Eidukaite et al. World Allergy Organization Journal (2023) 16:100827
http://doi.org/10.1016/j.waojou.2023.100827

WORLD ALLERGY ORGANIZATION JOURNAL

Open Access

Molecular sensitization patterns to cat and dog allergens in Lithuanian children population

Audrone Eidukaite, MD, PhD[a], Evelina Gorbikova, MSc[a]*, Monika Miskinyte, MSc[c], Ieva Adomaite, MD[b], Odilija Rudzeviciene, MD, PhD[b], Almantas Siaurys, PhD[a] and Asta Miskiniene, PhD[c]

ABSTRACT

Background: Over the last few decades, there was observed an increase of asthma and allergic rhinitis cases caused by allergy to pets.

Objective: This study aimed to analyze molecular sensitization patterns to dog and cat allergens in Lithuanian children who were experiencing allergy-like symptoms.

Materials and methods: A total of 574 children (0–18 years) were tested for allergen-specific immunoglobulin E (sIgE) levels with ALEX^2 (ALEX$^{2®}$, Allergy Explorer Test System). Positive sera were further analyzed for sensitization to cat (Fel d 1, Fel d 2, Fel d 4, and Fel d 7) and dog (Can f 1, Can f 2, Can f 3, Can f 4, Can f 5, and Can f 6) allergen components.

Results: Two hundred forty-seven children tested positive (sIgE $\geq$0.3 kUA/L) to at least 1 dog or cat allergen component. There were 61.1% children sensitized to components from both sources, 29.2% – exclusively to cat, and 9.7% – to dog components. The major sensitizers were Fel d 1 (84.8%) and Can f 1 (59.4%). There were 42.9% patients sensitized to 3 or more different mammalian protein families and 40.4% – to 3 or more lipocalins. There were 5.7% of children sensitized both to Fel d 1 + Fel d 4 and Can f 1/2 + Can f 5, indicating the high risk of severe asthma. Monosensitization to Fel d 1 was the dominant pattern among Lithuanian children (26.3%).

Conclusion: The majority of children were cat/dog-polysensitized, although sensitization only to cat allergens was most observed. Extensive molecular profiling can be an useful tool for accurate true sensitization diagnosis and prognosis of disease severity.

Keywords: Allergens, Pets, Cross-reactivity, Component-resolved diagnostics, Sensitization

INTRODUCTION

In recent decades the number of allergic rhinitis and asthma cases caused by pet allergens has been increasing.[1–3] Allergens from these sources are among the most common inhalant allergens that cause allergic diseases.[4] Furry animal allergens affect not only pet owners but also those around them through indirect exposure.

[a]Centre for Innovative Medicine, Vilnius, Lithuania
*Corresponding author. E-mail: evelina.gorbikova@santa.lt
Full list of author information is available at the end of the article

http://doi.org/10.1016/j.waojou.2023.100827
Received 8 September 2022; Received in revised from 28 July 2023; Accepted 21 September 2023

Online publication date xxx

The distribution of pet allergens depends on factors, such as production, aero-dispersion, sedimentation, and passive transport with clothes and other items. These variables determine the presence of pet allergens in environments without pets and those where furry animals have not been present for a long time.[5] That said, animal allergens can be found in all furry animal-free environments. The amount of allergens in these environments may be sufficient to cause allergic symptoms in susceptible individuals.[1] Furry animal allergens can cause a great variety and severity of symptoms, ranging from minor discomfort associated with rhinitis and conjunctivitis to severe asthma that can develop into a life-threatening condition.

In Lithuania, more than half (52%) of the population and 60% of families with children are pet owners, most of which are dogs and cats. Such a high prevalence of furry animal ownership is suggestive of the high distribution of dog and cat allergens in all, even furry animal-free, environments and raises a question of the importance of sensitization to cats and dogs in our population.

The diagnosis of allergy to furry animals conventionally starts with a medical history, physical examination of the patient, and skin prick tests, followed by detection of sIgE in the sera.

Allergen extracts used to diagnose sensitization to cats and dogs have been used for decades. Still, they are not capable of differentiating the primary sensitization from cross-reactions; thus, they may lead to false positive results.[6] Moreover, allergen extracts may lack important allergen molecules, therefore providing false negative results.[7] In recent years, component resolved diagnostics proved to be a valuable tool in diagnosing allergic diseases.[8] Molecular allergy diagnosis provides an opportunity to know the comprehensive sensitization profile to pet allergens. Determination of sensitizing molecules can reliably explain the complex molecular background in polysensitized patients, distinguishing between true sensitization and cross-sensitization and providing physicians with valuable information on the risk of disease severity.[9,10]

Sensitization profiles to cat and dog allergen molecular components have not yet been explored in the Lithuanian population. This study aimed to analyze the molecular sensitization patterns to dog and cat allergens in Lithuanian children.

MATERIALS AND METHODS

A retrospective study of 574 children (0–18 years old) sIgE results, obtained via ALEX^2 (ALEX$^{2®}$ Allergen Explorer Test System), (Macro Array Diagnostics GmbH [MADx], Austria) technology, was conducted in Vilnius, Lithuania. The study participants were suspected of an atopic condition such as atopic dermatitis, food allergy, allergic rhinitis, or asthma. ALEX^2 Allergen Explorer was prescribed to further investigate these suspicions by the treating physician as a routine diagnostic testing solution. Our study sample included all sera samples that were tested in Vilnius University Children's Hospital outpatient clinics laboratory during the 2019–2020 time period. No specialized patient recruitment was conducted. The analysis of the 574 study participants ALEX^2 Allergen Explorer test results determined that 433 children's samples were positive for at least 1 allergen extract or any allergen component. Samples were considered positive if the sIgE level was $\geq$0,3 kUA/L. Data were categorized according to age groups: 0–4, 5–9, 10–14, and 15–18 years to enable sensitization pattern analysis in different age groups.

All positive samples were then selected for further analysis of patient sensitization patterns. The ALEX^2 Allergy Explorer is a novel and powerful multiplex sIgE detection platform, enabling simultaneous detection of sIgE antibodies against 117 allergen extracts and 178 molecular allergens. The ELISA based macroarray is the most extensive sIgE detection test to date. That being said, the ALEX^2 platform does not include cat and dog extracts. This allowed us to perform sensitization pattern analysis to the following allergens: cat allergen components Fel d 1, Fel d 2, Fel d 4, and Fel d 7; dog allergen components Can f 1, Can f 2, Can f 3, Can f 4, Can f 5, Can f 6, and Can f Fel d 1 like.

Statistical analysis was carried out by SPSS 28.0 statistics program and Microsoft Excel. Baseline and demographic characteristics were summarized by standard descriptive summaries. Chi-square test was used to identify statistically significant differences between age groups. Spearman's correlation analysis was used to identify possible trends of sensitization frequency. Interferential test results

were considered to be statistically signifficant with confidence interval was observed at 95% ($p \leq 0.05$). Our study did not involve any sensitive patient data (such as names, anamnesis, suspected allergic disease type, etc), and did not require any additional medical procedures; therefore, no specialized consent from the guardians was required. The study was approved by Vilnius Regional Biomedical Research Ethics Committee.

RESULTS

Four hundred thirty-three (75%) of study participants (age 0–18 years, 255 females and 178 males) were sensitized to at least 1 allergen extract or any allergen component. A high sensitization frequency to different inhalant allergens in Lithuanian children was observed: pollen (n = 287; 66.3%), dust mites (n = 191; 44.1%), and molds (n = 105; 24.3%). More than half of the children (n = 247; 57%) were sensitized to dog and/or cat allergens (146 M/101F). Children up to 4 years of age (aged 0–4 years) were more frequently sensitized to cat and/or dog allergens (n = 61; 41.8%) than to house dust mites (n = 23; 15.8%; $p < 0.01$) and molds (n = 25; 17.1%; $p < 0.01$). One hundred seventy-five (70.85%) out of 247 dog and/or cat sensitized children were sensitized at least 1 of the dog molecular allergens, and 223 (90.28%) were sensitized to at least 1 of the cat molecular allergens. Twenty-four (9.71%) children were sensitized exclusively to dog molecular allergens, 72 (29.14%) – to cat, and the vast majority of patients (n = 151; 61.13%) were sensitized to both cat and dog molecular allergens (Fig. 1).

One hundred eighty-nine (84.8%) out of Fel d positive children had elevated sIgE to Fel d 1 – cat molecular component. Can f 1 dog molecular allergen was present in 59.4% of the Can f positive patients. Forty-seven (26.9%) children were independently positive to Can f 4, 59 (33.7%) to Can f 5, 57 (32.6%) to Can f 6, and 83 (37.2%) to Fel d 7. The remaining allergens (Can f 2, Can f 3, Fel d 2, and Fel d 4) were verified as minor allergens (prevalence below 20%) in the whole study population (Fig. 2).

Sensitization to dog allergen molecules Can f 2, 3, and 4 was found to be highest in the youngest group of children 0–4 years (22.95%; 19.67%; 24.59% respectively), compared to the 15–18 year-old group (3.70%; 3.70%; 14.81% respectively). Statistically significant differences between sensitization rates in these age groups was found for Can f 2 and Can f 3 allergens ($p \leq 0.05$). The difference between these groups in regards to sensitization to Can f 4 was determined to be statistically insignificant ($p = 0.3$). In contrast, sensitization to Can f 5 molecular allergen increased with age and reached 44.44% in the 15–18-year-old group. A very weak, yet statistically significant correlation (rho = 0.32, $p < 0.01$) was observed. Although sensitization to Can f 5, Can f 2, Can f 3, and Can f 4 varies with age group, we cannot confirm that this variation is determined by age and not by individual patient characteristics and allergen exposure. This is an interesting observation in a retrospective study, but a prospective cohort study is required to determine the association of these allergens by age, especially for the Can f 5 allergen.

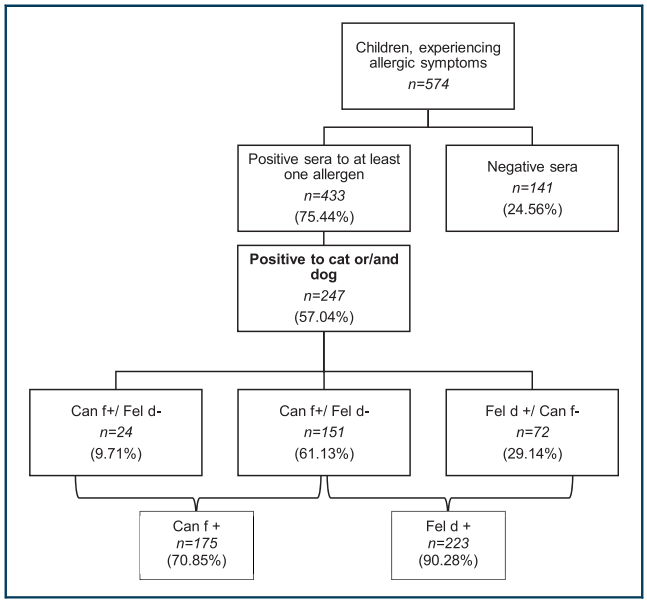

Fig. 1 Flow chart of the study design and sample sizes used as denominators for sensitization pattern determination

Prevalence of individual molecular profiles in dog and/or cat positive patients

In relation to the aggregation of allergens, the repertoire of molecules recognized by sIgE was widely pleomorphic for cat and dog allergens, including 96 distinct profiles in 247 subjects. The most frequently identified molecular profile (26.3%) was monosensitization to Fel d 1 molecular allergen

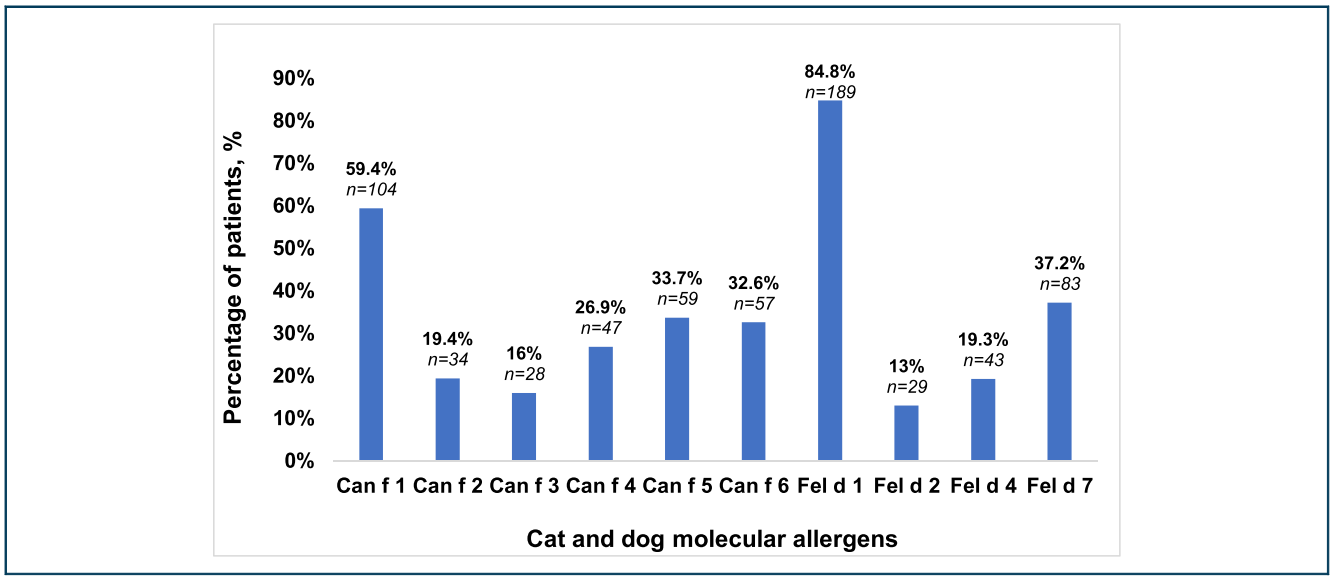

Fig. 2 Sensitization rates to cat and dog molecular allergens obtained from samples determined to be positive to Can f (n = 175) and Fel d (n = 223)

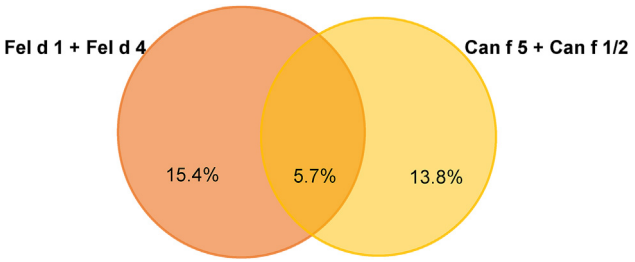

Fig. 3 Venn diagram of sensitization to cat and dog allergens associated with more severe asthma. Section A means co-sensitization to two cat allergens Fel d 1 and Fel d 4. Section B means co-sensitization to dog allergens Can f 5 and Can f1/2. Section C means co-sensitization to both cat and dog allergens

followed by sensitization to Fel d 1 and Can f 1 with cross-reaction to Fel d 7 (4.5%). Third place was taken by 2 quite different sensitization profiles: sensitization to species specific Can f 5 and Fel d 1 molecular allergens as well as sensitization to Can f 1 with cross-reaction to Fel d 7 was detected in 3.6% of all cat and dog sensitized individuals in our study. It is worth noting that a great number of patients 88 (37.6%) displayed sensitization profiles that were prevelant in <1% of the studied population, highlighting the pleomorphicity of sensitization profiles to these allergens in our population. Out of all dog sensitized patients, 17 (9.7%) were monosensitized to Can f 5 molecular allergen. True sensitization to dog allergens (positive to Can f 1 and/or Can f 5) was observed in 122 (69.7%) children sensitized to dog allergens. True sensitization to cat allergens (children positive to Fel d 1) was observed in 189 (84.8%) patients. True sensitization to both cat and dog allergens (positive to Can f 1 and/or Can f 5 and Fel d 1) was detected in 89 (36%) children sensitized to cat and/or dog allergens. Co-sensitization to Fel d 1 + Fel d 4 was found in 38 (15.4%) children sensitized to cat allergens. Co-sensitization to Can f 5 and Can f 1/2 was found in 34 (13.8%) children sensitized to dog allergens. Interestingly, co-sensitization to Fel d 1 + Fel d 4 + Can f 5 + Can f 1/2 was observed in 14 (5.7%) children sensitized to cat and dog allergens (Fig. 3).

The majority of all patients that were deemed to be sIgE positive (72.1%) were positive to 1 to 3 allergen components of cat and/or dog allergens (average = 2.96). Sensitization to 3 or more animal-derived components (kallikrein, lipocalins, secretoglobins, and serum albumins) was found in 42.9% of our positive study population (Fig. 4). One hundred forty-six (59.1%) children were sensitized to lipocalins. Most mammalian-sensitive patients (74.7%) were positive to 1 to 3 of the tested lipocalin components (average = 2.52). Of those positive to lipocalins – 59 (40.4%) children were sensitized to 3 or more cat and dog lipocalins (Fig. 5).

DISCUSSION

Although molecular allergy diagnostics has been commercially available for quite some time, it is still

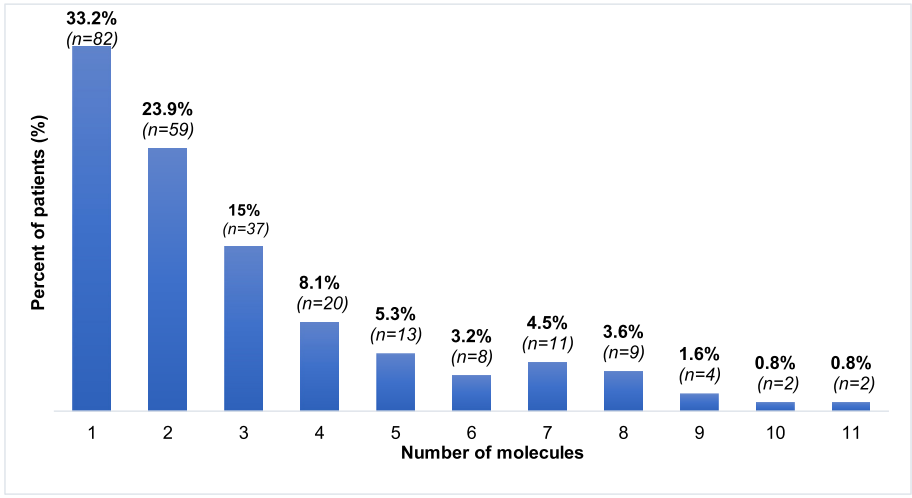

Fig. 4 Frequency of sensitized subjects to a different number of cat and dog molecules. Specific IgE ≥0.3 kUA/L were considered positive

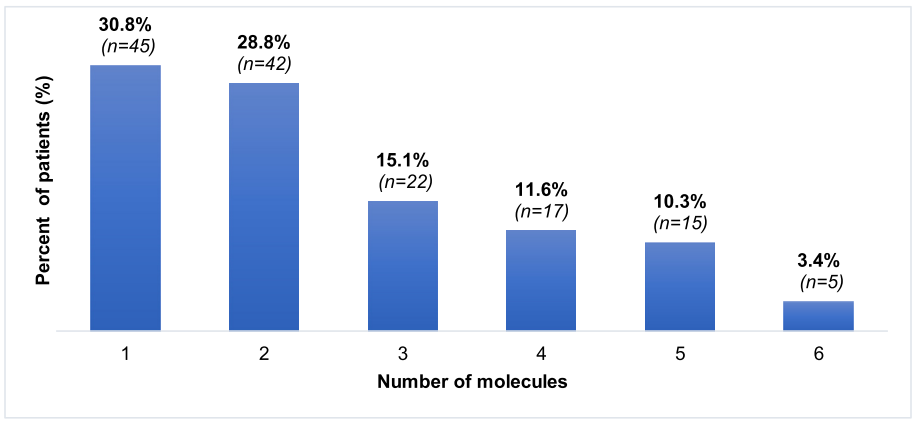

Fig. 5 Frequency of sensitized subjects to a different number of cat and dog lipocalin molecules. Specific IgE ≥0.3 kUA/L were considered positive

finding its footing in the standard clinical practice. Since the first commercial allergen component testing became available, it has significantly advanced the knowledge on differences of allergic disease manifestation and its dependence on patient molecular sensitization profile, as well as possible allergen immunotherapy (AIT) treatment outcomes.[6,17] Critical observations were made on regional differences in patient sensitization profiles that allow physicians to take a more targeted approach on patient diagnosis and treatment algorithms.[11] However, it is widely accepted that even with all the recent advances in knowledge and fast pace evolvement of the field of molecular allergology, the data on regional sensitization patterns, that would serve, at the very least, as a starting point of gaining more in depth understanding of the manifestation and evolution of allergic disease for the upcoming years, is still severely lacking.[13] The Lithuanian population is still one of the most understudied populations from a molecular sensitization point of view, with no published data on its molecular sensitization patterns. Our research paper is the first one, describing this understudied population, as well as one of the few analyzing sensitization to such a broad range of molecular pet allergens in a pediatric population.

In this study, we investigated sensitization status and molecular sensitization profiles of children experiencing allergy-like symptoms. Our study was focused on sensitization to pet allergens, such as cats and dogs. The ALEX^2 Allergen Explorer

multiplex allergy test system is a powerful diagnostic tool capable of simultaneously measuring total IgE and sIgE to 295 allergens (117 allergen extracts and 178 molecular components). This test provides an opportunity to view each patient's comprehensive sensitization profile in a clinical setting, distinguishing true sensitization from cross-reactions and giving much-needed insights into population-specific sensitization patterns. This test allowed us to analyze the frequency of sensitization to animal allergens compared to sensitization to other allergens in our population, as well as to identify the most common sensitization profiles to cat and dog allergens and determine the frequencies of sensitization to particular cat and dog allergen protein families.

It is common to assume that allergies to inhalant allergens are more likely to develop in older children.[11] However, we have determined a high sensitization rate to cat and/or dog allergens in children during early childhood. There were 41.8% small children (aged 0–4 years) sensitized to cat and/or dog allergens, and this sensitization rate exceeded sensitization rates to other important sensitizers such as mites and molds. Sensitization to cat and/or dog allergens was almost equivalent to sensitization to pollen allergens (41.8% vs. 42.5% in the 0–4-year-old group). Sensitization to cat and dog allergens increased with age but did not exceed pollen sensitization rates among older children. The highest sensitization rate to cat and/or dog allergens was observed in children in the 10–14-year-old group (70.2%).

Sensitization profiles in different populations vary, as sensitization to allergen components depends on cultural differences, environmental factors, rate of pet ownership, allergen source materials used for testing, and detection methods.[12,13]

Cat molecular allergens: sensitization rates and patterns

Our study showed similar results of rates and sensitization patterns to those observed in other research studies. In a comprehensive population-based study of animal component sensitization in Swedish schoolchildren, sensitization rates to cat and dog allergens were determined, and sIgE to Fel d 1 was detected in 83.7% of subjects, of which 67.8% were monosensitized.[14] Sensitization to Fel d 1 was observed in 84.8% of children, but fewer patients were monosensitized (26.3%) to this allergen component. The discrepancy in the results might be due to different numbers of cat allergen components tested in these studies (Fel d 1 and Fel d 4, versus Fel d 1, Fel d 2, Fel d 4, and Fel d 7 in our study). Results of the beforementioned study also showed a 31.3% sensitization rate to Fel d 4.86.2% of those sensitized to Fel d 4 displayed co-sensitization to Fel d 1. Our results slightly differ from the ones of Bjerg et al. Sensitization to Fel d 4 was observed only in 19.3% of children, but a similar percentage of these patients had Fel d 4 + Fel d 1 co-sensitization (88.4%).[14] Furthermore, our study's sensitization rate to Fel d 4 was significantly lower than the literature portrayed average of 61–63%.[4] We cannot confidently speculate if such a difference is seen because the portrayed average was determined from more adult sensitization studies, and analysis of Lithuanian adults would show similar results, or if lower sensitization rates to Fel d 4 reflect the uniqueness of the whole Lithuanian population.

Dog molecular allergens: sensitization rates and patterns

As with cat molecular allergens, our study's dog molecule sensitization rates and patterns also shared similarities to those found by other research groups. A Swedish study showed sensitization frequency to Can f 1 to be 40.7%. We observed a slightly higher sensitization rate to Can f 1 in our children population (59.4%). Thus, our study would suggest Can f 1 to be a major allergen component. In contrast, in the above-mentioned Swedish study, no single dog allergen component reached a major allergen concept (prevalence more than 50%). There were 22% of Sweden's child population sensitized to Can f 2. Similar Can, f 2 sensitization frequency results were determined in our study at 19.4%. In our study, 33.7% of patients were sensitized to Can f 5, while the already discussed study found a rate of 46% in a Swedish pediatric population.[14]

Furthermore, an adult population study showed the sensitization rate to Can f 5 to be as high as 70%.[4] Our study did show sensitization to Can f 5

frequency rates increase with age. Studies showing a significantly higher prevalence among adults also support this theory.

However, we cannot confirm that this variation is determined by age and not by individual patient characteristics and allergen exposure. A prospective cohort study would be helpful to decide if Can f 5 is genuinely a later-onset sensitization molecule. Can f 5 is prostatic kallikrein isolated from the urine of male dogs; this allergen is significant as it can be associated with more severe symptoms of the disease. Sensitization to Can f 5 is related to symptoms of asthma and rhinoconjunctivitis and may contribute to polysensitization in individuals with sIgE response to many animal allergen molecules. Due to cross-reactivity with prostate-specific human sperm antigen, sensitization to Can f 5 may lead to sexual dysfunction, such as infertility or allergic symptoms, including local or even anaphylactic reactions.[15]

Sensitization patterns as predictors of disease severity

Over recent years, molecular sensitization profiling has also been linked to disease severity. That being said, extensive molecular profiling might become an even more critical tool for physicians in predicting possible disease development and the need for preventive preclinical actions. A study analyzing 294 children and adults with suspected allergic rhino-conjunctivitis or asthma and sensitization to cats, dogs, and horses showed that the clinical relevance of sensitization has increased with the number of recognized dog allergens.[16] Progression of allergic sensitization, described as detecting sIgE to an increasing number of molecular components from the same sensitizing allergen source, has been shown to correlate with disease severity.[17] Käck et al showed an association between sensitization to an increasing number of dog allergen components and a positive nasal challenge result.[18] Nordlund et al showed that polysensitization to 3 or more mammalian protein families (kallikrein, lipocalins, secretoglobins, and serum albumins) was associated with severe asthma and increased bronchial inflammation, and a trend toward more courses of oral corticosteroid treatment.[19] Our study showed as many as 42.9% of our study population to be sensitized to 3 or more animal-derived molecular components. According to the studies mentioned above, these 42.9% of patients might be at an increased risk of developing asthma. Later studies by Nordlund et al, where children were recruited from the Swedish Nationwide Study on severe childhood asthma, extended and refined those results by exploring the sensitization pattern exclusively in children allergic to furry animals. According to their research, sensitization to lipocalins seemed to be associated with this cohort's most severe disease exhibition. Polysensitization to 3 or more lipocalins was more frequently detected in patients with severe asthma.[9] Our study results showed that 59 (40.4%) children were sensitized to 3 or more cat and dog lipocalins. It might be that these patients are at higher risk for more severe diseases. However, these speculations need further prospective studies.

In assessing the risk of more severe allergic disease, some associations with the diagnosis of the more severe disease have been observed with molecular component co-sensitization cases. A West Sweden Asthma Study of a random population-representative sample of adults showed that sensitization to furry animal allergen components is an essential predictor of asthma, rhinitis and an indicator of disease severity. Polysensitization patterns and clusters were associated with a substantially increased risk of asthma, rhinitis, and concomitant asthma and rhinitis. Sensitization to Fel d 1, Can f 1, Can f 2, and Can f 3 and polysensitization were markers of asthma severity with increased blood eosinophils, fractional exhaled nitric oxide, and airway hyperreactivity.[20] A recent study reported that asthmatic children with sensitization to Fel d 2 or Fel d 4 or Fel d 4 + Fel d 7 were more likely to have persistent type 2 inflammation; on the other hand, sensitization to Fel d 7 molecular allergen on its own has shown no independent associations with inflammatory outcomes.[21] In our study population, 29 (13%) of the patients had sensitization to Fel d 2, 43 (19.3%) to Fel d 4, and 26 (11.7%) had co-sensitization to Fel d 4 + Fel d 7. As sensitization to these allergens overlaps, susceptibility to Fel d 2 or Fel d 4 or Fel d 4 + Fel d 7 with a risk of persistent type 2 inflammation has been identified in 61 (27.4%) individuals.

A study of 696 Swedish children allergic to cats found that diagnosis of asthma and asthma

symptoms after contact with cats was associated with Fel d 1 + Fel d 4 co-sensitization.[14] In our study, 38 (15.4%) children were identified as Fel d 1 + Fel d 4 co-sensitized and might have an increased risk of asthma based on this study. In the same study, most dog-sensitized children were sensitized to more than 1 dog component, and Can f 5 + Can f 1/2 co-sensitization conferred the greatest risk for asthma.[14] Our study results revealed that 34 (13.8%) children that were co-sensitized to Can f 5 and Can f 1/2 might have an increased risk of asthma. Considering these 2 risk factors of co-sensitization, we believe that patients displaying sensitization to both of these profiles may have an even greater risk of asthma. Fourteen (5.7%) patients in our population were co-sensitized Fel d 1 + Fel d 4 + Can f 5 + Can f 1/2. Although currently, there is no literature data to support or reject our theory, we believe it would be worthwhile to investigate the severity of the disease in patients with this particular sensitization profile. After evaluating all the risk factors presented in the available literature, we found that an average of 24.7% of our population might have an increased risk of asthma among patients sensitized to cat and/or dog allergens.

CONCLUSION

This is the first study examining cat and dog molecular component sensitization patterns in the Lithuanian population. To our knowledge, this is also the first study in which sensitization to such a broad spectrum of allergen components (Can f 1, Can f 2, Can f 3, Can f 5, Can f 6, Can f Fel d 1 like Fel d 1, Fel d 2, Fel d 4, and Fel d 7) has been analyzed in children. Our study shows a high rate of children's sensitization to inhalant allergens, and sensitization to dog and/or cat allergens to be prevalent sensitizers. This study also supports the need for broad-spectrum molecular allergen sensitization testing, as the sensitization profiles of our patients proved to be unique and wide-scattered. In conclusion, *in vitro* molecular allergy diagnostics is a fast pace evolving field. Such diagnostics might also prove itself to be a useful tool of prediction of clinical disease severity development, even in pre-clinical stages of sensitization; however further studies are required before implementing such predicatory measures in to everyday clinical practice.

Abbreviations
sIgE, specific immunoglobulin E.

Acknowledgements
We are appreciated all the patients and investigators who participated in our study.

Funding
This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Availability of data and materials
This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal.

Author contributions
Gorbikova Evelina collected and analyzed the data. Eidukaite Audrone interpreted the data and drafted the article. Miskinyte Monika analyzed the data and drafted the article. Adomaite Ieva made substantial contributions to acquisition of the data. Rudzeviciene Odilija made substantial contributions to acquisition of the data. Siaurys Almantas made substantial contributions to conception and design of the article. Miskiniene Asta made substantial contributions to acquisition of the data.

All authors' consent for publication
All the authors listed have approved the manuscript.

Ethics approval
This study was approved by the ethics committee of Lithuanian Bioethics Committee.

Competing interest
JSC "In Novum" is a local distributor company of ALEX^2® Allergy Explorer tests.

Author details
[a]Centre for Innovative Medicine, Vilnius, Lithuania. [b]Vilnius University, Faculty of Medicine, Institute of Clinical Medicine, Vilnius, Lithuania. [c]JSC "In Novum", Vilnius, Lithuania.

REFERENCES

1. Munir AKM, Einarsson R, Schou C, Dreborg SKG. Allergens in school dust: I. The amount of the major cat (Fel d I) and dog (Can f I) allergens in dust from Swedish schools is high enough to probably cause perennial symptoms in most children with asthma who are sensitized to cat and dog. *J Allergy Clin Immunol*. 1993;91(5):1067–1074.
2. Portnoy JM, Kennedy K, Sublett JL, et al. Environmental assessment and exposure control: a practice parameter-furry animals Classification of recommendations and evidence. *Ann Allergy Asthma Immunol*. 2012;108(4):223.e1–223.e15.

3. Grönlund H, Saarne T, Gafvelin G, van Hage M. The major cat allergen, fel d 1, in diagnosis and therapy. *Int Arch Allergy Immunol*. 2010;151:265–274.
4. Dávila I, Domínguez-Ortega J, Navarro-Pulido A, et al. Consensus document on dog and cat allergy. *Allergy*. 2018;73(6):1206, 122.
5. Hentges F, Léonard C, Arumugam K, Hilger C. Immune responses to inhalant mammalian allergens. *Front Immunol*. 2014;5:234–241.
6. Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Gronlung H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy*. 1999;29(7):896–904.
7. 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(3):258–263.
8. Barber D, Diaz-Perales A, Escribese MM, et al. Molecular allergology and its impact in specific allergy diagnosis and therapy. *Allergy*. 2021;76(12):3642–3658.
9. Konradsen JR, Nordlund B, Onell A, Borres MP, Grönlund H, Hedlin G. Severe childhood asthma and allergy to furry animals: refined assessment using molecular-based allergy diagnostics. *Pediatr Allergy Immunol*. 2014;25(2):187–192.
10. Schoos AMM, Nwaru BI, Borres MP. Component-resolved diagnostics in pet allergy: current perspectives and future directions. *J Allergy Clin Immunol*. 2021;147(4):1164–1173.
11. Ansotegui IJ, Melioli G, Canonica GW, et al. IgE allergy diagnostics and other relevant tests in allergy, a World Allergy Organization position paper. *World Allergy Organ J*. 2020;13(2):100080–100130.
12. 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.
13. 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(10):1498–1506.
14. Bjerg A, Winberg A, Berthold M, Mattsson L, Borres MP, Rönmark E. A population-based study of animal component sensitization, asthma, and rhinitis in schoolchildren. *Pediatr Allergy Immunol*. 2015;26(6):557–563.
15. Liccardi G, Caminati M, Senna G, Calzetta L, Rogliani P. Anaphylaxis and intimate behaviour. *Curr Opin Allergy Clin Immunol*. 2017;17(5):350–355.
16. Hemmer W, Sestak-Greinecker G, Braunsteiner T, Wantke F, Wöhrl S. Molecular sensitization patterns in animal allergy: relationship with clinical relevance and pet ownership. *Allergy*. 2021;76(12):3687–3696.
17. 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(4):894–901.
18. Käck U, Asarnoj A, Grönlund H, et al. Molecular allergy diagnostics refine characterization of children sensitized to dog dander. *J Allergy Clin Immunol*. 2018;142(4):1113–1120.
19. 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(5):661–669.
20. 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*. 2019;7(4):1230–1238.
21. 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;48(9):1186–1194.