Molecular allergy diagnostics refine characterization of children sensitized to dog dander

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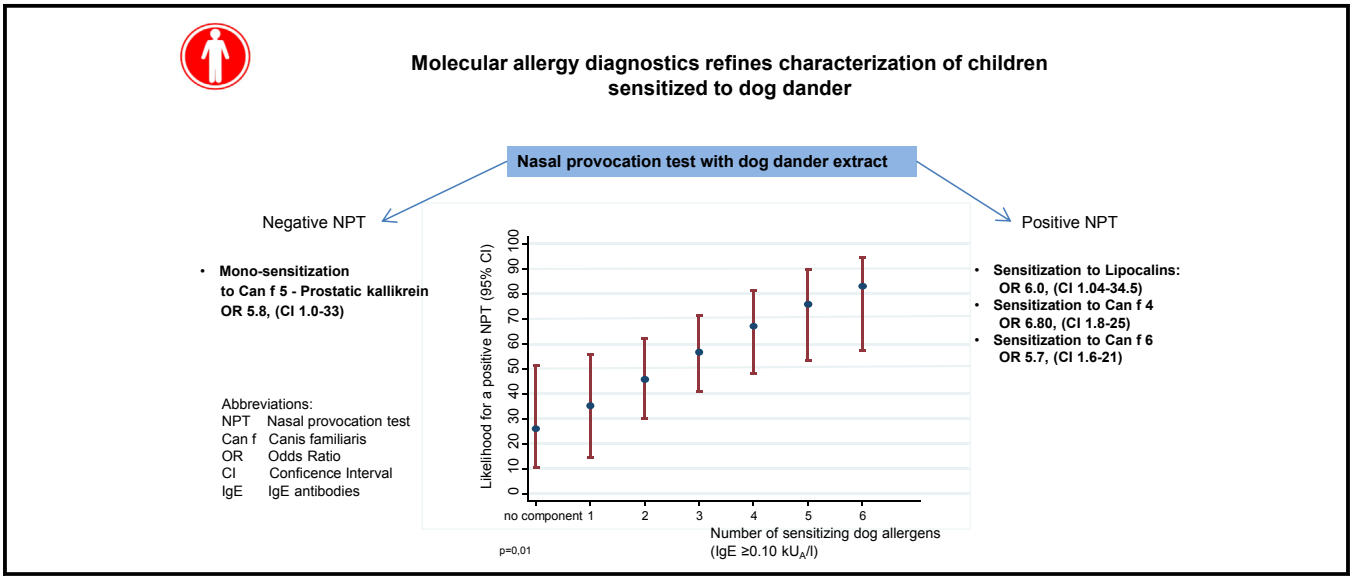
GRAPHICAL ABSTRACT



Background: Sensitization to dog dander is an important risk factor for rhinoconjunctivitis and asthma but is not sufficient for diagnosing dog allergy. Molecular allergy diagnostics offer new opportunities for refined characterization.

Objectives: We sought to study the association between sensitization to all presently known dog allergen components and clinical symptoms of dog allergy in children evaluated by using nasal provocation tests (NPTs).

Methods: Sixty children (age, 10-18 years) sensitized to dog dander extract underwent NPTs with dog dander extract. Measurement of IgE levels to dog dander and to Can f 1, Can f 2, Can f 3, and Can f 5 was performed with ImmunoCAP, and measurement of IgE levels to Can f 4 and Can f 6 was performed with streptavidin ImmunoCAP. An IgE level of 0.1 kU_A/L or greater was considered positive.

Results: There was an association between sensitization to an increasing number of dog allergen components and a positive nasal challenge result ($P = .01$). Sensitization to lipocalins (odds ratio [OR], 6.0; 95% CI, 1.04-34.5), in particular Can f 4 (OR, 6.80; 95% CI 1.84-25.2) and Can f 6 (OR, 5.69; 95% CI, 1.59-20.8), was associated with a positive NPT result. Monosensitization to Can f 5 was related to a negative NPT result (OR, 5.78; 95% CI, 1.01-33.0).

Conclusion: Sensitization to an increasing number of dog allergen components and to lipocalins is associated with dog allergy. Monosensitization to Can f 5 should not be regarded primarily as a marker for dog allergy. (J Allergy Clin Immunol 2018;■■■:■■■-■■■.)

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Dog allergy is one of the most common perennial airborne allergies among children.[1] Exposure to dogs causes not only rhinitis, asthma, or both but can also affect quality of life in the child with dog allergy.[2] Thus correct diagnosis and advice from the physician is essential.

Measurement of IgE antibodies to dog dander extract is an established diagnostic tool but has several limitations. Crude dog dander extracts have been shown to vary in allergen content, which can affect the accuracy of the test results.[3] Moreover, a positive test result might be due to cross-reactivity with allergens from other furry animals and hence might be of uncertain clinical relevance.[4]

The introduction of molecularly based allergy diagnostics offers new opportunities for improved characterization and management.[5] At the time of our investigation, there were 6 known dog allergens,[6] and sensitization seems to start early in life.[7] Four of these, Can f 1, Can f 2, Can f 4, and Can f 6, belong to the lipocalin protein family, which is present in dog dander, saliva, and urine.[8-10] Can f 1 is considered a major allergen and is detected in 50% to 90% of patients sensitized to dog.[5] Sensitization to Can f 2 and the more recently characterized Can f 4 and Can f 6 is less common. Can f 6 is highly cross-reactive with cat and horse lipocalins, whereas Can f 2 shows patient-dependent cross-reactivity with the cat lipocalin Fel d 4.[11-13] Can f 3, dog serum albumin, is a highly cross-reactive minor allergen detected in 15% to 35% of dog-sensitized patients.[4,14] IgE reactivity to Can f 5, the male dog's prostatic kallikrein, has been found in up to 70% of dog-sensitized subjects, among whom 38% were not sensitized to Can f 1, Can f 2, or Can f 3, suggesting that there might be subjects allergic specifically to male dogs.[15]

Can f 7, Canine NPC2 protein (dog epididymal secretory protein), was recognized as a dog allergen in 2016[16] after our study was conducted and is therefore not included in our analysis.

However, there is a need to further explore the clinical relevance of sensitization to different dog allergen components. The aim of this study was to analyze the patterns of IgE reactivity to dog allergen components among dog-sensitized children and, for the first time, to investigate the association between sensitization and clinical symptoms of dog allergy evaluated by using nasal provocation tests (NPTs) with dog dander extract.

Abbreviations used
NPT: Nasal provocation test
OR: Odds ratio
ROC: Receiver operating characteristic

METHODS

Patient population

A total of 105 patients were recruited from pediatric outpatient clinics in the Stockholm area. Inclusion criteria were age of 10 to 18 years and confirmed sensitization to dog dander based on a positive skin prick test response (wheal size > 3 mm), serum IgE antibodies to dog dander of greater than 0.10 kU_A/L, or both. The patients were included regardless of history of clinical symptoms to dog. Exclusion criteria were as follows: known impaired lung function of other cause than asthma and ongoing or completed allergen-specific immunotherapy to furry animals. Six patients did not meet the inclusion criteria, and 39 declined participation after receiving detailed information. A total of 60 patients were included.

Written informed consent was obtained from all participants and their parents. The study was approved by the regional board of ethics at Karolinska Institutet (Dnr 2014/1453-31/4).

Interviews and standardized questionnaires

All children and their parents were interviewed according to a modified version of the standardized questionnaire used in the Environmental and Childhood Asthma study.[17] The questionnaire included questions regarding demographic data, such as family history of allergy and asthma, exposure to dogs and other furry animals, history of asthma and rhinitis and other allergic manifestations, triggering symptoms, and medication and health care use.

NPTs

NPTs were performed with a commercially available dog dander extract, Aquagen 100,000 SQ-E/mL (ALK-Abelló, Copenhagen, Denmark), according to a modified Lebel protocol.[18] The dog dander extract used was analyzed concerning concentrations of specific allergens by using competitive inhibition ELISA and was found to contain all 6 investigated components (See Table E1 in this article's Online Repository at www.jacionline.org).

One spray dose, 0.1 mL of dog dander extract (10,000 SQ-E), was deposited in each nostril. Symptoms during NPTs were scored according to the Lebel scoring scale before and 5, 15, and 30 minutes after administration by a trained research nurse.[18] The scoring system identifies 3 cardinal symptoms: sneezing, rhinorrhea, and nose blockage, each graded on a scale from 0 to 3 points. In addition, nasal pruritus (1 point), ear pruritus (1 point), and eye symptoms (1 point) were registered. Maximum score was 12. According to Lebel et al,[18] a score of 5 or greater was considered positive, and a score of 2 or less was considered negative.

NPTs were performed in a 2-step manner. Patients scoring 4 or less and who were clinically unaffected/modestly affected 30 minutes after spray dose 1 proceeded to spray dose 2 (100,000 SQ-E) in each nostril, and the scoring procedure was repeated. Patients with a maximum score of 2 to 4 after the second step of the NPT procedure were considered to have indecisive results. Treatment with oral antihistamines was withheld for at least 72 hours, and nasal steroids were withheld for 2 weeks before the NPT. No patient showed symptoms of allergy or infectious rhinitis before the investigation.

Serologic analysis

Samples of venous blood were collected, and IgE antibodies against dog dander extract (e5), Can f 1, Can f 2, Can f 3, and Can f 5 were analyzed by using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). Can f 4 and Can f 6 were produced as recombinant proteins according to previously published data.[9,11] IgE levels to Can f 4 and Can f 6 were measured by coupling 5 μg of biotinylated antigen to streptavidin ImmunoCAP, as described by the manufacturer (Phadia AB/Thermo Fisher Scientific). All IgE determinations were analyzed by using the ImmunoCAP System (Phadia AB/Thermo Fisher Scientific), according to the manufacturer's instructions. Results are presented as kilounits of allergen per liter, where the cutoff for the presence of allergen-specific IgE was 0.10 kU_A/L or greater, which is the cutoff level in clinical settings.

Statistics

All statistical analyses were performed with Stata statistical software (release 14.2; StataCorp, College Station, Tex).

Categorical data were compared by using the χ^2 test or Fisher exact test, where appropriate. The t test on log-transformed values was used for group comparisons of IgE levels (continuous variables). Odds ratios (ORs) with 95% CIs for associations were calculated by using logistic regression analysis. Adjustments for the 3 dog allergen protein types (lipocalin, serum albumin, and kallikrein) were performed to determine possible independent markers