander extract (correlation coefficient to ImmunoCAP is 0.94 with PPV = 0.99 and NPV = 0.95). These predictive values appeared to be even more statistically significant than the divergence between the ISAC IgE test and the extract-based singleplex ImmunoCAP. The combination of the three investigated proteins (Fel d 1, Fel d 2 and Fel d 4) is suitable for *in vitro* molecular (serological) diagnosis of cat allergy in this region as a complement to cat dander extract. Moreover, with this method, we found distinction between Fel d 2 and other Feline sIgEs formation.

ARTICLE HISTORY

Received 4 May 2017
Revised 16 October 2017
Accepted 5 November 2017

KEYWORDS

Allergy diagnostics; antigen-specific IgE; cat allergy; Fel d 1; Fel d 2; Fel d 4; sensitization profiles

Introduction

According to a survey conducted by the Levada Center, 57% of Russian citizens have pets, and 37% of respondents have cats. Considering this high prevalence of cats and the fact that, worldwide, cat allergens are one of the most important indoor allergens that often cause IgE-mediated allergic diseases [1,2] the risk of developing cat allergy in Russia may be as high as that in Northern Europe or in the USA, where up to 15% of the population is sensitized to cat [3–7]. The most common manifestations of cat allergy are rhinitis or rhinoconjunctivitis; however, studies have shown that sensitization to cat allergens is also a strong risk factor for asthma development [8,9].

Thus far, no integrated study focusing on cat allergy and covering all regions of the country and all population groups has been conducted in the Russian Federation. Only limited data from different parts of the country are available on this topic. A study focusing on residents of the Republic of Tatarstan demonstrated that more than half of all examined subjects sensitized to animal epidermal allergens were sensitized to cat fur [10]. Cat epidermal allergens were shown to be the most common cause of allergic rhinitis (AR) (8.2–11.6%) among residents of the Penza region [11]. In addition, approximately 30% of children in Moscow (7–18 years old) had a positive allergic history in one study; 6.6% reported symptoms of allergic rhinitis, with more than

half reporting the onset/intensification of allergy symptoms after contact with pets (mainly cats) [12]. In Tomsk, every third child with symptoms of allergic rhinoconjunctivitis was sensitized to cat allergens [13]. A study conducted in Kolomna (Moscow region) involving the analysis of 130 serum samples collected from patients with bronchial asthma and/or AR identified specific IgEs (sIgEs) to cat epidermal antigens in 46.7% of samples collected from children and 42.8% of samples collected from adults [14]. Population studies have shown that more than half of Russian asthmatic patients are sensitized to cat allergens [15]. Thus, from available studies, it is evident that cat allergy is a frequent occurrence in the Russian population. The goal of this study was to determine the profile of sIgE prevalence for the three major cat allergens, Fel d 1, Fel d 2 and Fel d 4, in cat-allergic patients living in the Moscow region of Russia.

Materials and methods

Patients and serum samples

Serum samples were collected at the Center of Molecular Diagnostics of the Federal Budget Institution of Science 'Central Research Institute of Epidemiology' of The Federal Service on Customers' Rights Protection and Human Well-being Surveillance in Moscow, Russia. The subjects with cat allergy ($n = 174$) were volunteers who reported developing

allergy symptoms to cats and had serum levels of IgE to cat dander extract (e1) ≥ 0.35 IU/mL determined using the ImmunoCAP system (Phadia, Uppsala, Sweden). The mean age of the overall patient group was 17.3 years, and a significant difference in age was observed between genders (males: 14.33 ± 14.26 years; females: 21.23 ± 15.21 years; $p = 0.0019$). Serum samples from 56 subjects (32 females and 24 males; average age 20 years, range from 1 to 57 years) that were classified as 0 by ImmunoCAP (e1) were used as a negative control group. The use of anonymous sera was approved by the local ethics committee.

Production of allergens

Fel d 1 was expressed in *E. coli* as a fusion protein of two protein chains with the flexible linker GGSGGGG SGGGSGGG designed for the baculovirus system [16]. Fel d 1 was digested with EcoRI/XhoI and ligated into the pET24a vector with a GST-tag. Recombinant Fel d 4 was produced from fragments by one-step PCR as described previously [17]. Recombinant allergens were expressed in *E. coli* BL21 (DE3) and purified using Chelating Sepharose Fast Flow (GE Healthcare, Uppsala, Sweden) and DEAE Sepharose Fast Flow (GE Healthcare, Uppsala, Sweden). The Fel d 4 and Fel d 1 proteins were obtained at final concentrations of 1.4 mg/ml and 3 mg/ml, respectively, in buffer containing 50 mM Tris/HCl, pH 8.0, 0.3 M NaCl and 10% glycerol. Fel d 2 was purified as described elsewhere [18] from pooled cat blood plasma obtained from The All-Russian State Center for Quality and Standardization of Veterinary Drugs and Feed. Aliquots of protein solutions of interest were analysed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in a 10% gel. All allergens showed a single band with the expected molecular weight.

Cat component-specific IgE measurement

Total IgE levels and serum sIgE levels to Fel d 1, Fel d 2 and Fel d 4 were measured using a special microarray method developed in our laboratory [17]. The concentrations of Fel d 1-, Fel d 2- and Fel d 4 sIgEs were interpolated from the obtained goat anti-human IgE antibody 4-parameter logistic curve. The use of this more complex mathematical model improved the resolution of the positive and the negative results for low values compared with the use of polynomial quadratic regression [17]. Total IgE levels were expressed in international units per unit volume (IU/ml, 1 IU equals 2.4 ng). Sensitization to Fel d 1, Fel d 2 and Fel d 4 was considered significant when the sIgE level was ≥ 0.35 IU/ml.

Statistical analysis

The correlations of the IgE antibody responses between different analytical systems were assessed using Pearson's χ^2 test, and the correlations between total IgE and sIgEs were assessed using both Pearson's and Spearman's tests. To calculate 95% confidence intervals for the sensitivity, specificity, PPA and NPA values, the two-sided Clopper–Pearson method was used (assuming a beta distribution of the respective probabilities). Statistical analysis was performed using Minitab 17 and a significance level of 0.05.

Results

Prevalence of IgE reactivity to individual cat allergens

Most of the sensitized subjects had IgE responses to the major cat allergen Fel d 1 (161 patients; 92.5%). Fel d 2 and Fel d 4 sIgEs were found in 29.9% (52 serum samples) and 39.1% (68 serum samples) of cases, respectively (Figure 1). The average level of sIgE was significantly higher for Fel d 1 and Fel d 2 than for Fel d 4 (1717, 1383 and 479 IU/ml, respectively). Moreover, very high anti-Fel d 1 IgE levels

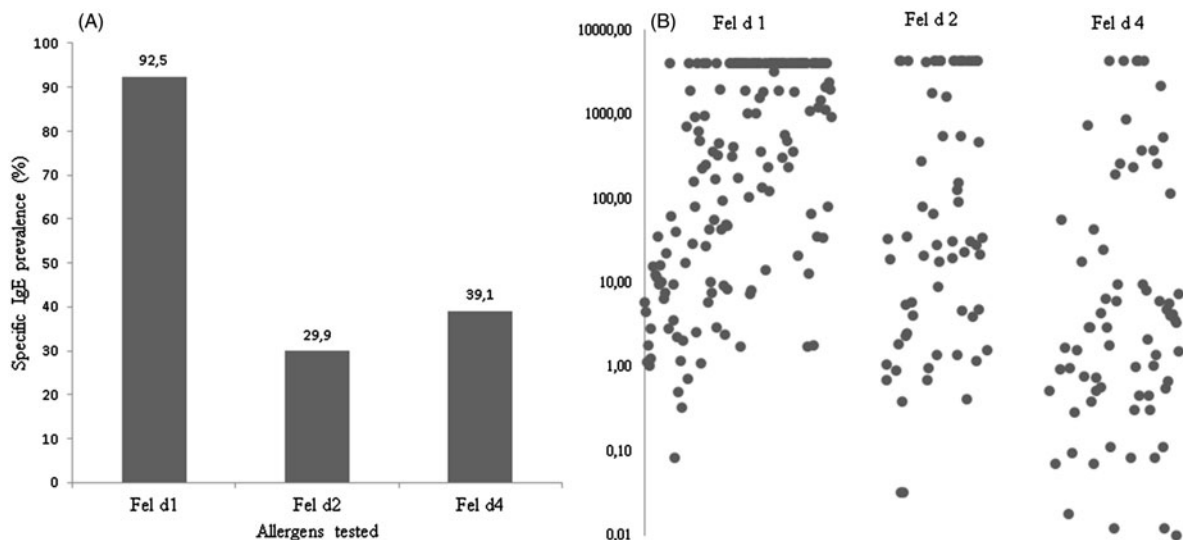


Figure 1. (A) Prevalence of individual cat allergen-specific IgEs (sIgEs) in serum samples from 174 patients determined by ImmunoCAP as having positive reactions to cat dander extract. (B) Levels of sIgEs to the cat allergenic components Fel d 1, Fel d 2 and Fel d 4.

(more than 4000 IU/ml) were detected in 59 samples (37% of all Fel d 1-positive patients), while similarly high IgE levels to Fel d 2 and Fel d 4 were obtained in 16 and 6 serum samples, respectively.

Different combinations of immune responses to cat allergenic components were analysed (Figure 2). Among the serum samples that were positive in the ImmunoCAP test, exclusively anti-Fel d 1 IgE was detected in 47% (82 of 174); only anti-Fel d 2 IgE was detected in 2% (4 of 174), and only anti-Fel d 4 IgE was detected in <2% (3 of 174). In 16% of the serum samples (28 subjects), IgEs to all three investigated proteins were detected. In 19% of the samples (34 patients), a combination of anti-Fel d 1 and anti-Fel d 4 IgEs were detected, and 10% (17 patients) were responsive to a combination of Fel d 1 and Fel d 2. Three of 10 samples negative for Fel d 1 had IgE reactions for both minor proteins, Fel d 2 and Fel d 4.

Among all Fel d 1-positive patients ($n=161$), 51% had anti-Fel d 1 IgE only, 17% also had anti-Fel d 2 IgE, 21% also had anti-Fel d 4 IgE, and 17% had all three groups of IgE. Among all Fel d 2-positive patients ($n=52$), 7% had anti-Fel d 2 IgE only, 33% also had anti-Fel d 1 IgE, 6% also had anti-Fel d 4 IgE, and 54% had all three groups of IgE. Among all Fel d 4-positive patients ($n=68$), 5% had anti-Fel d 4 IgE only, 50% also had anti-Fel d 1 IgE, 4% also had anti-Fel d 2 IgE, and 41% had all three IgEs.

Correlation between total IgE and specific IgEs to cat allergenic components

As the data were not normally distributed, to identify correlations between total IgE and sIgEs to cat allergenic components, Spearman's rank correlation test was used. We observed a low positive correlation between total IgE and sIgE to Fel d 1 (0.36; $p < .0005$) that was approximately the same in both gender groups (0.396 for female, 0.349 for male, $p < .005$). There was no significant correlation between Fel d 2 sIgE and total IgE levels. The correlation coefficient between Fel d 4 sIgE and total IgE levels was 0.25 ($p < .05$), but a significant difference in this parameter was observed between genders. Among females, the correlation coefficient was 0.173 ($p = .32$), which is considered negligible, whereas among males, the correlation coefficient was 0.36 ($p < .05$), which is considered a low positive correlation. To confirm the values, the data were reduced to a normal distribution using a base natural logarithmic transformation to perform Pearson's test. The new calculations affirmed the results of Spearman's rank correlation test. No correlation of total IgE levels and Fel d 2 sIgE was observed (0.23; $p = .096$). The correlation coefficient was 0.36 for Fel d 1 ($p < .0005$) and 0.304 for Fel d 4 ($p < .01$).

The mean total and sIgE levels did not differ by sex or age. We observed no correlation between Fel d 2 sIgE levels and other feline antigens. There was a low positive correlation (0.33, $p = .0005$) between Fel d 1 and Fel d 4 sIgE levels.

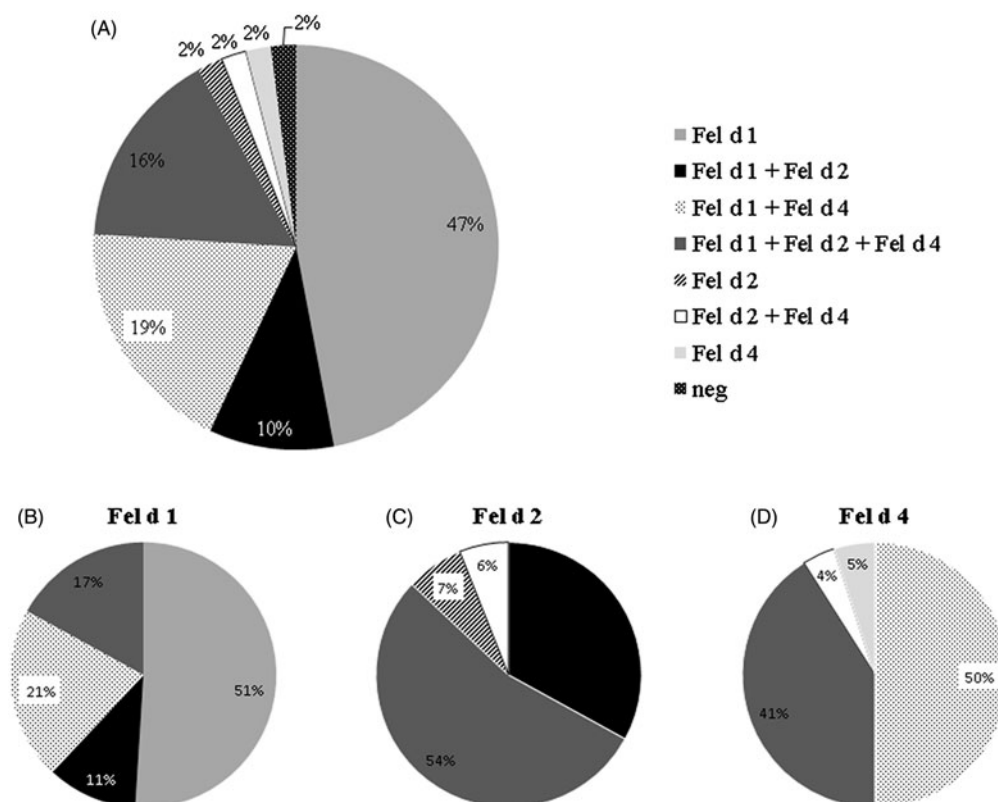


Figure 2. Combination of IgE responses to cat allergenic components. Circular diagrams representing the percentage of sIgE responses to different cat allergenic components and their combinations. (A) All positive samples (174); (B) Fel d 1-positive samples (161); (C) Fel d 2-positive samples (52); (D) Fel d 4-positive samples (68).

Table 1. Prevalence of specific IgEs against Fel d 1, Fel d 2 and Fel d 4 in sera from patients with cat allergy in different countries.

Fel d 1	Fel d 2	Fel d 4	Country			Year
95%	17%	–	The Netherlands	Competitive RIA	Positive RAST Result for cat dander	[35]
100%	35%	–	Austria	ELISA and immunoblotting	Cat-allergic patients suffering from atopic dermatitis or from allergic rhinoconjunctivitis	[26]
89%	–	63%	Australia	DELFA	Allergic to cats with Asthma and/or rhinoconjunctivitis	[36]
95%	–	–	Austria & Sweden	ELISA	Cat-allergic children with RC and/or asthma	[37]
45%	7%	17%	Australia	DELFA	Asthma and/or rhinoconjunctivitis, positive SPT or ImmunoCAP	[28]
100%	4%	34%	Sweden	ImmunoCAP ISAC	Children aged 6–18 years with severe or controlled asthma and positive ImmunoCAP	[38]
84%	2%	31%	Sweden	ImmunoCAP ISAC	Cat-allergic children (11–12 years) with ≥ 0.1 kUA/l	[22]
88%	22%	42%	Spain	ImmunoCAP	Patients with rhinitis/asthma who were sensitized to cat	[23]
94%	25%	49%	Poland	ImmunoCAP	Cat allergy	[31]
74%	3%	14%	Germany	ImmunoCAP	Positive SPT	[24]
91%	6%	17%			Positive SPT and positive anamnesis and/or a positive nasal allergen challenge	
~95%	~30%	~20%	Sweden	MeDALL chip	BAMSE study, positive ImmunoCAP	[32]
~90%	~11%	~37%	Northern Sweden	ImmunoCAP	19-year-olds, associations with asthma symptoms	[29]
93%	30%	39%	Russia	In-house microarray method	Positive ImmunoCAP	This study

Diagnostic characteristics

The diagnostic sensitivity for various combinations of allergens was determined compared with that of the ImmunoCAP system. Fel d 1 showed the highest diagnostic sensitivity, 0.93 (0.88; 0.96). The use of Fel d 1 in combination with Fel d 4 increased the sensitivity to 0.96 (0.92; 0.98), whereas the combination of Fel d 1 with Fel d 2 yielded a sensitivity of 0.97 (0.93; 0.99). The use of all three allergens resulted in 0.98 (0.95; 1.00) diagnostic sensitivity (171/174). Of 56 samples in the non-allergic group, 55 were characterized as negative, corresponding to 0.98 (0.90;1.00) diagnostic specificity. Thus, when all three proteins were used, the component-resolved test achieved diagnostic characteristics comparable to those of ImmunoCAP with a correlation coefficient of 0.94 and Fisher's exact test p -value = .91. Moreover, according to Pearson's chi-squared test (χ^2), the differences between the methods were not significant, with positive predictive value PPV = 0.99 (0.97; 1.00) and negative predictive value NPV = 0.95 (0.86; 1.00).

Discussion

The commonly used methods of allergy diagnosis are based on the application of allergen extracts. The disadvantage of allergen extracts is that the primary source of sensitization cannot be differentiated from cross-reactivity to allergens from different allergen sources [19]. Component-resolved allergy diagnostics may represent a solution to these problems. Furthermore, the application of extracts in AIT treatment also has a number of caveats. There is a high probability that patients sensitized only to minor allergens would be unable to receive sufficient amounts of allergen for a successful outcome of allergen immunotherapy [20]. Thus, molecular based-allergy diagnostics has the potential to be

performed before initiating treatment to prevent these problems in the future.

Cat dander extract consists of several allergens. Fel d 1 is the major cat allergen [21]. Although Fel d 1 is responsible for the majority of IgE reactivity in cat-allergic patients, additional individual allergens should be used to increase the sensitivity of *in vitro* diagnostics. Moreover, cosensitization of different allergen molecules may serve as an indicator of asthma (Fel d 1 and Fel d 4) [22,23] or atopic dermatitis (Fel d 4 and/or Fel d 2) [24–26]. In the current study, we investigated the patients' sensitization profiles to the three proteins of interest (Fel d 1; Fel d 2 and Fel d 4). The percentage of patients suffering from cat allergy that are sensitive to other cat proteins is considerably lower than the percentage of patients that are sensitive to these three. The total prevalence of sensitization was 92.5% for Fel d 1, 29.9% for Fel d 2, and 39.1% for Fel d 4 (Figure 1, Table 1). The average IgE reactivity to Fel d 1 and Fel d 2 was significantly higher than that to Fel d 4. However, there was no strong correlation between total IgE and sIgEs to Fel d 1, Fel d 2 and Fel d 4, in contrast to some other allergy types, such as house dust mite allergy [27]. There was absolutely no correlation between total IgE and sIgE towards Fel d 2 and a low positive correlation between Fel d 1 sIgE and total IgE (0.36; $p < .0005$). An interesting pattern was observed for the Fel d 4 protein. There was no obvious correlation between Fel d 4 sIgE and the total IgE level (0.25; $p < .05$), but the correlation increased to 0.36 when only males were considered ($p < .05$). The apparent correlation in males but not females is attributable not only to gender but also differences in age. The mean ages of the two groups differed significantly, with values of 14 years for males and 21 years for females ($p = .0019$), and the difference between the median values was even larger (7 and 23 years, respectively). The correlation between sIgE levels for each protein was also rather low. However, consistent with other component-

resolving cat allergy studies, the best correlation was between Fel d 1 and Fel d 4 sIgE levels, with a correlation coefficient of 0.33 ($p = .0005$) (0.4 in [28]; 0.33 in [29] studies).

Although we did not observe significant differences in mean total and sIgE levels by sex or age, other studies have shown that total IgE and sIgE levels increase with age until a certain age and then slowly decrease, with specific trend patterns for each allergen. For example, the maximum level of sIgE to cat dander extract decreases from 15–30 years of age [30]. In addition, between 4 and 16 years of age, levels of sIgEs to the individual cat antigens Fel d 1, Fel d 2 and Fel d 4 increase [31]. The sample sizes in these studies were larger than that of the present study, and thus, our sample size may have been insufficient to observe such a correlation.

We identified IgE responses to different combinations of cat allergens (Figure 2). Among positive samples in our study, 47% contained only anti-Fel d 1 sIgE, consistent with previous data (46.2%; [31]). The percentage of serum samples containing sIgEs to the single allergens Fel d 2 or Fel d 4 did not exceed 2%, and no more than 6% of samples contained sIgEs to both Fel d 2 or Fel d 4 in the absence of Fel d 1 sIgE, consistent with previous research [32]. Fel d 2 and Fel d 4 sIgEs were more commonly identified together with Fel d 1 sIgE. According to the literature, approximately 86% of Fel d 4 sIgE-positive samples also contain sIgE to Fel d 1 [22,23], consistent with the value of 91% obtained in the current study. Anti-Fel d 1 sIgE was found in combination with anti-Fel d 4 IgE nearly twice as often as with anti-Fel d 2 (19% and 10%, respectively). sIgEs for all three antigens were detected in 16% of the samples. These data will be useful for future studies of the association between the component-specific IgE profile and diagnosis of allergic disease. The MeDALL chip study demonstrated that sIgEs to individual cat allergen molecules in serum samples collected from preschool- or school-age children are a more specific predictive risk marker for the development of allergy to cat at 16 years of age than IgE to cat dander extract [32].

Our data indicate that the profile of sensitization to cat dander components in Russia is quite similar to those observed in studies conducted mainly in countries with temperate climates and in which samples were collected from a broad age range group rather than only from children and adolescents (Table 1). In our study, we showed that Fel d 1 protein alone is far from optimal for high diagnostic sensitivity, but the of all three allergens results in 98% sensitivity (compared with ImmunoCAP) (Figure 2). According to statistics analysis, the differences between the diagnostic characteristics of microarray method developed during this study and the ImmunoCAP method were not significant, with PPV = 0.99 and NPV = 0.95. These predictive values appeared to be even more statistically significant than the divergence between the ISAC IgE test and the extract-based singleplex ImmunoCAP [33]. Seven of 56 patients with a negative reaction to cats had a positive reaction to dog (class ≥ 1 , ImmunoCAP). This result suggests a lack of cat–dog cross-reactivity [34].

In conclusion, this demonstrates that the combination of all three proteins (Fel d 1, Fel d 2 and Fel d 4) can be used as a marker of sensitization to cat dander, and the diagnostic value of this combination in *in vitro* molecular (serological) diagnostics is equivalent to that of cat dander extract. This study is also the first to examine the sIgE prevalence for the three individual cat allergens among Russian cat-allergic patients sample. The component-resolved approach used in this study provides an opportunity to predict disease course and outcome and may also be useful to prescribe adequate AIT treatment and for monitoring patients undergoing immunotherapy in the future. Moreover we found that total IgE production correlates with sIgE anti Fel d 1 and Fel d 4 (only in male group) but not with Fel d 2. So it is seems that there is distinction between Fel d 2 and other Feline sIgEs formation.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- [1] Ichikawa K, Iwasaki E, Baba M, et al. High prevalence of sensitization to cat allergen among Japanese children with asthma, living without cats. *Clin Exp Allergy*. 1999;29:754–761.
- [2] Lau S, Illi S, Sommerfeld C, et al. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group. *Lancet*. 2000;356:1392–1397.
- [3] Plaschke P, Janson C, Norrman E, et al. Skin prick tests and specific IgE in adults from three different areas of Sweden. *Allergy*. 1996;51:461–472.
- [4] Freidhoff LR, Meyers DA, Bias WB, et al. A genetic-epidemiologic study of human immune responsiveness to allergens in an industrial population: I. Epidemiology of reported allergy and skin-test positivity. *Am J Med Genet*. 1981;9:323–340.
- [5] Freidhoff LR, Meyers DA, Marsh DGA. Genetic epidemiologic study of human immune responsiveness to allergens in an industrial population. 2. The associations among skin sensitivity, total serum IgE, age, sex, and the reporting of allergies in a stratified random sample. *J Allergy Clin Immunol*. 1984;73:490–499.
- [6] Roost HP, Kunzli N, Schindler C, et al. Role of current and childhood exposure to cat and atopic sensitization. European Community Respiratory Health Survey. *J Allergy Clin Immunol*. 1999;104:941–947.
- [7] Elholm G, Linneberg A, Husemoen LLN, et al. The Danish urban-rural gradient of allergic sensitization and disease in adults. *Clin Exp Allergy*. 2015;46:103–111.
- [8] Perzanowski MS, Rönmark E, Platts-Mills TA, et al. Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. *Am J Respir Crit Care Med*. 2002;166:696–702.
- [9] Lau S, Illi S, Platts-Mills TA, Multicentre Allergy Study Group, et al. Longitudinal study on the relationship between cat allergen and endotoxin exposure, sensitization, cat-specific IgG and development of asthma in childhood: report of the German Multicentre Allergy Study (MAS 90). *Allergy*. 2005;60:766–773.
- [10] Amirov NH, Tsibulkina VN, Hakimova RF. Regional peculiarities of allergic diseases in children. *Kazanskiy Meditsinskiy Zhurnal*. 2004;5:327–331. [In Russian]
- [11] Manzhos MV, Zheltikova TM, Fedenko ES, Shkadov SA, Batanova VA. The prevalence and etiological characteristics of

- the allergic rhinitis in the Penza region. *Kazanskiy Meditsinskiy Zhurnal*. 2008;5:627–630. [in Russian]
- [12] Macharadze DSH, Sepiashvili RI. Pilot-screening of allergic diseases at the medical examination of schoolchildren in Moscow. *Allergol Immunol*. 2007;8:187–190. [in Russian]
- [13] Kamaltinova EM, Belonogova EG, Deev IA, et al. The prevalence of rhinitis and sensitization among schoolchildren living in Tomsk and Tomsk region. *Mother and Baby in Kuzbass*. 2012;1:38–42. [in Russian]
- [14] Macharadze DSH, Beridze VD. Allergies to pets: diagnosis and treatment. *Attending Doctor*. 2009;11:72–75. [in Russian]
- [15] Gusareva ES, Bragina EJ, Deeva EV, et al. Cat is a major allergen in patients with asthma from west Siberia, Russia. *Allergy*. 2006;61:509–510.
- [16] Vailes LD, Sun AW, Ichikawa K, et al. High-level expression of immunoreactive recombinant cat allergen (Fel d 1): targeting to antigen-presenting cells. *J Allergy Clin Immunol*. 2002;110:757–762.
- [17] Dolgova AS, Stukolova OA. High-fidelity PCR enzyme with DNA-binding domain facilitates de novo gene synthesis. *3 Biotech*. 2017;7:128.
- [18] Odunuga OO, Shazhko A. Ammonium sulfate precipitation combined with liquid chromatography is sufficient for purification of bovine serum albumin that is suitable for most routine laboratory applications. *Bio Chem Comp*. 2013;1:3.
- [19] Borres MP, Ebisawa M, Eigenmann PA. Use of allergen components begins a new era in pediatric allergology. *Pediatr Allergy Immunol*. 2011;22:454–461.
- [20] Barber D, Moreno C, Ledesma A, et al. Degree of olive pollen exposure and sensitization patterns. Clinical implications. *J Investig Allergol Clin Immunol*. 2007;17(Suppl 1):11–16.
- [21] Grönlund H, Saarne T, Gafvelin G, et al. The major cat allergen, Fel d 1, in diagnosis and therapy. *Int Arch Allergy Immunol*. 2010;151:265–274.
- [22] 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;26:557–563.
- [23] Uriarte S, Sastre J. Clinical relevance of molecular diagnosis in pet allergy. *Allergy*. 2016;71:1066–1068.
- [24] Eder K, Becker S, San Nicolás M, et al. Usefulness of component resolved analysis of cat allergy in routine clinical practice. *Allergy Asthma Clin Immunol*. 2016;12:58.
- [25] 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;43:1160–1170.
- [26] Reininger R, Swoboda I, Bohlew B, et al. Characterization of recombinant cat albumin. *Clin Exp Allergy*. 2003;33:1695–1702.
- [27] Kim HS, Kang SH, Won S, et al. Immunoglobulin E to allergen components of house dust mite in Korean children with allergic disease. *Asia Pac Allergy*. 2015;5:156–162.
- [28] Hales BJ, Chai LY, Hazell L, et al. IgE and IgG binding patterns and T-cell recognition of Fel d 1 and non-Fel d 1 cat allergens. *J Allergy Clin Immunol Pract*. 2013;1:656.
- [29] 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;138:1582–1590.
- [30] De Amici M, Ciprandi G. The age impact on serum total and allergen-specific IgE. *Allergy Asthma Immunol Res*. 2013;5:170–174.
- [31] Ukleja-Sokołowska N, Gawrońska-Ukleja E, Żbikowska-Gotz M, et al. Analysis of feline and canine allergen components in patients sensitized to pets. *Allergy Asthma Clin Immunol*. 2016;12:61.
- [32] Asarnoj A, Hamsten C, Wadén 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;137:813–821.
- [33] Huss-Marp J, Gutermuth J, Schäffner I, et al. Comparison of molecular and extract-based allergy diagnostics with multiplex and singleplex analysis. *Allergo J Int*. 2015;24:46–53.
- [34] Reininger R, Varga EM, Zach M, et al. Detection of an allergen in dog dander that cross-reacts with the major cat allergen, Fel d 1. *Clin Exp Allergy*. 2007;37:116–124.
- [35] van Ree R, van Leeuwen WA, Bulder I, et al. Purified natural and recombinant Fel d 1 and cat albumin in in vitro diagnostics for cat allergy. *J Allergy Clin Immunol*. 1999;104:1223–1230.
- [36] Smith W, Butler AJL, Hazell LA, et al. Fel d 4, a cat lipocalin allergen. *Clin Exp Allergy*. 2004;34:1732–1738.
- [37] Grönlund H, Adédoyin J, Reininger R, et al. Higher immunoglobulin E antibody levels to recombinant Fel d 1 in cat-allergic children with asthma compared with rhinoconjunctivitis. *Clin Exp Allergy*. 2008;38:1275–1281.
- [38] 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;25:187–192.