

Skin test sensitivity to mouse predicts allergic symptoms to nasal challenge in urban adults

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ABSTRACT

Epidemiologic studies have shown an association between mouse allergen exposure and asthma morbidity among urban populations, but confirmatory challenge studies in community populations have not been performed. This study was designed to examine the clinical relevance of mouse sensitization using a nasal challenge model. Forty-nine urban adults with asthma underwent skin-prick testing (SPT) and intradermal testing (IDT) with mouse epithelia extract. A positive SPT was defined as a net wheal size ≥ 3 mm and a positive IDT was defined as a net wheal size ≥ 6 mm using a 1:100 dilution of extract (1:10 w/v was obtained from Greer Laboratories (Lenoir, NC) as a single lot [Mus m 1 concentration = 2130 ng/mL]). Mouse-specific IgE (m-IgE) was measured by ImmunoCAP (Phadia, Uppsala, Sweden). Nasal challenge was performed with increasing concentrations of mouse epithelia extract and symptoms were assessed by visual analog scale. A positive challenge was defined as a 20-mm increase in the scale. The age range of the 49 participants was 18–50 years; 41% were men and 86% were black. Fourteen participants were SPT⁺ to mouse, 15 participants were SPT[−] but (IDT⁺), and 20 participants were negative on both SPT[−] and IDT[−] (SPT[−]/IDT[−]). Sixty-four percent of the SPT⁺ group, 40% of the IDT⁺ group, and 20% of the SPT[−]/IDT[−] group had a positive nasal challenge. Sixty-seven percent (10/15) of those who were either SPT⁺ or m-IgE⁺ had a positive nasal challenge. SPT or the combination of SPT plus m-IgE performed best in diagnosing mouse allergy. The great majority of mouse-sensitized urban adults with asthma appear to have clinically relevant sensitization. Urban adults with asthma should be evaluated for mouse sensitization using SPT or SPT plus m-IgE testing.

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Community mouse allergen exposure is widespread in the United States, with particularly high levels in inner-city homes.^{1–4} In addition, epidemiologic studies have found associations between higher mouse allergen exposure and poorer asthma outcomes among mouse-sensitized inner-city children.^{1,3} Thus, assessment of mouse sensitization may be an important part of the evaluation of patients with asthma, particularly those living in urban communities.

Although epidemiologic evidence suggests that mouse sensitization is associated with worse asthma outcomes in urban populations, whether direct airways exposure to mouse allergen elicits allergic symptoms in urban populations is unclear. In addition, there is little information about the diagnostic test performance of skin testing or specific IgE testing to mice in community populations

because the clinical relevance of mouse sensitization in urban populations is unclear. Furthermore, direct challenge studies showing clinical reactivity to mice have not been performed in this population. Because urban communities are the precise population in which assessment of mouse sensitization would be indicated, we evaluated the diagnostic test performance of skin-prick testing (SPT), intradermal testing (IDT), and mouse-specific IgE (m-IgE) testing in a population of Baltimore City adults with asthma.

METHODS

Study Population and Design

Baltimore City adults with asthma were recruited by advertisement and word of mouth. Recruitment occurred between April 2007 and September 2008. Two hundred three participants were screened for this study. The initial screening took place over the telephone. Participants who reported physician-diagnosed asthma, were between the ages of 18 and 50 years, and who had no other major exclusion criteria were scheduled for a screening clinic visit. Exclusion criteria included (1) active lung disease other than asthma; (2) current smoker; (3) severe or unstable asthma defined as requiring hospitalization in the previous 6 months, intubation in the previous 2 years, high-dose inhaled corticosteroids, or chronic oral steroids; and (4) preg-

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nancy or breast-feeding. During the screening clinic visit, a questionnaire was administered to collect sociodemographic information, pulmonary and allergic history, smoking history, occupational and environmental history, and family history. Screening study procedures included a questionnaire to assess mouse exposure and related allergic symptoms, spirometry, SPTs, end point skin test titration, and measurement of serum levels of m-IgE. Participants with forced expiratory volume in 1 second (FEV₁) of <70%, and FEV₁/forced vital capacity of <0.7, and positive SPT to a current pet were excluded from the study. Baltimore City participants were screened for symptoms and sensitization with the goal of identifying participants for each of the following categories: (1) positive mouse SPT (SPT⁺), (2) negative mouse SPT and a positive IDT (SPT⁻IDT⁺), (3) negative mouse SPT and a negative IDT (SPT⁻/IDT⁻). The study was approved by the Johns Hopkins School of Medicine Institutional Review Board, and written informed consent was obtained.

Evaluation of Mouse Sensitization

SPTs were performed with the MultiTest II device (Lincoln Diagnostics, Decatur, IL) to 14 allergens, including mouse epithelia, rat epithelia, cat, dog, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, German cockroach, American cockroach, oak, grass mix, *Alternaria* species, *Aspergillus* species, common ragweed, and *Cladosporidium* species. Histamine and glycerinated saline controls were also applied. A skin test response was considered positive if the net orthogonal wheal diameter was at least 3 mm. The net orthogonal wheal was calculated as the average of the longest diameter and the perpendicular diameter ([longest + perpendicular]/2).

For participants with a negative SPT response to mouse allergen, intradermal skin tests were applied with a 1:100 dilution. A positive IDT response was defined as a >6-mm wheal based on the definition used in previous study populations.^{5,6} For participants with a positive mouse SPT response, serial end point titration testing was performed to determine the starting dose for the nasal challenge.⁵ Aqueous mouse epithelia extract (1:10 w/v) was obtained from Greer Laboratories (Lenoir, NC), all of a single lot (Mus m 1 concentration = 2130 ng/mL; endotoxin concentration; 50 EU/mL). The m-IgE was quantified by using the ImmunoCap System (e72; Phadia, Uppsala, Sweden). A level of ≥ 0.35 kU/L was considered positive.

Nasal Challenge

The nasal challenge consisted of the intranasal delivery of diluent control and mouse allergen by means of 2 metered-dose sprays/nostril, 0.1 mL/spray (VP3 metered-dose spray device; Valois of America, Congers,

NY).⁵ After the starting dose, participants were then exposed every 15 minutes to twofold increasing extract concentrations until either a positive challenge or the maximum dose (full strength extract) was reached. Spirometry was performed before the nasal challenge and after completion of each nasal challenge dose. Nasal symptoms were assessed using a visual analog scale (VAS) 15 minutes after each dose. Our VAS rated overall nasal allergy symptoms. A positive challenge result was defined as a 20-mm increase above the baseline VAS value, as in a previous mouse nasal challenge study in a laboratory worker population.⁵

Statistical Analysis

Participants were stratified by skin test results into one of three groups: (1) SPT⁺, (2) IDT⁺, or (3) SPT⁻/IDT⁻. The primary outcome variable, nasal challenge outcome, was compared between groups using cross-tabulation and the χ^2 -test. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each diagnostic test. Receiver operator curves were used to evaluate test performance. All analyses were performed with StataSE 8.0 (Stata Corp., College Station, TX), and a two-tailed value of $p < 0.05$ was considered statistically significant.

RESULTS

Study Population

Forty-nine Baltimore City adults with asthma underwent nasal challenges: 14 were SPT⁺, 15 were SPT⁻ and IDT⁺, and 20 were SPT⁻ and IDT⁻ to mouse allergen (Table 1). The median (interquartile range) net wheal diameter among the SPT⁺ group was 4.5 mm (4.0–5.5 mm). Five of the study participants were m-IgE⁺. Eighty-six percent of the subjects were atopic; 54% had a positive skin test to cat, 47% to dust mite, 53% to cockroach, and 53% to at least one pollen. All study participants had physician-diagnosed asthma and 49% reported short-acting β -agonist use during this time period. Lung function indices were consistent with obstructive lung disease because the mean percent predicted FEV₁ was 75%. None of the participants had occupational exposure to mice. However, 77% of subjects reported seeing mice in their home, and 84% reported seeing evidence of mice such as droppings or chewed materials. A history of symptoms associated with mouse exposure was rare: two participants (4%) reported rhinitis and two participants (4%) reported itchy eyes.

Nasal Challenge Outcomes

Nineteen of the participants had a positive nasal challenge (Fig. 1). Symptoms elicited during positive challenge included nasal and ocular symptoms. No

Table 1 Study population characteristics (*n* = 49)

Characteristic	<i>n</i> (%)
Age (yr), mean ± SD	36 ± 8.5
Sex	
Male	20 (41)
Female	29 (59)
Race	
Black	42 (86)
White	7 (14)
Home mouse exposure	
Mouse infestation,* >1 yr	37 (77)
Evidence of mice,# >1 yr	37 (84)
Skin test and specific IgE status	<i>n</i>
SPT ⁺	14
SPT [−] /IDT ⁺	15
SPT [−] /IDT [−]	20
m-IgE ⁺	5
SPT ⁺ or m-IgE ⁺	15

**n* = 48.

#*n* = 44.

m-IgE = mouse-specific IgE; SPT = skin-prick testing; IDT = intradermal testing.

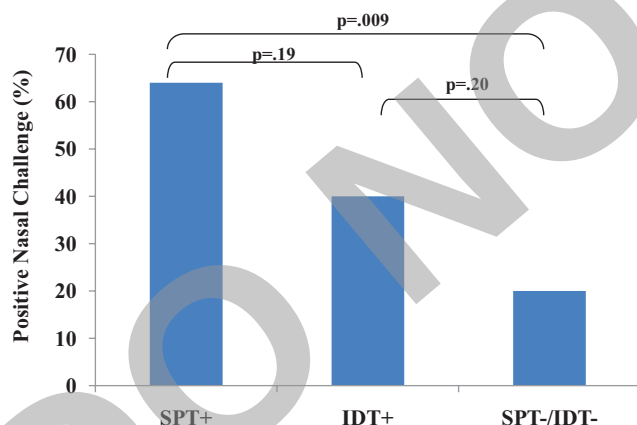


Figure 1. Nasal challenge outcome stratified by skin test status. The percentages in each skin test subgroup with a positive nasal challenge result are depicted on the y-axis. A positive nasal challenge is defined as a minimum 20-mm increase on the visual analog scale. SPT⁺ versus SPT[−]/IDT[−] (*p* = 0.009); SPT[−]/IDT⁺ versus SPT[−]/IDT[−] (*p* = 0.20); and SPT⁺ versus SPT[−]/IDT⁺ (*p* = 0.19).

systemic allergic symptoms were observed, but one patient experienced cough and a decrease in FEV₁ of >15% that was reversible with short-acting β -agonist. Of the two participants who reported having mouse-associated allergic symptoms, one had a positive nasal challenge.

Sixty-four percent of the participants who were SPT⁺ had a positive nasal challenge. Forty percent of participants who were IDT⁺ had a positive challenge (40%),

and 20% of those who were SPT[−] and IDT[−] had a positive nasal challenge. Only five participants were m-IgE⁺, three of whom had a positive challenge.

Comparison of Diagnostic Tests for Mouse Allergy

SPT had a sensitivity of 47% and specificity of 83% for mouse allergy, defined as a positive nasal challenge (Table 2). In this community-based population with asthma, the PPV of SPT was 64% and the NPV was 71%. IDT had a sensitivity of 60% and a specificity of 64%, and the PPV was 40% and the NPV was 80%. Compared with SPT, IDT had a higher false positive rate but a lower false negative rate.

The m-IgE had excellent specificity (93%) but poor sensitivity (16%) for the diagnosis of mouse allergy. The PPV for m-IgE was 60% and the NPV was 64%. However, these estimates are less reliable than those for SPT and IDT because a small number of participants were m-IgE⁺. A combination of SPT and m-IgE testing had a sensitivity of 53% and a specificity of 83% and a PPV of 67% and NPV of 74%. Comparison of the percentage correctly classified among diagnostic tests indicated that either SPT or the combination of SPT and m-IgE were the tests of choice for diagnosis of mouse allergy. SPT testing alone correctly classified 69% of study participants and the combination of SPT and m-IgE correctly classified 71% of study participants as mouse allergic or not mouse allergic.

DISCUSSION

This is the first study, to our knowledge, to assess the clinical relevance of mouse sensitization in a nonoccupational population using an allergen challenge model. Although previous epidemiologic studies have established strong associations between mouse allergen exposure and asthma morbidity among mouse-sensitized urban populations with asthma,^{1–3} there are scant allergen challenge data in this population that lend support to the epidemiologic findings. In this study, 67% of participants who were SPT⁺ or m-IgE⁺ developed allergic symptoms with a nasal challenge, providing strong corroboration of the epidemiologic findings. The results of this study also indicate that SPT or the combination of SPT and m-IgE are the tests of choice when assessing urban patients with asthma for mouse allergy.

Because mouse allergen exposure typically occurs indirectly in the community setting, it is very difficult for individuals from this setting to provide a reliable history of mouse-associated allergic symptoms (or absence of allergic symptoms). Despite the fact that our study population was composed almost entirely of individuals who denied mouse-associated symptoms, 67% of participants who were either SPT⁺ or m-IgE⁺ had allergic symptoms with nasal challenge. Although

Table 2 Comparison of diagnostic tests for mouse allergy in urban adults with asthma

	<i>n</i>	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Correctly Classified (%)	ROC
SPT ⁺ *	14	47	83	64	71	69	0.68
SPT ⁻ /IDT ⁺ #	15	60	64	40	80	63	0.60
SPT ⁺ or IDT ⁺ §	29	79	53	52	80	63	0.66
m-IgE ⁺	5	16	93	60	64	63	0.62
SPT ⁺ or m-IgE ⁺	15	53	83	67	74	71	0.70

*SPT⁺ versus IDT⁺ or IDT⁻.

#Versus IDT⁻.

§versus IDT⁻.

m-IgE = mouse-specific IgE; SPT = skin-prick testing; IDT = intradermal testing; NPV = negative predictive value; PPV = positive predictive value; ROC = area under the receiver operator curve.

a PPV of 67% is suboptimal performance for a diagnostic test, the fact that two-thirds of mouse-sensitized adults with asthma have allergic symptoms in a challenge setting highlights the importance of evaluating urban asthma patients for mouse sensitization.

Our findings that SPT or SPT plus m-IgE performed best in diagnosing mouse allergy are similar to findings from previous studies of diagnostic tests for cat allergy.⁶ In general, however, the diagnostic tests for mouse allergy in this urban community population performed less well overall than diagnostic tests for cat allergy⁶ or mouse allergy in a laboratory worker population.⁵ This is likely because of the fact that these previous studies have focused on populations that reported cat- or mouse-associated allergic symptoms, so that the underlying prevalence of true disease in these previous studies was likely higher than in our study population. Although PPVs and NPVs can vary depending on the prevalence of disease (*i.e.*, mouse allergy) in the population in which the diagnostic tests are being evaluated, sensitivity and specificity should be similar across study populations.

Although challenge studies have typically used patient-reported allergic symptoms as the gold standard comparator,⁵ this history is very difficult, if not impossible, to obtain in a community-based population with chronic, indirect mouse allergen exposure. It is important to note, however, that the nasal challenge methods used in this urban population were the same as those used in the laboratory worker population that reported mouse-associated symptoms, and for whom SPT performed well in predicting a positive challenge.⁵ In the clinical evaluation of urban community populations with asthma, assessment of mouse sensitization, and interpretation of these results, is done in the absence of a reliable history and our study was designed to reflect this scenario.

Four of the 19 subjects with a positive nasal challenge did not have any evidence of sensitization because they

had negative SPT, IDT, and m-IgE. The positive challenge responses among this subset of participants could be caused by an irritant effect or a nonspecific inflammatory response. Although our study used aqueous extracts for all skin testing and nasal challenges, so that any irritant effects could not be due to glycerin, other extract components might be responsible. Endotoxin has been shown to be associated with allergic-type symptoms in nonsensitized laboratory animal workers,⁷ so it is possible endotoxin in the extract may have resulted in the positive nasal challenges observed in the nonsensitized population. However, the endotoxin concentration in the lot of mouse epithelia extract used in this study was quantified and found to be low (50 EU/mL). This concentration resulted in a total delivered dose of endotoxin (20 EU in a total of 0.4 mL) that is ~200-fold lower than doses found to elicit nasal responses in other studies.⁸ Another potential explanation for the apparent false negative rate of SPT is local IgE production. Participants may not have detectable IgE to mouse allergen in the skin or blood but may have local IgE production to mouse allergen in the nose. However, another possibility is that these participants had false positive nasal challenges. This explanation is very unlikely because the false positive rate appears to be extremely low in this challenge model. Specifically, none of the nonsensitized laboratory workers who reported no mouse-associated symptoms had a positive nasal challenge in the earlier study.⁷

It is also possible that participants without any evidence of sensitization had a positive challenge because the extract was of a low potency, so that it had limited sensitivity for detecting low levels of IgE sensitization. The Mus m 1 content of the extract used in this urban population was similar to the content of the extract used in the laboratory worker population in which none of the SPT⁻/IDT⁻ participants had a positive challenge (2130 ng/mL versus 2369 ng/mL, respectively). It is important to note, however, that the neg-

ative control group in the laboratory worker population had acute and discrete periods of mouse exposure and reported no symptoms associated with the exposure. In contrast, in the current study's urban community population, the negative control group had asthma and resided in a community with endemic mouse allergen exposure, so they may have had asthma symptoms that were caused by chronic and indirect exposure. Furthermore, they may have been unable to identify exposure-related symptoms because of the indirect and chronic nature of their exposure. Development of a more potent extract and evaluation of diagnostic test performance using challenge models with objective outcomes are needed to resolve this discrepancy fully.

An unexpected finding in this study was that only 29% of subjects with a positive SPT had a positive m-IgE. In the laboratory worker population, a greater proportion of SPT⁺ participants had a positive m-IgE (67%), but this proportion was still less than reported for other allergens.^{6,9} One potential source of the discrepancy between prevalence of positive m-IgE and SPT results is the differences in the substrates used in these tests. For SPT, mouse epithelia extract was used, and for m-IgE testing, the mouse urine CAP (e72) was used. It is possible that the epithelia extract includes less well-characterized allergenic proteins that are not present or are present in lower concentrations in urine extracts and that this accounts for these differences in sensitivity. It is also possible that these differences in sensitivity between SPT and m-IgE are a result of the modest sample sizes. However, even with the modest sample size, these results suggest that the use of m-IgE testing alone may fail to identify a substantial proportion of patients with mouse sensitization, particularly among the urban populations with asthma. Specific IgE testing may still have a role in the diagnosis of mouse allergy, however, because it provides some benefit when used in conjunction with SPT.

In summary, urban patients with asthma should be evaluated by SPT or both SPT and m-IgE for mouse sensitization. Most patients with evidence of IgE sensitization using these tests will have clinically relevant sensitization and should be educated about environmental control practices to reduce mouse allergen exposure if they reside in mouse-endemic areas or report mouse infestation. Future studies should evaluate relationships between mouse sensitization and lower airways responses in environmental allergen challenges to develop a better understanding of the implications of mouse sensitization with respect to asthma.

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