Determinants of asthma after severe respiratory syncytial virus bronchiolitis

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Abstract

Background—The development of asthma after respiratory syncytial virus (RSV) bronchiolitis has been demonstrated in case-control studies, although the determinants of post-RSV asthma remain undefined.

Objectives—We sought to evaluate the potential determinants of physician-diagnosed asthma after severe RSV bronchiolitis during infancy.

Methods—We enrolled 206 children during an initial episode of severe RSV bronchiolitis at 12 months of age or less in a prospective cohort study and followed these children for up to 6 years. In a subset of 81 children, we analyzed CCL5 (RANTES) mRNA expression in upper airway epithelial cells.

Results—Forty-eight percent of children had physician-diagnosed asthma before the seventh birthday. Independent determinants significantly associated with increased risk for physician-diagnosed asthma by the seventh birthday included maternal asthma (odds ratio [OR], 5.2; 95% CI, 1.7-15.9; $P= .004$), exposure to high levels of dog allergen (OR, 3.2; 95% CI, 1.3-7.7; $P= .012$), aeroallergen sensitivity at age 3 years (OR, 10.7; 95% CI, 2.1-55.0; $P= .005$), recurrent wheezing during the first 3 years of life (OR, 7.3; 95% CI, 1.2-43.3; $P= .028$), and CCL5 expression in nasal epithelia during acute RSV infection (OR, 3.8; 95% CI, 1.2-2.4; $P< .001$). White children (OR, 0.19; 95% CI, 0.04-0.93; $P= .041$) and children attending day care (OR, 0.18; 95% CI, 0.04-0.84; $P= .029$) had a decreased risk of physician-diagnosed asthma.

Conclusions—Approximately 50% of children who experience severe RSV bronchiolitis have a subsequent asthma diagnosis. The presence of increased CCL5 levels in nasal epithelia at the time of bronchiolitis or the development of allergic sensitization by age 3 years are associated with increased likelihood of subsequent asthma.

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Respiratory syncytial virus (RSV) infection is nearly ubiquitous in early life, with nearly all children seropositive by age 2 years.[1] Although most children are asymptomatic or have only mild illness, some children require hospitalization because of severe respiratory symptoms. RSV bronchiolitis was the most common diagnosis for hospitalized infants less than 1 year old from 1998 to 2001, accounting for more than 70,000 hospitalizations annually.[2]

Long-term prospective case-control and cohort studies have also linked RSV bronchiolitis to the development of wheeze[3-6] and asthma[5,7-9] later in childhood. Studies by Sigurs et al[7] have demonstrated a 7.2-fold increased risk of asthma 18 years after severe RSV bronchiolitis,[7] although this might be related in part to a very low incidence of asthma in the control subjects. Epidemiologic studies suggest that the risk of asthma after bronchiolitis is related to episode severity, with children receiving hospital-based care experiencing a 2.8-fold increased risk of asthma by 4.5 to 5.5 years of age.[10]

CCL5, previously known as RANTES, is a β-chemokine chemoattractant for inflammatory cells, including T-lymphocyte subsets associated with allergic inflammation,[11] as well as eosinophils.[12] Previous studies have demonstrated increased CCL5 expression in airway epithelial and submucosal inflammatory cells of subjects with asthma.[13,14] CCL5 expression and transcription in human tracheal epithelial cells is induced by RSV *in vitro*.[15] Furthermore, mice infected with a related paramyxovirus, Sendai virus, demonstrate increased CCL5 expression along with greater airway resistance and methacholine hyperresponsiveness.[16] Therefore we sought to evaluate whether CCL5 expression in airway epithelial cells obtained from children with severe RSV bronchiolitis was predictive of the subsequent development of asthma.

We have assembled a cohort of 206 infants during an episode of severe RSV bronchiolitis and followed these children closely through their seventh birthdays to describe the prevalence of asthma and evaluate potential determinants of asthma after a severe RSV-related lower respiratory tract infection during this vulnerable time period.

METHODS

Study population

From 1998 to 2001, we enrolled 206 infants 12 months of age or less in the RSV Bronchiolitis in Early Life (RBEL) prospective cohort study. Selection of the study population and characteristics of the cohort at study entry are described in detail elsewhere.[17] Included infants were required to have bronchiolitis severe enough to require emergency department care or hospitalization, a positive nasopharyngeal swab result confirming infection with RSV, and physician-documented wheezing during the acute illness. Exclusion criteria were a history of previous wheezing or a diagnosis of asthma, congenital abnormalities of the heart and lung, cystic fibrosis diagnosed in the patient or immediate family, regular use of anti–gastroesophageal reflux medication, bronchodilators, or anti-inflammatory medications. A study coordinator collected detailed information about demographic factors, environmental exposures, and family history of respiratory and atopic disease from the child's parent or parents. This study was approved by the Washington University School of Medicine Institutional Review Board. Informed consent was obtained from parents or guardians.

Parent/guardian interviews

Telephone interviews of parents or guardians were conducted every 3 months to monitor children's respiratory symptoms (wheeze, cough, chest tightness, and shortness of breath), physician-diagnosed respiratory disease, and any change in environmental exposure or family history.

Allergy evaluation

We performed percutaneous allergy skin tests during the third year of follow-up (n = 150; mean age, 3.6 ± 0.7 years of age) using the MultiTest device. Additional details are included in the Methods section in this article's Online Repository at www.jacionline.org.

Total serum IgE levels were measured at study entry by using nephelometry. The percentage of eosinophils among peripheral white blood cells was measured at study entry with automated cell count methodology.

Nasal epithelial CCL5 expression

We obtained nasal epithelial cells (>90% cytokeratin positive) from nasal brushings from 81 subjects based on their consent to provide samples at entry and at 1 month. Cytospin preparations were prepared and hybridized to anti-sense and sense ^{35}S-labeled CCL5 cRNA. The samples were then exposed to a Phosphor screen and quantified for ^{35}S-labeled anti-sense RNA by using ImageQuant (Molecular Dynamics Storm, Sunnyvale Calif). A CCL5 ratio was calculated by subtracting sense RNA from antisense RNA ^{35}S signal and correcting for the total number of cells as follows:

$$(\text{Antisense} - \text{Sense}) \times 10,000/\text{cell count}.$$

Environmental assessment

Dust samples (n = 174) were collected from enrolled infants' homes within the first year of enrollment and analyzed, as described previously (see the Methods section in this article's Online Repository).[17]

Asthma outcomes

We defined the occurrence of a wheezing episode each time a parent/guardian answered yes to either "Has your child had wheezing with colds?" or "Has your child had wheezing without colds?" during each follow-up contact every 3 months.

We examined 2 asthma outcomes of differing stringencies: physician-diagnosed asthma and active asthma. A child was classified as having physician-diagnosed asthma if the parent/guardian answered yes to "Has your child ever been diagnosed with asthma by a physician?" at any time before the child's seventh birthday. A persistent negative response defined children without physician-diagnosed asthma. All children with at least 1 follow-up contact (201/206) were included in analyses by using data obtained at baseline as predictors of physician-diagnosed asthma. To analyze factors associated with physician-diagnosed asthma after age 3 years, we excluded children given a diagnosis of asthma before age 3 years, as well as those children with no follow-up contact after age 3 years.

We defined active asthma as physician-diagnosed asthma at any time along with parent-reported wheezing during the last year of follow-up between the child's third and seventh birthdays. For example, for subjects with a follow-up at age 5 years, active asthma was defined based on the active wheezing records for the 12 months preceding that last follow-up. Children without follow-up contact between the third and seventh birthdays were

excluded from analyses examining predictors of asthma outcomes obtained at either baseline or by age 3 years.

Statistical considerations and data analysis

Data are reported as means ± SDs or percentages (frequencies), as appropriate. Univariate and multivariate logistic regression models were used to explore potential relationships among demographic factors, CCL5 expression, environmental exposures, and the 2 asthma outcomes (physician-diagnosed asthma and active asthma). Variables with P values of less than .1 in univariate analysis were entered into multivariate models, and stepwise procedures were used to identify the specific factors that were independently associated with the 2 asthma outcomes. P values of .05 or less were considered statistically significant in the multivariate predictive models. Odds ratios (ORs) are presented with their 95% CIs. The Hosmer and Lemeshow goodness-of-fit test was applied to all of the logistic regression models and established the fit of all models we present. Preliminary analysis found a strong association between nonwhite race and baseline annual household income of $20,000 or less ($P <$ .0001); we thus included only nonwhite race in the multivariate model. IgE levels and PC_{20} values were log-transformed.

The association between CCL5 expression and asthma outcomes was explored separately because of the smaller sample size. Receiver operating characteristic (ROC) curves were generated for CCL5 expression to evaluate the sensitivity and specificity for predicting the 2 asthma outcomes. All analyses were performed with SAS 9 software (SAS institute, Inc, Cary, NC).

RESULTS

Of 206 children enrolled in the RBEL cohort, 201 (98%) had at least 1 follow-up contact during the study period. Fig 1 and the Results section in this article's Online Repository at www.jacionline.org show recruitment and retention over the course of the study. One hundred fifty-nine (77%) subjects provided data between ages 3 and 6 years. Children who did not provide data between ages 3 and 6 years did not differ from those with data in terms of age, sex, race, bronchiolitis severity, smoke exposure, IgE levels, eosinophil percentages, or nasal epithelial CCL5 levels, although there was a slightly higher, although not statistically significant, proportion of children with maternal asthma in the group followed through 6 years ($P =$ .054, see Table E1 in this article's Online Repository at www.jacionline.org). The baseline demographic characteristics of this population have been previously described (see Table E2 in this article's Online Repository at www.jacionline.org).[17]

Recurrent wheezing after severe RSV bronchiolitis was common —92% of participants experienced 1 or more additional wheezing episode before 3 years of age (Fig 2), and only 27% of participants did not experience another wheezing episode after 3 years of age. Nearly half (48%) of the study participants were given a diagnosis of asthma by a physician before the seventh birthday, and 35% were classified as having active asthma before the seventh birthday. Compared with children without asthma, children with physician-diagnosed asthma had similar measures of lung function (prebronchodilator FEV_1 percent predicted and FEV_1/forced vital capacity [FVC] ratio) and bronchodilator response at age 6 years (Table I). Although the prevalence and degree of airways hyperresponsiveness (defined as PC_{20} 3.2 mg/mL) were high in the population as a whole (n = 109 [84%]; mean PC_{20}, 1.80 ± 2.39 mg/mL), the prevalence of airways hyper-responsiveness did not differ between those with and without physician-diagnosed asthma (87.5% vs 81.5%, $P =$.35), and children with physician-diagnosed asthma demonstrated statistically greater mean airway hyperresponsiveness than those without physician-diagnosed asthma (PC_{20}, 1.42 ± 1.86 vs

2.19 ± 2.78 mg/mL; $P=.016$). There were no significant differences between participants with or without active asthma in terms of lung function or airway hyperresponsiveness by the seventh birthday (Table I).

Factors related to physician-diagnosed asthma by the seventh birthday

Univariate analyses—We examined the factors associated with a physician's diagnosis of asthma by the seventh birthday based on data available at 2 time points: baseline (enrollment) and age 3 years (Table II). By using unadjusted data obtained at baseline, factors significantly associated with physician-diagnosed asthma included white race (OR, 0.5; $P=.022$), longer birth length (OR, 0.91; $P=.025$), day care attendance before study enrollment (OR, 0.50; $P=.042$), maternal asthma (OR, 4.28; $P<.001$), and exposure to high levels of dog allergen in the home (OR, 2.05; $P=.035$). Among this cohort of children who required hospital care for bronchiolitis, neither factors reflective of severity of the initial bronchiolitis episode (lowest oxygen saturation, length of hospital stay, presence of hyperexpansion, or opacity on chest radiography) nor baseline laboratory data (peripheral blood eosinophil numbers or total IgE levels) were significantly associated with physician-diagnosed asthma. The nasal epithelial CCL5 ratio at baseline (in a subset of 81 participants) was significantly associated with physician-diagnosed asthma (OR, 3.81 per unit increase in CCL5 ratio; $P<.001$). In addition, at age 3 years, the occurrence of repeated wheezing episodes (3 episodes; OR, 9.14; $P=.005$) and evidence of sensitization to 1 or more aeroallergens (OR, 5.81; $P=.004$) were each significantly associated with physician-diagnosed asthma.

Multivariate analyses—Two separate multivariate models were developed incorporating patient characteristics available at the time of enrollment (baseline) and age 3 years (Table III). These models demonstrated the following factors to be significantly associated with physician-diagnosed asthma with adjustment for the other factors: white race (baseline and 3-year models), longer birth length (baseline model), and day care attendance (3 year model) were associated with decreased risks of physician-diagnosed asthma, whereas maternal asthma (baseline model), allergic sensitization (3-year model), recurrent wheeze (3-year model), and exposure to high levels of dog allergen (baseline model) were associated with increased risks. In separate models incorporating nasal epithelial CCL5 levels at baseline, higher CCL5 ratios (OR of 3.81 [$P<.001$] in the baseline model and OR of 6.52 [$P=.028$] in the 3-year model) were significantly associated with physician-diagnosed asthma by the seventh birthday. In addition, the CCL5 ratio from samples obtained 1 month after RSV bronchiolitis was also significantly associated with physician-diagnosed asthma (OR, 1.89; 95% CI, 1.04-3.4; $P=.038$).

Factors related to active asthma by the seventh birthday

Univariate analyses—To increase the stringency of the asthma outcome diagnosis, we also examined factors associated with active asthma by the seventh birthday (Table IV). Characteristics available at enrollment that were significantly associated with active asthma before the seventh birthday in univariate unadjusted analyses included maternal asthma (OR, 4.0; $P<.001$), a high level of cockroach allergen (Bla g 1) in the home (OR, 3.2; $P=.037$), day care attendance (OR, 0.5; $P=.047$), and white race (OR, 0.3; $P<.001$). Neither the severity of the initial infection nor baseline laboratory data were significantly associated with development of active asthma.

Multivariate analyses—Two separate multivariate models were developed incorporating patients' characteristics available at the time of enrollment (baseline) and age 3 years (Table III). These models demonstrated the following factors to be significantly associated with active asthma with adjustment for the other factors: white race (baseline and 3-year models)

and day care attendance (3-year model) were associated with decreased risks of active asthma, whereas maternal asthma (baseline model), allergic sensitization (3-year model), and recurrent wheeze (3-year model) were associated with increased risks. In separate models incorporating nasal epithelial CCL5 levels at baseline, higher CCL5 ratios (OR of 1.67 [$P = .003$] in the baseline model and OR of 15.1 [$P = .021$] in the 3-year model) were significantly associated with active asthma by the seventh birthday. In contrast, CCL5 ratios from samples obtained 1 month after RSV bronchiolitis were not significantly associated with active asthma (OR, 1.29; 95% CI, 0.71-2.34; $P = .4$).

In an effort to develop a post-RSV Asthma Predictive Index with the best overall sensitivity and specificity for predicting asthma outcomes, we analyzed potential predictors using ROC curves. The model that incorporated the clinically derived predictors that were significant in the univariate analyses (race, birth length, maternal asthma history, and exposure to high levels of dog allergen) provided the highest combination of sensitivity (64%) and specificity (73%) for physician-diagnosed asthma (ROC area under the curve [AUC], 0.75; Fig 3, *A*). However, the model that provided the overall highest combination of sensitivity and specificity for predicting asthma status by the seventh birthday included only the CCL5 ratio at enrollment (physician-diagnosed asthma: sensitivity, 87%; specificity, 84%, ROC AUC, 0.90; positive predictive value [PPV], 89%; negative predictive value, 82%; active asthma: sensitivity, 79%; specificity, 90%; ROC AUC, 0.88; PPV, 88%; negative predictive value, 82%; Fig 3, *B*).

DISCUSSION

We have demonstrated that severe RSV bronchiolitis in the first year of life is followed by a diagnosis of childhood asthma by the seventh birthday in nearly half of children. Factors assessed around the time of bronchiolitis that were associated with increased risk of physician-diagnosed asthma in our cohort included maternal asthma, exposure to high levels of dog allergen, and increased upper airway expression of CCL5, whereas white race and longer birth length decreased the risk. The subsequent occurrence of repeated wheezing episodes, aeroallergen sensitization by age 3 years, or both was associated with an increased risk of physician-diagnosed asthma, whereas day care attendance by age 3 years was associated with a significantly lower risk of physician-diagnosed asthma. Similar characteristics were associated with increased risk for both active asthma and physician-diagnosed asthma (maternal asthma, nasal CCL5 ratio, and nonwhite race at baseline and repeated wheezing episodes and aeroallergen sensitization by age 3 years), whereas day care attendance by age 3 years was protective for physician-diagnosed and active asthma.

Prior prospective studies[5,7,9] of children after severe RSV bronchiolitis have had lengthy intervals between follow-up assessments and have been unable to evaluate within-group differences because of generally small sample sizes. Our prospective cohort of 206 children enrolled during an initial episode of severe RSV bronchiolitis during infancy is the largest such cohort reported to date, affording us the ability to examine prospectively the interrelationships of multiple demographic, familial, environmental, and biological characteristics associated with asthma development after severe RSV bronchiolitis.

Our finding of a high prevalence of asthma diagnosis (48% by age 7 years) after RSV bronchiolitis is consistent with previous studies,[3,5,10] although the proportion of RBEL children with physician-diagnosed asthma is higher than previously described in children of similar age and history of severe RSV infection.[3,5,18,19] Our prospective data collection with frequent interviews might have increased the identification of children with a reported asthma diagnosis. Additionally, a substantial proportion of the RBEL cohort exhibited known risk factors for asthma, including African American race,[20,21] low socioeconomic

status,[21] and family history of asthma and other atopic diseases,[22-24] although some of these factors were less common in earlier study populations.

We found that participants without physician-diagnosed asthma exhibited a heightened degree of airway hyperresponsiveness approximately 6 years after severe RSV bronchiolitis, with mean PC_{20} to methacholine approximating 2 mg/mL. These findings are consistent with a previous study showing that 62% of children at 8 to 9 years of age after hospitalization for bronchiolitis during the first 2 years of life exhibited airway hyperresponsiveness (defined as a 20% decrease in peak expiratory flow with <4.9 mg/mL methacholine).[25] In contrast, an earlier study of mild bronchiolitis demonstrated no evidence of heightened airway hyperresponsiveness 8 to 12 years later.[26] Therefore it appears that severe bronchiolitis is associated with subsequent airway hyperresponsiveness but not necessarily related to the diagnosis of asthma. The long-term consequences of this finding might be the development of asthma in additional subjects because airway hyperresponsiveness at age 6 years has been demonstrated to be a risk factor of newly diagnosed asthma in early adulthood.[27]

Many children in the RBEL cohort had a family history of asthma and other atopic diseases, and a maternal history of asthma at the time of RSV bronchiolitis was associated with persistence in the study, as well as a significantly increased risk of physician-diagnosed asthma, which is consistent with previous studies.[23,24,28] Mothers with asthma could have greater awareness of asthma symptoms, leading to earlier diagnosis of asthma in their children. This effect is specific to maternal asthma because maternal atopy (other than asthma) was not significantly associated with study retention or asthma diagnosis.

Longer birth length was associated with a lower rate of asthma, potentially related to larger airway caliber at birth, and is consistent with recent studies demonstrating a lower risk of persistent wheezing among children with longer length at birth[29] and lower risk of asthma at age 10 years among children with longer first-trimester fetal size.[30]

Aeroallergen sensitization at age 3 years was associated with the diagnosis of asthma after severe RSV bronchiolitis, which is consistent with the findings of 2 other prospective cohort studies.[31,32] Previous research regarding environmental allergens and asthma has found no consistent relationship between pet or pet allergen exposure and later asthma, with some studies demonstrating that early dog ownership might be protective against subsequent recurrent wheezing[32,33] and allergic sensitization but not against asthma by age 6 to 7[34] and 18[35] years. Our finding of dog allergen exposure augmenting the risk of physician-diagnosed asthma might be due to exposure to allergen in the setting of lower airway injury associated with severe RSV bronchiolitis, suggesting that there might be an interaction between RSV infection and dog allergen exposure. We could not determine whether the effect of dog exposure was mediated through an increase in allergic sensitization to dog because only 5 subjects had evidence of allergic sensitization to dog at age 3 years, resulting in very low statistical power. However, other studies have reported the absence of a relationship between dog exposure in the home during infancy and allergic sensitization to dog during the first 6 years of life.[33,34,36,37] Alternatively, our differing results might be due to our definition of dog exposure (increased allergen level in household dust), whereas others have used the presence of a pet in the home to define exposure. However, this intriguing link between exposure to allergen during a time of immune system development and later respiratory disease deserves further attention.

We observed that day care attendance by 3 years of age was associated with significantly reduced likelihood of physician-diagnosed asthma and active asthma, which is consistent with previous studies,[38,39] although others have demonstrated increased airway symptoms in

early life but no difference in asthma at age 8 years.[40] This observation is supportive of the hygiene hypothesis, which argues that repeated exposure to infections, as in day care institutions, might promote T_H1 immune response early in life that counterbalances a T_H2 propensity, leading to the development of atopic disease.[41]

Repeated wheezing (3 episodes before age 3 years) was significantly associated with both asthma diagnosis and active asthma in our cohort. Asthma is a clinical diagnosis usually ascribed after repeated wheezing episodes, and we observed this to be the case because the asthma diagnosis lagged behind the first several wheezing episodes (Fig 2). In addition, after severe RSV bronchiolitis, these children continue to experience recurrent respiratory symptoms because two thirds have had at least 3 wheezing episodes by the end of their second year of follow-up.

Airway inflammation, as reflected by CCL5 mRNA expression in upper airway epithelial cells from children during RSV bronchiolitis, was strongly predictive for the development of physician-diagnosed asthma and active asthma, whereas CCL5 expression 1 month after resolution of the bronchiolitis episode was only associated with physician-diagnosed asthma but not active asthma. The expression of CCL5 during RSV bronchiolitis is likely genetically determined, and thus it is possible that a predisposition to an exuberant CCL5 response to RSV results in acute RSV bronchiolitis of greater severity acutely and with risk of symptomatology throughout early life. A case-control study provided evidence supporting a hereditary basis for susceptibility to severe lower respiratory tract infection,[42,43] whereas Janssen et al[44] reported genes related to innate immunity (vitamin D receptor, *JUN, NOS2A*, and *IFNA5*) that were strongly associated with bronchiolitis. Whether genetic variations explain the increased risk of asthma in those children who expressed the highest levels of CCL5 after severe RSV bronchiolitis is being evaluated. We have previously reported that peripheral blood T-cell production of the cytokines IL-4, IFN-γ, and IL-2 at study entry and at ages 2, 4, and 6 years did not differ between those children who had physician-diagnosed asthma and those without asthma, whereas those with physician-diagnosed asthma did demonstrate lower IL-13 expression at 6 years.[45] These findings, along with the current finding of nasal epithelial CCL5 expression, confirm the complex interplay of immunologic pathways that contribute to post-RSV asthma.

In an effort to provide a clinically useful tool for predicting asthma after severe RSV bronchiolitis, we developed an index using both clinical measures and the biomarker nasal epithelial CCL5 ratio. Maternal history of asthma alone was poorly predictive of physician-diagnosed asthma (ROC AUC, 0.55). The models developed include factors that have not been previously reported (epithelial CCL5) and are unexpected (dog allergen). The model incorporating the biomarker of nasal epithelial CCL5 during the acute bronchiolitis episode alone (Fig 3, *B*) exhibited very strong performance characteristics for predicting physician-diagnosed asthma (sensitivity, 87%; specificity, 84%; and PPV, 89%) compared with the model incorporating clinical features alone (Fig 3, *A*). The addition of clinical features (including maternal asthma) to the CCL5 level–containing model did not improve the performance of the model (ROC AUC of 0.9 for models of CCL5 with or without maternal asthma). The PPV of 89% approaches a potentially useful clinical model for pediatricians to use for children hospitalized with RSV bronchiolitis. These findings suggest that a single biomarker, nasal epithelial CCL5 at the time of acute bronchiolitis, is a more robust predictor of physician-diagnosed asthma and active asthma after severe RSV bronchiolitis than other often-cited risk factors for childhood asthma. Prospective validation of this model is necessary, as is exploration of other biomarkers that have greater availability to practicing physicians.

A limitation that could affect the interpretation of our findings includes the absence of a control group of infants without severe RSV infection, making us unable to firmly conclude whether severe RSV bronchiolitis causes asthma or whether RSV bronchiolitis is seen more frequently in children at high risk of asthma later in life. This is a prospective cohort study following subjects with a common initial exposure (severe RSV bronchiolitis) and assessing for the development of the primary outcome (asthma and related phenotypes). Because nearly all children are infected with RSV by age 2 years, establishing a control group of unexposed children is not practical. A case-control study has been previously done.[3,7-9,46] Furthermore, a prospective cohort study is a more robust epidemiologic method to establish causation of disease and is not subject to many of the limitations of case-control studies. The directionality of the relationship between RSV infection and asthma has been questioned for many years, and a recent study of twins concluded that severe RSV infection that leads to hospitalization does not cause asthma but serves as an indicator of genetic predisposition to asthma,[47] although the definitions of RSV infection and asthma in this study were less stringent than in the RBEL cohort. The major focus of this research is to determine the significant factors for the development of asthma once severe RSV bronchiolitis has occurred; thus we cannot determine whether these findings are specific to asthma after RSV infection or represent risk factors for asthma in general. Although the fact that the children were hospitalized for bronchiolitis might indeed indicate a predisposition to recurrent wheezing and subsequent asthma, we were careful to exclude children with clinically relevant prebronchiolitis respiratory tract morbidity. However, it is possible that a greater (presumably genetic) susceptibility to severe bronchiolitis, including CCL5 response to severe RSV infection, contributed to the episode's severity. Additionally, we relied on parental report followed by medical record confirmation of physician-diagnosed asthma, which might have introduced bias into our results because parental report of diagnosed asthma is only moderately concordant with a physician's diagnosis obtained from records.[48,49] Asthma is a clinical diagnosis, and physicians' criteria to diagnose pediatric asthma varies by ethnicity,[50,51] with African American children with recent wheezing being more likely to receive a diagnosis of asthma by a physician than white children.[52] This could account for some of the lower rates of physician-diagnosed asthma and active asthma seen in white children in the RBEL cohort.

Our results demonstrate that children who experience severe RSV bronchiolitis in infancy are at significantly increased risk for asthma during the first 6 years of life and that symptoms generally persist as children grow toward school age. Aeroallergen sensitization, repeated wheeze, and maternal asthma were strongly associated with the development and persistence of asthma in our cohort, whereas white race and day care attendance were associated with decreased likelihood of asthma. The presence of increased CCL5 expression in the nasal epithelia during bronchiolitis is strongly predictive of asthma at school age, with much greater sensitivity and specificity than clinical features, including maternal asthma. Physicians are encouraged to closely monitor children after severe RSV bronchiolitis, to provide additional guidance to children and families with additional risk factors (genetic and environmental) for asthma, and to test at a young age for sensitization to common allergens. Additional research is necessary to evaluate whether these factors remain significant as these children grow older because our data and those of several previous studies[3,6,7,9] have shown that characteristics associated with persistence of asthma after RSV bronchiolitis vary with the child's age.

METHODS

Allergy evaluation

We performed percutaneous allergy skin tests during the third year of follow-up (n = 150; mean age, 3.6 ± 0.7 years of age) using the MultiTest device. Children were instructed to

avoid antihistamine medications for 72 hours before skin testing was performed. Allergens tested included tree mix, grass mix, weed mix, mold mix, dust mite mix, cockroach, cat and dog, and 3 common food allergens (egg, milk, and peanut; Greer Laboratories, Lenoir, NC). We evaluated for allergic sensitization in 4 children who were unable to undergo skin testing with ImmunoCAP (Phadia, Uppsala, Sweden). An allergen-specific IgE level of greater than 0.35 kU/L was considered a positive response.

Environmental assessment

We examined levels of 5 allergens: cat (Fel d 1), dog (Can f 1), house dust mite (Der p 1 and Der f 1), and cockroach (Bla g 1). If the child moved in the first year, a repeat dust sample was collected, and average allergen levels were calculated. High levels of measured allergen were defined as a Fel d 1 level of greater than 8000 ng/g of dust, a Can f 1 level of greater than 8000 ng/g of dust, a Bla g 1 level of greater than 1 U/g of dust, and a Der f 1 or Der p 1 level of greater than 2000 ng/g of dust.[E1]

Procurement of upper airway samples

Eighty-one subjects consented to provide nasal epithelial cell samples at entry and 1 month. A cytobrush was used to obtain a sample from below the middle turbinate by using direct visualization. The brush was then agitated to remove cells in 10% formalin for permanent fixation. The cells were then washed, and cytospin preparations performed. The formalin-fixed slides were dehydrated and fixed in 70% ethanol. The identity of epithelial cells from the brushings was confirmed by means of immunohistochemical staining with cytokeratin antibody (R&D Systems, Minneapolis, Minn).

Measurement of CCL5 from upper airway epithelial cells

CCL5 riboprobe was synthesized by using a 0.45-kb human cDNA fragment (nt 1-410, ATCC) positionally cloned into the *EcoRI* and *HindIII* sites of pBluescript (Promega, Madison, Wis). Radiolabeled ^{35}S-UTP sense and anti-sense cRNA transcripts were *in vitro* transcribed by T3 and T7 RNA polymerases, respectively, with the Gemini Riboprobe system (Promega). Riboprobes were then subsequently precipitated with ethanol/acetate, washed, and counted, and an average of 3.0×10^6 cpm/μL was generated for each probe. *In situ* hybridization was performed by incubating fixed cytospin slides with 50 μL of hybridization solution containing ^{35}S-labeled CCL5/pBluescript riboprobe (2×10^4 cpm/μL) at 60°C for 18 hours in a humidified chamber. Slides were subsequently washed and processed for quantitation.

Quantitation of *in situ* RNA signal

Slides were exposed to phosphor screens for 1 week and subsequently read on a Storm PhosphorImager system (Molecular Dynamics, Sunnyvale, Calif). ImageQuant software was used to measure ^{35}S-labeled anti-sense and sense RNA signal from each slide. A CCL5 ratio was calculated by subtracting sense RNA from antisense RNA ^{35}S signal and correcting for the total number of cells as follows:

$$([\text{Antisense} - \text{Sense}] \times 10{,}000/\text{cell count}).$$

RESULTS

Details of inclusion of data from subjects who did not contribute data at the seventh birthday

Two hundred six subjects were enrolled in the RBEL cohort. Five subjects did not contribute any data after enrollment, and these subjects were removed from all analyses. If asthma was diagnosed at any time, the participant was considered to have physician-diagnosed asthma (n = 16). If asthma was not diagnosed before the participant was lost to follow-up, the participant was defined as nonasthmatic (n = 26). For active asthma (n = 62), only participants with data after age 3 years were included. For the analyses using predictors obtained at age 3 years, only participants with follow-up data after age 3 years were included.

Abbreviations used

AUC	Area under the curve
OR	Odds ratio
PPV	Positive predictive value
RBEL	RSV Bronchiolitis in Early Life
ROC	Receiver operating characteristic
RSV	Respiratory syncytial virus

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Key messages

- Nearly 50% of children have physician-diagnosed asthma 6 years after severe RSV bronchiolitis.

- Asthma after severe RSV bronchiolitis is positively associated with maternal asthma, exposure to high levels of dog allergen, aeroallergen sensitization, and recurrent wheezing, whereas day care attendance and white race are associated with decreased asthma risk.

- The biomarker CCL5 in nasal epithelium during RSV bronchiolitis is strongly predictive of physician-diagnosed asthma.

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        │  RSV + swab eligible for         │
        │  enrollment (N=1,222)            │
        └─────────────────────────────────┘
                      │
                      │         ┌──────────────────────────────────────────┐
                      │         │ Excluded (N=1,016)                        │
                      │         │ •>12 months old (n=520)                   │
                      │────────▶│ •Previous wheezing (n=235)                │
                      │         │ •Previous asthma medication (n=193)       │
                      │         │ •MD-diagnosed asthma (n=144)              │
                      │         │ •Never wheezed (n=125)                    │
                      │         │ •Refused to participate (n=116)           │
                      │         │ •Parent unavailable (n=106)               │
                      │         │ •Discharge prior to contact (n=77)        │
                      │         │ •Congenital anomalies (n=63)              │
                      │         │ •Bronchopulmonary dysplasia (n=49)        │
                      │         │ •RSV+ post-discharge (n=49)               │
                      │         │ •Anti-GE reflux meds (n=45)               │
                      │         └──────────────────────────────────────────┘
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        ┌─────────────────────────────────┐
        │ Enrolled in RBEL cohort (N=206)  │
        └─────────────────────────────────┘
                      │         ┌──────────────────────────────────────────┐
                      │────────▶│ Lost follow up before age 1 (N=13)        │
                      ▼         └──────────────────────────────────────────┘
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        │ Subjects at age 1 (N=193)        │
        └─────────────────────────────────┘
                      │         ┌──────────────────────────────────────────┐
                      │────────▶│ Lost follow up before age 3 (N=14)        │
                      ▼         └──────────────────────────────────────────┘
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        │ Subjects at age 3 (N=179)        │
        └─────────────────────────────────┘
                      │         ┌──────────────────────────────────────────┐
                      │────────▶│ Lost follow up before age 6 (N=20)        │
                      ▼         └──────────────────────────────────────────┘
        ┌─────────────────────────────────┐
        │ Subjects at age 6 (N=159)        │
        └─────────────────────────────────┘
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FIG 1.
Enrollment and follow-up of participants through the RBEL study. Patients were enrolled during an episode of severe RSV bronchiolitis and followed prospectively through the first 6 years of life. *GE*, Gastroesophageal.
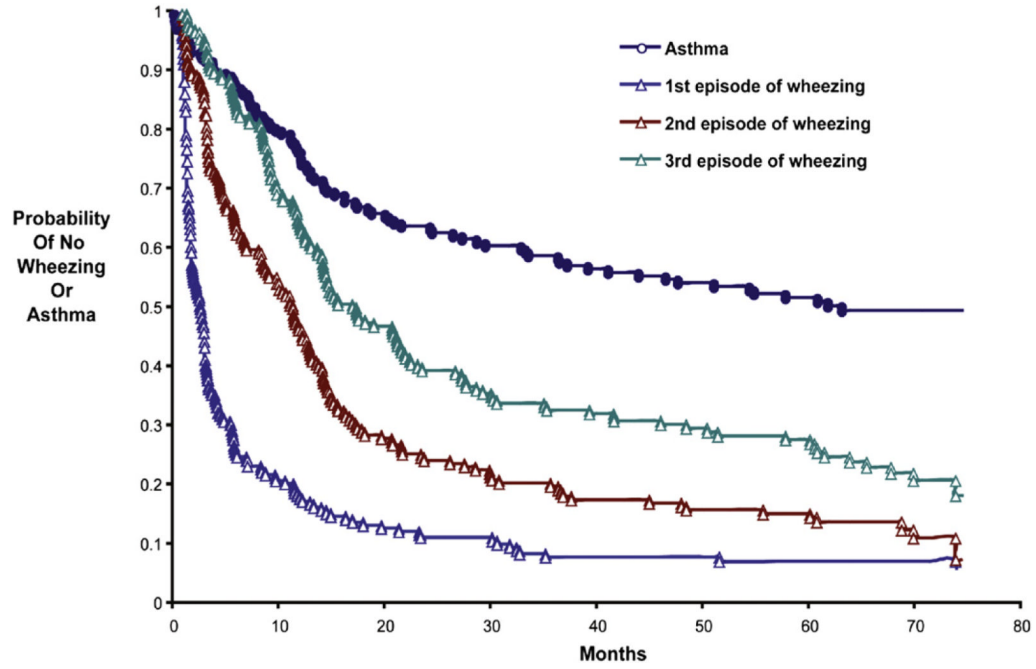
FIG 2.
Survival curves for time to development of the first 3 wheezing illnesses and physician's diagnosis of asthma subsequent to the index RSV bronchiolitis episode. The *vertical axis* represents the probability of not wheezing (for times to first, second, and third wheezing episodes) or not having physician-diagnosed asthma (*blue circles*). *Blue triangles* represent the first subsequent parent-reported wheezing episode, *brown triangles* represent the second subsequent parent-reported wheezing episode, *green triangles* represent the third subsequent parent-reported wheezing episode, and *blue circles* represent a physician's diagnosis of asthma.

FIG 3.
ROC curves for a post-RSV Asthma Predictive Index for physician-diagnosed asthma and active asthma. **A**, The model for physician-diagnosed asthma, incorporating the clinically derived predictors that were significant in the univariate analyses. **B**, The model for physician-diagnosed asthma (*red*) and active asthma (*blue*) incorporating nasal CCL5 levels alone at enrollment.

TABLE I

Characteristics by outcome group

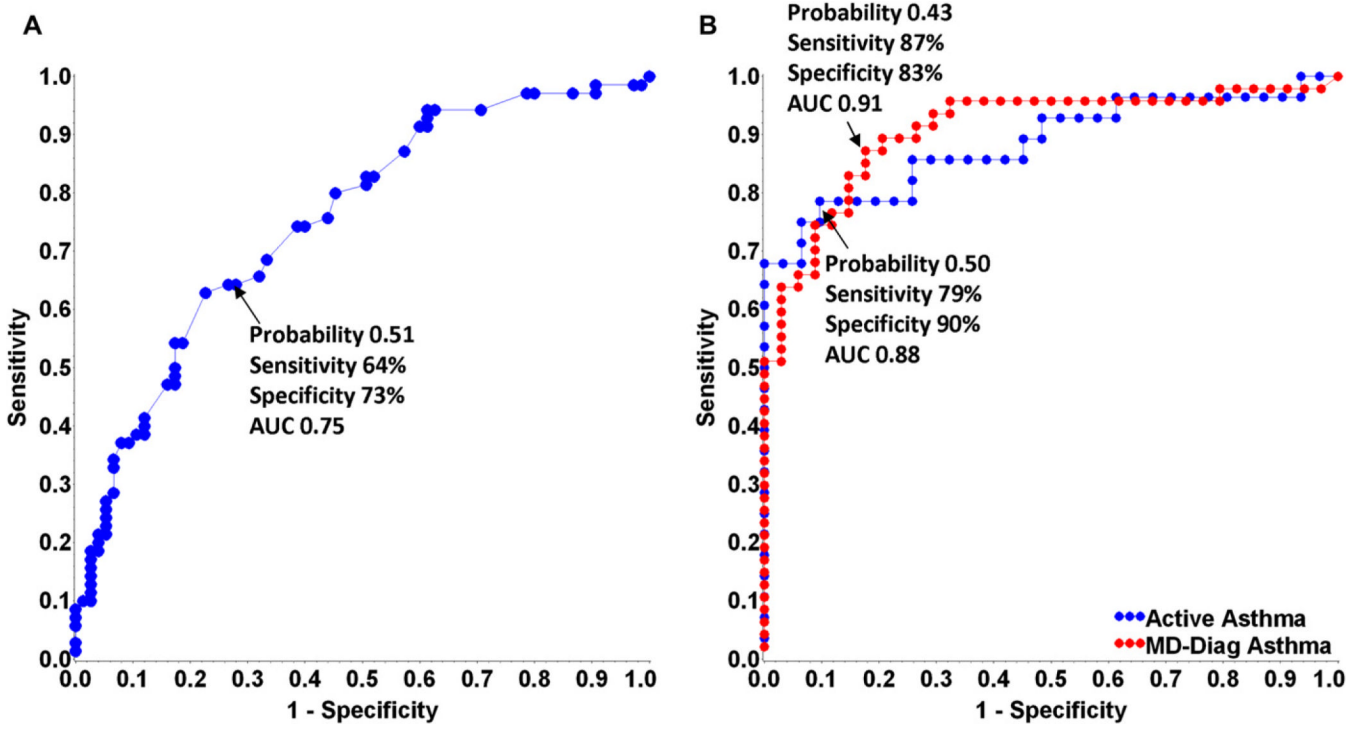|	Physician-diagnosed asthma by seventh birthday (n = 97)	No physician-diagnosed asthma by seventh birthday (n = 104)	P value	Active asthma by seventh birthday (n = 62)	No active asthma by seventh birthday (n = 117)	P value
Demographics						
Age at RSV (study entry [d])	123 ± 90	147 ± 107	.091	95 ± 14	150 ± 105	.059
Male sex (%)	52.6 (51)	63.5 (66)	.118	51.6 (32)	63.3 (74)	.132
White race (%)	44.3 (43)	60.6 (63)	.021	37.1 (23)	63.3 (74)	.001
Hospitalization data						
Length of stay in hospital (d)	2.60 ± 2.46	2.32 ± 2.57	.207	2.60 ± 2.34	2.35 ± 2.55	.196
Lowest SaO_2 (%)	92 ± 6	91 ± 8	.271	92 ± 6	92 ± 7	.688
Family history						
Maternal history of asthma (%)	28.9 (28)	8.7 (9)	<.001	35.5 (22)	12.0 (14)	<.001
Maternal history of eczema (%)	12.4 (12)	12.5 (13)	.978	9.7 (6)	15.4 (18)	.286
Maternal history of allergic rhinitis (%)	23.7 (23)	17.3 (18)	.260	30.7 (19)	18.0 (21)	.052
Clinical history and other potential exposures						
Smoking in home during first year of life (%)	51.0 (49)	55.5 (56)	.536	52.5 (32)	53.5 (61)	.895
Dog in home (%)	42.3 (41)	42.0 (42)	.970	38.7 (24)	46.0 (52)	.351
Any positive skin test response to aeroallergen at 3 y (%)	44.0 (33)	18.7 (14)	.001	46.3 (25)	22.9 (22)	.003
Laboratory tests at baseline						
Baseline IgE (IU/mL)	25.2 ± 52.1	19.6 ± 30.8	.917	30.5 ± 60.5	19.2 ± 32.3	.306
Baseline eosinophils (%)	1.75 ± 2.40	1.87 ± 2.65	.587	2.03 ± 2.71	1.83 ± 2.60	.944
Nasal CCL5 (ratio) at enrollment (n = 81)	5.34 ± 1.63 (n = 47)	3.14 ± 1.05 (n = 34)	<.001	5.28 ± 1.87 (n = 29)	3.89 ± 1.55 (n = 49)	<.001
Lung function at age 6 y						
FEV_1 (% predicted, before BD)	98 ± 13	100 ± 15	.439	98 ± 13	100 ± 15	.444
FEV_1/FVC ratio (before BD)	0.91 ± 0.09	0.89 ± 0.11	.213	0.91 ± 0.09	0.90 ± 1.11	.451
Change in FEV_1 after BD (%)	6.2 ± 15.2	4.2 ± 10.3	.857	6.8 ± 16.4	4.2 ± 10.5	.890
Methacholine PC_{20} (mg/mL)	1.42 ± 1.86	2.19 ± 2.78	.016	1.52 ± 2.02	1.95 ± 2.56	.181

Data are expressed as means ± SDs, except as noted.

BD, Bronchodilator; *FVC*, forced vital capacity; *SaO_2* lowest oxygen saturation measured during index hospitalization.

TABLE II

Characteristics at entry and age 3 years associated with physician-diagnosed asthma after severe RSV bronchiolitis

	Baseline (n = 201)		Age 3 y (n = 104)	
	OR for physician-diagnosed asthma (95% CI)	*P* value	OR for physician-diagnosed asthma (95% CI)	*P* value
Demographics				
Age at study entry (d)	0.998 (0.995-1)	.092	1.0 (0.995-1.01)	.920
Male sex	0.64 (0.36-1.12)	.119	0.49 (0.16-1.48)	.207
White race	0.52 (0.30-0.91)	.022	0.28 (0.09-0.89)	.031
Annual household income <$20,000	1.80 (1.00-3.26)	.051	1.09 (0.31-3.89)	.891
Personal history				
Duration of pregnancy (wk)	0.96 (0.84-1.11)	.597	0.95 (0.87-1.04)	.597
Birth weight (g)	1.0 (0.99-1.0)	.265	1.0 (0.999-1.002)	.511
Birth length (cm)	0.91 (0.83-0.99)	.025	1.04 (0.83-1.29)	.764
Family history				
Maternal asthma	4.28 (1.90-9.65)	<.001	3.95 (1.12-13.91)	.033
Maternal eczema	0.91 (0.30-2.82)	.875	1.38 (0.45-4.25)	.575
Maternal hay fever	1.49 (0.74-2.96)	.262	1.06 (0.27-4.17)	.935
Exposure to allergens				
Exposure to 1 allergen at high levels	1.51 (0.81-2.81)	.200	1.35 (0.38-4.84)	.648
Fel d 1 >8000 ng/g	0.81 (0.41-1.61)	.551	0.81 (0.20-3.24)	.761
Can f 1 >8000 ng/g	2.05 (1.05-3.98)	.035	3.32 (0.96-11.49)	.059
Bla g 1 >1 U/g	1.43 (0.51-4.03)	.501	1.14 (0.13-10.35)	.910
Der f 1 >2000 ng/g	1.81 (0.85-3.85)	.123	1.06 (0.21-5.42)	.943
Der p 1 >2000 ng/g	0.70 (0.19-2.57)	.591	NA for mathematical reasons	
Exposure to other potential respiratory irritants				
Maternal smoking during pregnancy	0.57 (0.30-1.10)	.096	0.37 (0.08-1.77)	.214
Smoking in home at each time point	1.17 (0.64-2.12)	.617	0.85 (0.28-2.55)	.774
Day care at each time point	0.50 (0.26-0.98)	.042	0.24 (0.07-0.77)	.017
Breast-feeding	0.67 (0.38-1.17)	.158	0.78 (0.26-2.33)	.213
Clinical history				
Wheezing (3 reported episodes before age 3 y)	NA		9.14 (1.94-42.92)	.005
Physician-diagnosed eczema before age 3 y			1.38 (0.45-4.25)	.575
Sensitivity to allergens at age 3 y				
Positive response to 1 any allergen	NA		5.81 (1.74-19.43)	.004
Positive response to 1 food allergen			3.94 (0.60-26.13)	.155
Positive response to 1 environmental allergen			5.81 (1.74-19.43)	.004
Laboratory tests at baseline				
IgE	1.03 (0.74-1.41)	.881	1.21 (0.68-2.15)	.517
Blood eosinophils (%)	0.98 (0.86-1.12)	.778	0.94 (0.69-1.27)	.670
CCL5	3.81 (2.07-7.01)	<.001	6.52 (1.67-25.5)	.007

NA, Not applicable.

TABLE III

Multivariate model for factors associated with physician-diagnosed and active asthma by the seventh birthday after severe RSV bronchiolitis

Baseline data			Age 3 y data		
Variables	**OR (95% CI)**	***P* value**	**Variables**	**OR (95% CI)**	***P* value**
			Physician-diagnosed asthma		
			Without CCL5 included		
n = 201			n = 104		
White race	0.25 (0.11-0.57)	.001	White race	0.19 (0.04-0.93)	.041
Longer birth length per cm	0.86 (0.77-0.97)	.013	Day care by age 3 y	0.18 (0.04-0.84)	.029
History of maternal asthma at entry	5.21 (1.71-15.9)	.004	Positive response to ≥1 environmental allergen	10.7 (2.08-55.1)	.005
Exposure to levels of dog allergen >8000 ng/g dust	3.15 (1.29-7.68)	.012	Wheezing (≥3 episodes before age 3 y)	7.31 (1.24-43.3)	.028
			With CCL5 included		
n = 81			n = 40		
CCL5	3.81 (2.07-7.01)	<.001	CCL5	6.52 (1.67-25.5)	.007
			Active asthma		
			Without CCL5 included		
n = 179			n = 104		
White race	0.39 (0.20-0.76)	.005	White race	0.15 (0.03-0.76)	.022
History of maternal asthma at entry	3.49 (1.59-7.65)	.002	Day care by age 3 y	0.14 (0.03-0.70)	.017
			Positive response to ≥1 environmental allergen	9.0 (1.66-48.8)	.046
			Wheezing (≥3 episodes before age 3 y)	6.12 (1.03-36.3)	.011
			With CCL5 included		
n = 78			n = 40		
CCL5	1.67 (1.19-2.36)	.003	CCL5	15.1 (1.51-152)	.021
White race	0.24 (0.08-0.71)	.010			

TABLE IV

Characteristics at baseline and age 3 years associated with active asthma after severe RSV bronchiolitis

	Baseline (n = 179)		Age 3 y (n = 104)	
	OR for active asthma (95% CI)	*P* value	OR for active asthma (95% CI)	*P* value
Demographics				
Age at study entry (d)	0.997 (0.994-1)	.061	1.0 (0.995-1.01)	.940
Male sex	0.62 (0.33-1.16)	.133	0.58 (0.19-1.80)	.344
White race	0.34 (0.18-0.65)	<.001	0.22 (0.06-0.76)	.017
Annual household income <$20,000	2.02 (1.05-3.87)	.035	1.25 (0.34-4.54)	.735
Personal history				
Duration of pregnancy (wk)	1.07 (0.90-1.27)	.439	1.09 (0.80-1.48)	.580
Birth weight (g)	1.00 (0.99-1.00)	.739	1.0 (0.999-1.02)	.398
Birth length (cm)	0.94 (0.85-1.03)	.184	1.08 (0.86-1.37)	.505
Family history				
Maternal asthma	4.05 (1.89-8.68)	<.001	4.45 (1.24-15.9)	.022
Maternal eczema	0.59 (0.22-1.57)	.291	0.79 (0.09-6.83)	.829
Maternal hay fever	2.02 (0.99-4.14)	.055	1.17 (0.29-4.66)	.823
Exposure to allergens				
Exposure to 1 allergen at high levels	1.16 (0.31-4.26)	.716	1.16 (0.31-4.26)	.829
Fel d 1 >8000 ng/g	0.52 (0.24-1.10)	.100	0.52 (0.10-2.56)	.418
Can f 1 >8000 ng/g	1.19 (0.60-2.39)	.616	2.63 (0.72-9.53)	.142
Bla g 1 >1 U/g	3.20 (1.07-9.51)	.037	1.27 (0.14-11.6)	.834
Der f 1 >2000 ng/g	1.62 (0.75-3.50)	.222	1.20 (0.23-6.18)	.830
Der p 1 >2000 ng/g	0.19 (0.02-1.54)	.120	NA for mathematical reasons	
Exposure to other potential respiratory irritants				
Maternal smoking during pregnancy	0.55 (0.26-1.18)	.125	0.41 (0.09-1.96)	.264
Smoking in home at each time point	1.15 (0.59-2.25)	.680	0.86 (0.23-3.19)	.818
Day care at each time point	0.47 (0.22-0.99)	.047	0.20 (0.06-0.67)	.009
Breast-feeding	0.58 (0.31-1.09)	.091	0.67 (0.22-2.06)	.482
Clinical history				
Wheezing (3 reported episodes before age 3 y)	NA		8.21 (1.74-38.8)	.008
Physician-diagnosed eczema before age 3 y			1.11 (0.34-3.61)	.861
Sensitivity to allergens at age 3 y				
Positive response to 1 any allergen	NA		4.75 (1.39-16.2)	.013
Positive response to 1 food allergen			4.36 (0.65-29.1)	.128
Positive response to 1 environmental allergen			4.75 (1.39-16.2)	.013
Laboratory tests at baseline				
IgE	1.21 (0.86-1.70)	.281	0.94 (0.48-1.82)	.843
Blood eosinophils (%)	1.03 (0.90-1.18)	.678	0.95 (0.71-1.29)	.756
CCL5	1.66 (1.21-2.28)	.002	15.1 (1.51-152)	.021

NA, Not applicable.

TABLE E1

Clinical characteristics of subjects with and without follow-up between 3 and 6 years of age

	Subjects without follow-up (n = 47)	Subjects with follow-up (n = 159)	*P* value
Demographics			
Age at study entry (d)	122 ± 71	139 ± 105	.976
Male sex (%)	63.8 (30)	56.6 (90)	.378
White race (%)	51.1 (24)	52.8 (84)	.831
Hospitalization data			
Length of stay in hospital (d)	2.30 ± 2.53	2.47 ± 2.51	.416
Lowest SaO_2 (%)	92 ± 9	92 ± 7	.239
Family history			
Maternal history of asthma (%)	8.51 (4)	21.4 (34)	.054
Maternal history of eczema (%)	8.51 (4)	5.66 (9)	.499
Maternal history of allergic rhinitis (%)	25.5 (12)	30.8 (49)	.486
Clinical history and other potential exposures			
Smoking in home during first year of life (%)	76.6 (36)	64.5 (100)	.122
Dog in home (%)	51.3 (20)	43.5 (60)	.387
Laboratory tests at baseline			
Baseline IgE (IU/mL)	13.7 ± 16.0	24.3 ± 46.4	.452
Baseline eosinophils (%)	1.67 ± 2.00	1.81 ± 2.65	.604
Nasal CCL5 (ratio)	4.60 ± 2.19	4.39 ± 1.72	.445

SaO2, Lowest oxygen saturation measured during index hospitalization.

TABLE E2

Baseline characteristics of the RBEL cohort

Characteristic	Included (n = 206)
Demographics	
Age at study entry (d)	135 ± 99
Male sex (%)	58.3
Race/ethnicity (%)	
White	52.4
Black	43.7
Other	3.9
Annual household income $20,000 (%)	60.8
Pregnancy history	
Duration of pregnancy (wk)	38.5 ± 2.1
Birth weight (kg)	3.19 ± 0.56
Birth length (cm)	50.4 ± 3.8
Bronchiolitis severity	
Lowest SaO_2 (n = 191 [%])	91.6 ± 7.3
Length of stay (n = 199 [d])	2.5 ± 2.5
Presence of hyperexpansion on CXR (n = 131 [%])	63.4
Atopic features	
IgE level (n = 186 [IU/mL])	22.1 ± 42.1
Eosinophils (n = 152 [%])	1.8 ± 2.5
Family history	
Maternal history of asthma (%)	18.5
Maternal history of eczema (%)	6.3
Maternal history of allergic rhinitis (%)	20.4
Home environment	
Dog in home (%)	42.1
Cat in home (%)	26.7
Rodent in home (%)	6.3
Cockroaches in home (%)	7.8
Home allergen levels (n = 130)	
Fel d 1 >8000 ng/g (%)	25.8
Can f 1 >8000 ng/g (%)	29.3
Bla g 1 >1 U/g (%)	9.5
Der p 1 and Der f 1 >2000 ng/g (%)	22.4

Values are presented as means ± SDs.

CXR, Chest radiograph; *SaO2*, lowest oxygen saturation measured during index hospitalization.