Methods

Study Population

From March 2009 to June 2010, children in San Juan (Puerto Rico) were chosen from randomly selected households using a scheme similar to that of a prior study[1]. In brief, households in San Juan were selected by a multistage probability sample design. Primary sampling units (PSUs) were randomly selected neighborhood clusters based on the 2000 U.S. census, and secondary sampling units were randomly selected households within each PSU. A household was eligible if one or more residents was a child aged 6 to 14 years. In households with more than one eligible child, one child was randomly selected for screening[1]. On the basis of the sampling design, 7,073 households were selected, and 6,401 (90.5%) were contacted. Of these, 6,401 households, 1,111 had one or more children who were within the age range of the study and had four Puerto Rican grandparents. In an effort to reach a target sample size of approximately 700 children, we attempted to enroll a random sample (n=783) of these 1,111 children. Parents of 105 of these 783 eligible households refused to participate or could not be reached. There were no significant differences in age, sex, or area of residence between eligible children who did (n=678, ~87%) and did not (n=105, ~13%) agree to participate. Because we focused on allergic rhinitis and mouse allergen exposure, only those with non-missing data on allergy skin testing and Mus m 1 levels (n=511) were included in this analysis.

Study Procedures

Study participants completed a protocol that included questionnaires, allergy skin testing, and collection of dust samples. The child's parents completed two questionnaires used in

the Genetics of Asthma in Costa Rica Study[2]. These questionnaires were used to obtain information about the child's general and respiratory health, family history, socio-demographic characteristics, in utero smoke exposure, family history, and household characteristics.

Skin test reactivity (STR) to aeroallergens, histamine and saline diluent, was assessed using a Multi Test device (Lincoln Diagnostics, Decatur, IL) on the skin of the forearm (in a site free of eczema). Aeroallergens tested included house dust mites (*D. pteronyssinus* and *D. farinae*), *B. tropicalis*, German cockroach (*B. germanica*), mouse pelt, dog dander, cat dander, mold mix, *Alternaria tenuis*, mixed tree pollen, mixed grass pollen, mugwort sage, and ragweed (Alk-Abello, Round Rock, TX). A skin test was considered positive if the maximum wheal diameter exceeded the diluent wheal diameter by ≥3 mm.

Dust samples were obtained from three areas in the home: the one in which the child sleeps (usually his/her bedroom), the living room/television room, and the kitchen. The dust was sifted through a 50-mesh metal sieve, and the fine dust was reweighed, extracted, and aliquoted for analysis of allergens from mouse (mouse urinary protein [Mus m 1]), dust mite (*Dermatophagoides pteronyssinus* [Der p 1]), cockroach (*Blatella germanica* [Bla g 2]), dog dander (Can f 1), and cat dander (Fel d 1), as well as measurement of microbe-associated molecular patterns (MAMP) levels for glucan and peptidoglycan using two-site monoclonal antibody ELISA assays, and endotoxin using a modification of the Limulus Amebocyte Lysate assay[4]. Levels of allergens and MAMPs

in house dust samples were transformed to a log-10 scale.The highest level of each log-transformed allergen or MAMP was then used for the statistical analysis.

Written parental consent was obtained for participating children, from whom written assent was also obtained. The study was approved by the Institutional Review Boards of the University of Puerto Rico (San Jan, Puerto Rico), Brigham and Women's Hospital (Boston, Massachusetts), and the University of Pittsburgh (Pittsburgh, Pennsylvania).

Outcome Definition

A child was considered to have current symptoms suggestive of allergic rhinitis (AR) if there were affirmative answers to two questions (taken from the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire[4]): 1) Has your child ever had hay fever or a runny or stuffy nose accompanied by sneezing and itching at a time when he/she did not have a cold or flu?, and 2) Has your child had these symptoms in the last 12 months? AR was defined by 1) current symptoms suggestive of AR, and 2) skin test reactivity (STR) to ≥1 allergen. This definition is consistent with Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 guidelines[5]. Asthma was defined as physician-diagnosed asthma and wheeze in the previous year.

Statistical Analysis

Bivariate analyses of the covariates of interest and AR were conducted using Fisher's exact tests for categorical variables, and two-sample two-sided *t* tests for continuous variables. Logistic regression was used for the multivariable analyses. Because of their

known relation to AR, all models included age, sex, and type of health insurance (private/employer-based vs. others). Other covariates (see eTable 1) were also included in the initial multivariate models if $p \leq 0.20$ in bivariate analyses. These additional covariates remained in the final models if they were associated with AR at $p < 0.05$ or if they changed the parameter estimate (β) by $\geq 10\%$. The Pearson correlation coefficient was used to assess the relationship between levels of MAMPs and mouse allergen.

The covariates used in eTable 1 included baseline demographic characteristics, asthma status, atopic history, family history, home environment, allergen levels, and MAMP levels (see eTable 1). Parental education was dichotomized into at least one parent completed high school vs. none. Household income was dichotomized into above or below $15,000/year, as that was the approximate median income in Puerto Rico during the study. Eczematous rash referred to ever having a prolonged, itchy, scaly or weepy skin rash. All analyses were done using Stata/SE 12.1 for Windows (College Station, TX).

eTable 1. Characteristics of children included and excluded from analysis.

	Included (n=511)	Excluded (n=167)
Age, yrs (SD)	10.3 (.11)	9.9 (.21)
BMI, z-score (SD)	.62 (.05)	.43 (.12)
Male sex	53.1%	53.1%
Private / employer-based health insurance	33.1%	38.9%
Asthma	52.5%	49.4%
Parental history of allergic rhinitis	19.3%	17.6%
Shared bedroom	54.1%	56.2%
History of daycare attendance	22.0%	23.1%
Cat reported in home	11.1%	15.4%
Visible mold reported	40.6%	35.8%
Rats/mice reported	26.7%	29.0%
Leukotriene receptor antagonist use	13.2%	12.4%

*P<0.05 for included vs excluded.

References

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