

Severe bronchiolitis profiles and risk of recurrent wheeze by age 3 years



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Background: A better understanding of bronchiolitis heterogeneity might help clarify its relationship with the development of recurrent wheezing and asthma. **Objectives:** We sought to identify severe bronchiolitis profiles using a clustering approach and to investigate for the first time their association with allergy/inflammatory biomarkers, nasopharyngeal microbiota, and development of recurrent wheezing by age 3 years.

Methods: We analyzed data from a prospective, 17-center US cohort study of 921 infants (age <1 year) hospitalized with bronchiolitis (2011–2014 winters) with posthospitalization follow-up. Severe bronchiolitis profiles at baseline (hospitalization) were determined by using latent class analysis based on clinical factors and viral etiology. Blood biomarkers and nasopharyngeal microbiota profiles were determined by using samples collected within 24 hours of hospitalization. Recurrent wheezing by age 3 years was defined based on parental report of breathing problem episodes after discharge. **Results:** Three severe bronchiolitis profiles were identified: profile A (15%), which was characterized by a history of breathing problems/eczema during infancy and non-respiratory syncytial virus (mostly rhinovirus) infection; profile B (49%), which has the largest probability of respiratory syncytial virus infection and resembled classic respiratory syncytial virus-induced bronchiolitis; and profile C (36%), which was composed of the most severely ill group. Profile A infants had higher eosinophil counts, higher cathelicidin levels, and increased proportions of *Haemophilus*-dominant or *Moraxella*-dominant

microbiota profiles. Compared with profile B, we observed significantly increased risk of recurrent wheezing in children with profile A (hazard ratio, 2.64; 95% CI, 1.90–3.68) and, to a lesser extent, with profile C (hazard ratio, 1.51; 95% CI, 1.14–2.01). **Conclusion:** Although longer follow-up is needed, our results might help identify, among children hospitalized for bronchiolitis, subgroups with particularly increased risk of asthma. (J Allergy Clin Immunol 2019;143:1371–9.)

Key words: Respiratory tract infections, cluster analysis, respiratory syncytial virus, rhinovirus, asthma

Early-life viral respiratory tract infections are associated with increased risk of recurrent wheezing and asthma.^{1–3} Bronchiolitis is the most common acute lower respiratory tract infection in infancy.^{4,5} Although most children with bronchiolitis experience mild-to-moderate illness, symptoms can be severe enough to require hospitalization.⁶ Severe bronchiolitis is a strong predictor of long-term respiratory morbidity; indeed, 30% to 40% of infants hospitalized for bronchiolitis will experience recurrent wheezing or asthma.^{7,8} However, the factors predicting which infants will have chronic respiratory illness remain incompletely characterized.

Bronchiolitis is generally considered a single disease; however, recent studies have underlined its heterogeneity.^{9,10} Beyond variability in the clinical course of the disease, patients with bronchiolitis are heterogeneous with regard to viral etiology and clinical history.^{9,11} Respiratory tract infections with respiratory syncytial virus (RSV) and rhinovirus, the most commonly identified pathogens in patients with bronchiolitis, have both been associated with later development of recurrent wheezing and asthma, although stronger associations have been reported for those with rhinovirus.^{1,2,7,8,12,13} Moreover, among patients with bronchiolitis or early-life wheezing illnesses, rhinovirus infection is most often seen in children with clinical histories suggesting susceptibility to asthma (eg, history of wheezing and sensitization).^{14,15}

Using a statistical clustering approach in multicenter cohorts of US and Finnish children hospitalized with bronchiolitis, we recently identified distinct severe bronchiolitis profiles.⁹ Profiles differed according to prior history of wheezing/eczema, wheezing during acute infection, levels of acute severity, and viral etiology. Interestingly, the results suggested the existence of a subgroup of children presenting with early signs of asthma during a severe bronchiolitis episode. However, respiratory outcomes after the severe bronchiolitis episode were not examined in this previous cross-sectional analysis.

The observed bronchiolitis heterogeneity might reflect the existence of several disease entities with specific pathobiology (endotypes) and varying propensity for later asthma development.¹⁰

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Abbreviations used

CPAP:	Continuous positive airway pressure
ED:	Emergency department
ICS:	Inhaled corticosteroid
ICU:	Intensive care unit
LCA:	Latent class analysis
MARC-35:	35th Multicenter Airway Research Collaboration
MCA:	Multiple correspondence analysis
25(OH)D:	25-Hydroxyvitamin D
OTU:	Operational taxonomic units
RSV:	Respiratory syncytial virus

There is increasing evidence for a complex interplay between viral pathogens, host immune response, and respiratory microbiota in the pathogenesis and severity of acute respiratory tract infections, as well as their link with asthma-related outcomes.¹⁶⁻¹⁹ Thus examining the heterogeneity of bronchiolitis in relation to allergy markers, immune response parameters, nasopharyngeal microbiota, and long-term respiratory outcomes might provide insight into asthma susceptibility pathways. However, to date, no study has investigated the association of severe bronchiolitis profiles defined by using a clustering approach with objectively measured biomarkers and long-term respiratory outcomes.

The 35th Multicenter Airway Research Collaboration (MARC-35) study is an ongoing, prospective multicenter cohort study of US infants (age <1 year) hospitalized with bronchiolitis and followed up after hospitalization. In the current study we aimed to identify severe bronchiolitis profiles using a clustering approach and to investigate, for the first time, their association with (1) allergy, inflammatory, and immune response biomarkers; (2) nasopharyngeal microbiota profiles; and (3) the risk of recurrent wheezing by age 3 years.

METHODS

Study design and data collection

The MARC-35 study is coordinated by the Emergency Medicine Network (<http://www.emnet-usa.org/>).¹⁸ During the 2011-2014 winter seasons (November-March), investigators at 17 sites across 14 US states enrolled 1016 infants hospitalized with bronchiolitis (study baseline). Bronchiolitis was diagnosed by the attending physician in accordance with American Academy of Pediatrics guidelines.²⁰ Researchers conducted a structured interview to collect data on patients' demographic characteristics, medical history, and details of the acute illness. Further clinical data were obtained from emergency department (ED) and inpatient medical records. Researchers collected blood samples and nasopharyngeal aspirates on hospitalization by using a standardized protocol. Testing was performed for a large panel of respiratory tract viruses by using real-time PCR assays and microbiota by using 16S rRNA gene sequencing (see the [Methods](#) section in this article's Online Repository at www.jacionline.org).

Among the 921 (91%) children eligible for long-term follow-up (the chronic cohort), we conducted telephone interviews with families approximately 1 week after hospital discharge and approximately 3 weeks after hospital admission and then every 6 months from age 6 months onward. We collected detailed data about any new breathing problem episodes, including date, duration, use of medication, and whether this problem affected the child's sleep. Written informed consent was obtained from a parent or guardian of all patients included in the study. The institutional review boards at each participating hospital approved the study.

Severe bronchiolitis profiles at baseline (infancy)

Severe bronchiolitis profiles were determined by using latent class analysis (LCA) based on medical history, clinical course of bronchiolitis, and viral etiology. Selection of variables and number of classes were performed, as described earlier (see the [Methods](#) section, [Tables E1](#) and [E2](#), and [Fig E1](#) in this article's Online Repository at www.jacionline.org).⁹ Each child was assigned to the class for which he had the highest membership probability.

Biomarkers and nasopharyngeal microbiota profiles at baseline (infancy)

Complete blood counts were performed locally at each hospital. Serum allergy (total and allergen-specific IgE), 25-hydroxyvitamin D (25[OH]D), and innate immune response (cathelicidin [LL-37]) biomarkers were measured from blood samples.¹⁷ We dichotomized total IgE levels, eosinophil counts, and 25(OH)D levels according to standard cutoffs (100 kU/L, 300 cells/ μ L, and 30 ng/mL, respectively) and LL-37 levels according to median values (46 ng/mL).¹⁷

To examine nasopharyngeal microbiota, we identified 4 distinct microbiota profiles (*Moraxella*-dominant, *Haemophilus*-dominant, *Streptococcus*-dominant, and mixed profiles), as previously described,¹⁸ by using partitioning around medoids with weighted UniFrac distance.

Respiratory outcomes by age 3 years

Recurrent wheezing by age 3 years was defined by parental report of at least 2 corticosteroid-requiring breathing problems in 6 months or at least 4 breathing problem episodes in 1 year that last at least 1 day and affect sleep.²¹ Parent-reported use of inhaled corticosteroids (ICSs) or montelukast by age 3 years was also examined. Only breathing problem episodes and medication use reported after hospital discharge were included in outcome definitions. Asthma by age 3 years was defined based on parental report that the child received a diagnosis of asthma by a doctor or other health professional.

Statistical analyses

Associations of severe bronchiolitis profiles with biomarkers and nasopharyngeal microbiota profiles at baseline were evaluated by using multinomial logistic regression. The association between severe bronchiolitis profiles at baseline and development of recurrent wheezing by age 3 years was evaluated by using Kaplan-Meier survival curves and Cox models. Multivariable models were adjusted for age, sex, and race/ethnicity. Nasopharyngeal microbiota models were further adjusted for antibiotic and corticosteroid use. A 2-sided *P* value of less than .05 was considered statistically significant.

RESULTS

Follow-up data were available for more than 80% of children at each follow-up interview from age 6 months to 3 years (range, 82% to 88%). The mean age of the children at baseline was 4.1 months (SD, 3.0 months), and 40% were female; 50% of children were non-Hispanic black or Hispanic.

Severe bronchiolitis profiles

Three severe bronchiolitis profiles, labeled A to C, were identified by using LCA. The mean class membership probability range was 81% to 84%. In [Table I](#) the profiles are described based on the proportion of children presenting with each characteristic (variables used for classification) according to the class to which they were assigned by the LCA. A summary of the profiles is presented in [Fig 1](#), where the profiles are displayed according to their main distinctive characteristics. Further data are provided in the [Methods](#) section in this article's Online Repository.

TABLE I. Description of the infants hospitalized for severe bronchiolitis (baseline), according to the profiles (A-C) identified by using LCA

No. (%)	All 921	Profiles		
		A 136 (15)	B 452 (49)	C 333 (36)
History of breathing problems (%)	20	44	12	23
History of eczema (%)	15	23	14	13
Wheeze at ED presentation (%)	63	69	56	69
Cough at ED presentation (%)	96	92	100	94
Retractions at ED presentation (%)				
None	19	25	22	12
Mild	44	45	55	28
Moderate to severe	37	30	23	60
Hospital length-of-stay (d [%])				
<3	60	89	85	14
3-6	32	10	15	65
≥7	8	1	0	21
Highest respiratory rate during entire hospital stay ≥60 (breaths/min [%])	50	22	27	94
Viral etiology				
RSV (%)	82	5	99	83
Rhinovirus (%)	20	54	13	17

Results are presented as percentages (observed proportion in the study population and within profiles A-C). LCA was performed based on the 9 variables presented in the table selected from a larger set of variables, including many clinical factors (main features typically monitored in clinical practice, including data from ED presentation to inpatient discharge).

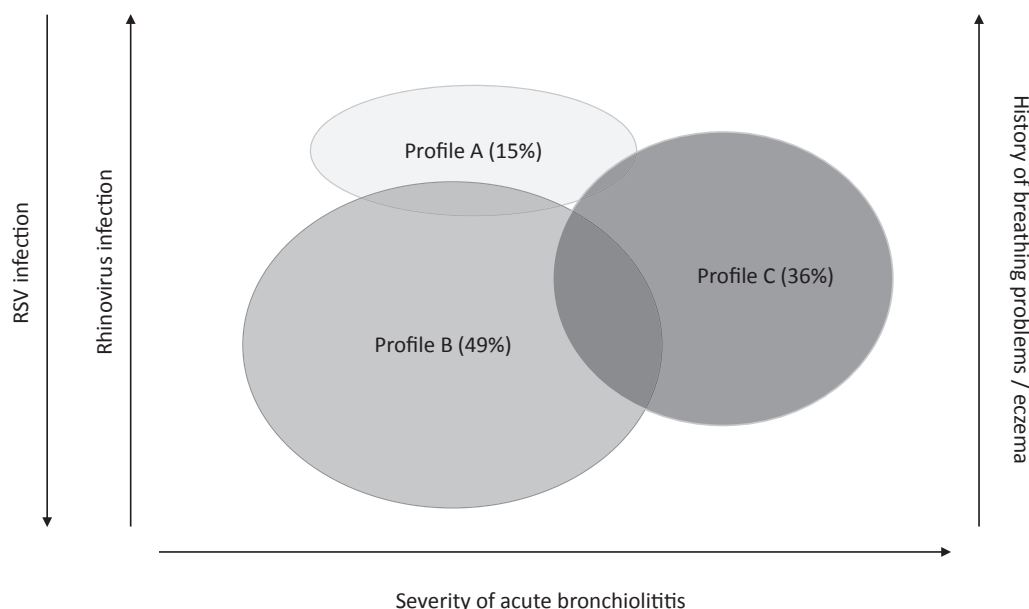


FIG 1. Graphic summary of the severe bronchiolitis profiles identified by using LCA. Profiles are displayed according to their main distinctive characteristics (bronchiolitis severity, viral etiology, and history of breathing problems/eczema). Overlaps between the profiles are proportional to class membership probabilities (eg, infants assigned to profile B had 5% probability to belong to profile A and 12% probability to belong to profile C).

Profile A infants (15%) were characterized by a higher proportion of history of breathing problems (44%) and eczema (23%); they also differed from other profiles by the low proportion of infants with RSV infection (5%) and higher proportion of rhinovirus infection (54%). In contrast with profile A, profile B (49%) had the largest proportion of infants with RSV infection (99%) and a low proportion of infants with history of breathing problems (12%) or eczema (14%). Profile C (36%) was the most severely ill group: 60% of profile C infants had

moderate-to-severe retractions at presentation (vs 23% to 30% in other profiles), and 21% were hospitalized for 7 days or longer (vs 0% to 1% in other profiles); they also had a higher respiratory rate during hospital stay. Most profile C infants (83%) were infected with RSV.

The distribution of sociodemographic and additional clinical characteristics across profiles is presented in [Table II](#). Compared with other profiles, profile A infants were older and more often Hispanic; they also more often had a history of antibiotics and

TABLE II. Characteristics of the children according to severe bronchiolitis profiles

No. (%)	All 921	Profiles			P value*
		A	B	C	
		136 (15)	452 (49)	333 (36)	
Sociodemographic characteristics					
Age (mo [%])					
<2	30	21	28	35	<.001
2-5.9	46	37	49	45	
6-11.9	25	43	23	20	
Girls (%)	40	37	41	39	.64
Race/ethnicity (%)					
Non-Hispanic white	45	35	47	46	.05
Non-Hispanic black	23	25	23	21	
Hispanic	30	38	28	29	
Other	2	1	2	4	
Clinical history					
Parental history of asthma (%)	33	38	32	33	.36
History of antibiotic use (%)	32	40	32	29	.04
History of corticosteroid use (%)	15	26	13	14	<.001
Characteristics of acute bronchiolitis					
ED presentation					
Fever (%)	26	22	28	24	.37
Inadequate oral intake (%)	61	48	59	68	<.001
ICU and intubation/CPAP (%)					
No ICU	85	96	96	65	<.001
ICU without intubation/CPAP	10	2	4	21	
ICU with intubation/CPAP	5	2	0	14	
Viral etiology (%)					
RSV only	59	0	74	62	<.001
Rhinovirus only	6	32	0	3	
RSV and rhinovirus	12	5	13	14	
RSV and other nonrhinovirus	11	0	12	13	
Rhinovirus and other non-RSV	3	16	0	1	
Non-RSV and nonrhinovirus	10	46	1	8	
No. of pathogens (%)					
No pathogen identified	3	15	1	2	<.001
1	70	57	74	69	
2	22	24	22	22	
≥3	4	4	3	6	

Results in boldface are statistically significant.

* χ^2 Tests.

corticosteroid use before bronchiolitis hospitalization. The most severe group (profile C) included younger infants and a greater proportion of infants with inadequate oral intake at ED presentation who required ICU admission and mechanical ventilation. Regarding viral etiology, it was notable that a large proportion (46%) of profile A infants were neither infected with RSV nor rhinovirus. Other pathogens identified in more than 5% of profile A infants were human metapneumovirus B (15%), adenovirus (11%), human bocavirus type 1 (10%), and parainfluenza virus type 3 (6%). For 15% of the profile A infants, no pathogen was identified. Because profile B was the largest group and most resembled “classic” RSV bronchiolitis, it was used as the reference category in subsequent analyses.

Severe bronchiolitis profiles and biomarkers

Overall, no significant difference was observed in biomarker levels between profiles B and C, but profile A differed for some biomarkers (Table III). Total IgE levels were not significantly different in profile A compared with profile B. In contrast, profile A infants had significantly greater eosinophil counts than profile B infants. No difference was observed between profiles for

allergic sensitization, as defined by any positive value to allergen-specific IgE testing. When examining monosensitization (only 1 allergen [15%]) and multisensitization (≥ 2 allergens [4%]), results suggested that profile A infants had lower odds of monosensitization (odds ratio, 0.56; 95% CI, 0.30-1.03; $P = .06$) and did not differ significantly for multisensitization (odds ratio, 1.34; 95% CI, 0.54-3.30; $P = .52$) compared with profile B infants. Profile C infants did not differ significantly from profile B infants for these specific IgE groupings (see Table E3 in this article's Online Repository at www.jacionline.org).

No differences in serum 25(OH)D levels were observed across the profiles. However, profile A infants had significantly greater levels of serum LL-37 than profile B infants.

Severe bronchiolitis profiles and nasopharyngeal microbiota

Nasopharyngeal microbiota composition differed across severe bronchiolitis profiles (Table IV). In adjusted analyses, when compared with profile B, both profile A ($P = .004$) and profile C ($P = .001$) children more often had a *Haemophilus*-dominant

TABLE III. Association of severe bronchiolitis profiles with atopy/allergy markers, vitamin D status, and serum cathelicidin levels

	All	Profile A	Profile B	Profile C
Food allergen or aeroallergen sensitization*				
Percentage	20	18	20	21
Adjusted† OR (95% CI)		0.68 (0.41-1.15)	1 (reference)	1.10 (0.76-1.57)
Total IgE (kU/L)				
GM (IQR)	5.8 (1.9-12.2)	8.7 (1.9-20.6)	5.4 (1.9-10.4)	5.4 (1.9-12.2)
>100 (%)	3	8	2	2
Adjusted† OR (95% CI)		2.13 (0.79-5.70)	1 (reference)	0.84 (0.27-2.63)
Blood eosinophil count‡ (cells/μL)				
Median (IQR)	114 (16-238)	167 (29-370)	123 (20-218)	152 (0-216)
>300 (%)	18	33	15	16
Adjusted† OR (95% CI)		3.75 (2.03-6.93)	1 (reference)	1.03 (0.61-1.74)
Serum 25(OH)D (ng/mL)				
Median (IQR)	26 (18-33)	26 (19-35)	27 (19-33)	26 (17-32)
≥30 (%)	37	40	38	34
Adjusted† OR (95% CI)		1.00 (0.66-1.51)	1 (reference)	0.96 (0.70-1.31)
Serum LL-37 (ng/mL)				
Median (IQR)	46 (34-62)	54 (40-71)	46 (34-58)	46 (34-60)
>46 (%)	49	62	47	46
Adjusted† OR (95% CI)		1.84 (1.23-2.75)	1 (reference)	1.10 (0.82-1.47)

Results in boldface are statistically significant.

GM, Geometric mean; IQR, interquartile range; OR, odds ratio.

*Defined as having 1 or more positive values for allergen-specific IgE (171 infants had ≥1 positive value for food allergens, and 15 infants had ≥1 positive value for aeroallergens).

†Adjusted for age, sex, and race/ethnicity.

‡Highest eosinophil count during inpatient stay available for 551 (60%) infants; rate of missing value did not differ according to profiles ($P = .81$).**TABLE IV.** Association of severe bronchiolitis profiles with nasopharyngeal microbiota profiles

Nasopharyngeal microbiota profile (n = 912)	All (%)	Severe bronchiolitis profiles					
		Profile A		Profile B (reference)		Profile C	
		Percentage	OR* (95% CI)	Percentage	OR* (95% CI)	Percentage	OR* (95% CI)
<i>Moraxella</i> dominant	22	29	1.44 (0.84-2.46)†	23	1	17	0.75 (0.49-1.14)
<i>Haemophilus</i> dominant	19	29	2.31 (1.31-4.06)	13	1	24	2.04 (1.32-3.16)
Mixed profile (reference)	31	27	1	32	1	31	1
<i>Streptococcus</i> dominant	28	16	0.61 (0.34-1.11)	32	1	29	0.93 (0.64-1.35)

Results in boldface are statistically significant for comparison of profile A or profile C versus profile B. For both microbiota profiles and severe bronchiolitis profiles, the largest group was used as the reference category in adjusted analyses.

OR, Odds ratio.

*Multinomial logistic regression model adjusted for age, sex, race/ethnicity, lifetime history of antibiotic use, history of corticosteroid use, and use of antibiotics during the ED visit.

†Statistically significant difference for comparison of profile A versus profile C.

microbiota profile. In addition, profile A was the group with the largest proportion of children with a *Moraxella*-dominant microbiota profile, whereas the proportion was the lowest in profile C children. This difference was significant when comparing profile A children with profile C children (odds ratio, 1.93; 95% CI, 1.08-3.45; $P = .03$).

Severe bronchiolitis profiles and respiratory outcomes by age 3 years

By age 3 years, 251 (27%) children had recurrent wheezing overall. Recurrent wheezing was present in 43% of profile A children, 21% of profile B children, and 29% of profile C children. The Kaplan-Meier plot (Fig 2) indicates increased risk of recurrent wheezing by age 3 years in profile A children and, to a lower extent, in profile C children compared with profile B children. These results were confirmed in multivariable Cox proportional hazards models (Table V): compared with profile B, we

observed significantly increased risk of recurrent wheezing associated with profile A (hazard ratio, 2.64; 95% CI, 1.90-3.68; $P < .001$) and profile C (hazard ratio, 1.51; 95% CI, 1.14-2.01; $P = .004$). We found similar associations with use of ICSs/montelukast by age 3 years. When examining doctor-diagnosed asthma, increased risk was also observed in profile A children compared with profile B children; in contrast, increased risk of asthma was not significantly associated with profile C. Significant differences for the risk of each outcome were also observed when comparing profile A children with profile C children.

Results were unchanged in sensitivity analyses (see Table E4 in this article's Online Repository at www.jacionline.org), where associations were further adjusted for day care attendance, exposure to environmental tobacco smoke, maternal smoking during pregnancy, number of siblings at home, gestational age, delivery mode (vaginal vs caesarian section), breast-feeding (from birth to age 3 months), and annual household income or

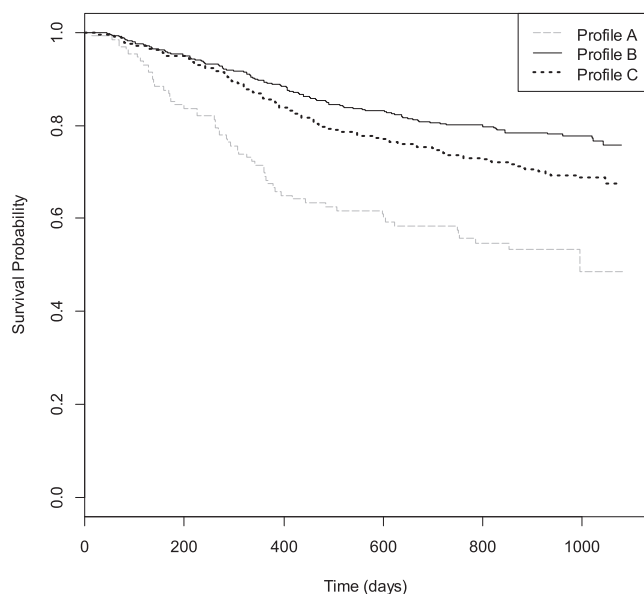


FIG 2. Kaplan-Meier survival curve for the risk of recurrent wheezing by age 3 years according to the severe bronchiolitis profile at baseline (severe bronchiolitis hospitalization). Time in days since hospital discharge. Survival indicates absence of recurrent wheezing. $P < .001$, log-rank test.

when we used a model accounting for a potential center effect (patient clustering at the hospital level) through a generalized estimating equations approach. Moreover, the association between severe bronchiolitis profiles and recurrent wheezing was not modified by age, sex, or race/ethnicity (no P values for interaction were statistically significant).

DISCUSSION

Three distinct clinical profiles of severe bronchiolitis were identified in a large sample of infants hospitalized for bronchiolitis. Profile A was characterized by a history of breathing problems/eczema during infancy and non-RSV (mostly rhinovirus) infection, profile B had the largest probability of RSV infection and resembled classic RSV-induced bronchiolitis, and profile C was the most severely ill group. Furthermore, for the first time, we showed significant differences across the profiles for biological markers and nasopharyngeal microbiota at baseline, as well as respiratory outcomes by age 3 years. Notably, compared with profile B, profile A children had a more than 2-fold increased risk of recurrent wheezing by age 3 years; to a lesser extent, the risk was also augmented in profile C children.

The severe bronchiolitis profiles identified by using LCA are consistent with earlier findings in similar US and Finnish cohorts of children hospitalized for bronchiolitis in which 3 to 4 profiles were identified by using the same clustering method.⁹ A larger number of profiles was found in the largest cohort ($n = 2207$), in which more heterogeneity might have been observed. In all 3 cohorts, including the current study, profile A, which was characterized by non-RSV (mostly rhinovirus) infection and history of breathing problems and eczema, was clearly identified as a separate group. The other profiles identified in the 3 cohorts all had a greater probability of RSV infection than profile A and differed according to level of acute severity. In addition to similarity with previous studies, the validity of the profiles is supported by the high value of mean class membership probability.

In the previous cross-sectional analysis of US and Finnish children hospitalized for bronchiolitis, we hypothesized that profile A was composed of children presenting with early signs of asthma during a severe bronchiolitis episode but did not examine long-term respiratory outcomes.⁹ Findings from the current study provide support for this hypothesis, especially the clear prospective association between profile A bronchiolitis and development of recurrent wheezing by age 3 years. Not all children with recurrent wheezing develop asthma. However, early (age ≤ 3 years) recurrent wheezing is a strong predictor of later asthma development.²² Moreover, although a longer follow-up is needed to ultimately confirm an association with development of childhood asthma, a prospective association between profile A bronchiolitis was also observed with development of doctor-diagnosed asthma by age 3 years.

Whether viral infections have a causal role in the development of recurrent wheezing and asthma, are a sign of susceptibility to respiratory illnesses, or correspond to their first manifestations is a long-running debate.^{1,2,23} The hypothesis that acute respiratory tract infections consist of distinct endotypes with different roles on the causal pathway to asthma might help clarify this important question. Profile A infants often had a clinical history suggestive of susceptibility to asthma or atopic diseases, including a history of breathing problems or eczema or prior use of corticosteroids. The hypothesis that some children with a greater propensity toward rhinovirus infection also have increased asthma susceptibility is consistent with a few earlier reports.¹ In particular, early signs of atopic conditions have been found to precede rhinovirus-induced infections in children at high risk for asthma or allergies.¹⁴ However, this susceptibility hypothesis might not be restricted to rhinovirus. An increased risk of asthma associated with the frequency of troublesome respiratory episodes in the first 3 years of life (suggestive of potential susceptibility or exaggerated response to infections) rather than with the specific viral etiologies was reported in a Danish birth cohort study.²³ In addition, both RSV- and rhinovirus-related illnesses share important susceptibility genetic variants with asthma.^{1,24} In the current study, although rhinovirus infection was markedly more common in profile A children, nearly half of them were not infected with rhinovirus; they either had other pathogens (eg, human metapneumovirus B, adenovirus, or bocavirus) or no pathogen was identified.

Profile C infants, who also had greater risk of recurrent wheezing (compared with profile B infants), were younger at bronchiolitis hospitalization and did not have a distinctive clinical history; however, they were the most severely ill group. Bronchiolitis severity has been found to increase asthma risk in a dose-response manner.²⁵ Although severe RSV infections and related inflammation have been suggested to cause long-term lung damage, evidence for a causal mechanism is lacking.² Nonetheless, differences in the features of profiles A and C and their differential association with biomarkers and nasopharyngeal microbiota suggest that they might be associated with recurrent wheezing through distinct pathways.

Higher blood eosinophil counts were observed in profile A children compared with the 2 other groups. Blood eosinophilia in childhood²⁶ or during bronchiolitis and early respiratory tract infection episodes^{8,27,28} has been associated with later development of recurrent wheezing or asthma. However, the exact role of eosinophils, both during acute respiratory tract infections and in patients with chronic asthma, remains poorly

TABLE V. Associations between severe bronchiolitis profile at baseline and respiratory outcomes at age 3 years

	No.	Recurrent wheezing*		ICS or montelukast use†		Doctor-diagnosed asthma‡	
		No. of events	HR (95% CI)	No. of events	HR (95% CI)	No. of events	OR (95% CI)
Profile A	136	59	2.64 (1.90-3.68)§	64	2.55 (1.85-3.51)§	49	2.79 (1.78-4.39)§
Profile B	452	97	1 (reference)	109	1 (reference)	78	1 (reference)
Profile C	333	95	1.51 (1.14-2.01)	123	1.84 (1.42-2.40)	72	1.38 (0.95-2.00)

Cox proportional hazard models (recurrent wheezing or ICS or montelukast use) or multinomial logistic regression model (doctor-diagnosed asthma) adjusted for age at baseline, sex, and race/ethnicity are shown. Results in boldface are statistically significant for comparison of profile A or profile C versus profile B.

HR, Hazard ratio; OR, odds ratio.

*Having at least 2 corticosteroid-requiring exacerbations in 6 months or having at least 4 wheezing episodes in 1 year that last at least 1 day and affect sleep.

†Parent-reported use of ICS or montelukast.

‡Parental report of doctor-diagnosed asthma available for 837 (91%) children.

§Statistically significant difference for comparison of profile A versus profile C.

understood.^{29,30} During acute respiratory tract infection, greater numbers of eosinophils have been found in patients with rhinovirus infection^{31,32}; on the other hand, in patients with RSV-induced bronchiolitis, a potential eosinopenic response to infection has been reported.^{27,28} In older children and adults with asthma, greater blood eosinophil counts have been associated with disease severity/activity and type 2 inflammation.³⁰ Thus our results might reflect either a different immune response to infection in profile A infants or higher eosinophil counts independently from infection in this group compared with others.

In contrast to the eosinophil findings, we did not observe a significant association between severe bronchiolitis profiles and allergy markers, although greater total IgE levels on the one hand and lower odds of monosensitization on the other hand were suggested in profile A infants. Eosinophilia and allergic sensitization in childhood both are associated with increased asthma risk and have been suggested to have a role in at least some common causal pathways.³³ However, these conditions have been independently associated with asthma and might relate to its development at different time windows.²⁶ Indeed, sensitization is highly dependent on age and is less common in infants³⁴; its relevance as a predictor of wheezing outcomes and asthma at such an early age is disputed.^{34,35} In future work, we will study associations of severe bronchiolitis profiles in infancy with phenotypes of allergic sensitization during childhood^{36,37} and asthma to provide further insight into potential causal pathways.

We also found greater cathelicidin levels in profile A children compared with those in the other groups. Human cathelicidin is an innate immunity host defense peptide with immunomodulatory properties.^{17,38} Although vitamin D is one of the factors regulating cathelicidin production and activation,³⁸ the profiles did not differ with regard to 25(OH)D levels. Direct antiviral activity of cathelicidin against both RSV and rhinovirus has been reported in experimental studies³⁹; however, lower cathelicidin levels were found in children with RSV infection compared with those with rhinovirus or other non-RSV infection in 2 cohorts of children hospitalized for severe bronchiolitis, including MARC-35.^{17,40,41} This is consistent with the lower cathelicidin levels found in profile B and C infants compared with profile A infants. Although the exact mechanisms and features of profile A that are driving this association are beyond the scope of our data, our results might reflect a different immune response during acute bronchiolitis in profile A children.

Nasopharyngeal microbiota also differed according to severe bronchiolitis profiles. Interestingly, although profiles B and C did

not differ for the investigated biomarkers, we observed distinct nasopharyngeal microbiota profiles in these groups. The *Haemophilus*-dominant microbiota profile was more common in profile C infants than in profile B infants, which is consistent with its association with increased bronchiolitis severity previously reported in MARC-35¹⁸ and with the severity of RSV-induced infection in young children.⁴² Profile A was characterized by increased proportions of children with either the *Haemophilus*-dominant or *Moraxella*-dominant profile. High abundance of *Haemophilus* and *Moraxella* has been reported in MARC-35 infants with rhinovirus infection.⁴³ Furthermore, in a Danish birth cohort specific bacterial colonization in the airways of neonates, including *Haemophilus influenza* and *Moraxella catarrhalis*, was associated with later development of pneumonia or bronchiolitis⁴⁴ and persistent wheeze and asthma.⁴⁵ In older children *Moraxella* abundance in the nasal microbiota was also associated with asthma.¹⁹ The timing and mechanisms of the interplay between viral infections, airway microbiota, and host immune response in relation to respiratory outcomes have yet to be determined.^{16,19} Although our findings are consistent with previous studies reporting associations between microbiota profiles and individual characteristics of bronchiolitis (severity and viral etiology),^{18,42,43} they question whether microbiota profiles are independently associated with each of these characteristics or are part of the features of distinct bronchiolitis endotypes. Overall, our findings add evidence to a contribution of both viruses and bacteria to the pathogenesis of acute and chronic respiratory illnesses.^{16,42}

This study had potential limitations. First, recurrent wheezing was defined by using parental reports of breathing problems episodes. Although “breathing problems” were clearly described to parents during interviews with intent to identify major respiratory symptoms, such as wheezing, cough, and shortness of breath (see details in the [Methods](#) section in this article’s Online Repository) and only those episodes that lasted at least 1 day and affected sleep,²¹ the outcome was not confirmed by a physician. However, our results were confirmed when examining ICS or montelukast use by age 3 years, a more objective outcome, and development of doctor-diagnosed asthma by age 3 years.¹³

Second, the severe bronchiolitis profiles identified in the current study have been defined in a cohort of hospitalized infants, which only represent a small proportion of all patients with bronchiolitis. It is likely that additional bronchiolitis profiles exist, including milder cases. However, it is relevant from a public health point of view to study patients with most severe bronchiolitis given that they are at high risk for asthma

development,²⁵ and bronchiolitis is the most common reason for hospitalization in US infants.⁶ For a more complete understanding of bronchiolitis, similar clustering approaches should be applied to other cohorts with different designs, in particular those including outpatient cases; indeed, beyond having distinct clinical presentations, outpatient cases can have differences in risk factors for bronchiolitis and sociodemographic characteristics.

Finally, the patient's profile, as defined based on a statistical clustering approach, cannot directly be assessed in clinical settings. However, we have identified in Fig 1 the main features of each profile to help the physicians and investigators identifying patients more likely to belong to each group. Blood eosinophilia might be an additional relevant marker to identify profile A children. Longer follow-up is needed to better determine the clinical significance of our results and before suggestions can be made about differential management of children with severe bronchiolitis according to clinical history, viral etiology, and levels of acute severity. Nonetheless, our findings might help identify, among children hospitalized for bronchiolitis, subgroups with particularly increased risk of recurrent wheezing and asthma.

In summary, our findings support the existence of several entities under the clinical diagnosis of bronchiolitis differentially associated with long-term outcomes, such as development of recurrent wheezing. Moreover, associations between the severe bronchiolitis profiles and several objectively measured biomarkers suggest a biological basis for the observed heterogeneity. Further work aiming at phenotyping and endotyping bronchiolitis through clinical, epidemiologic, and molecular approaches (including -omics data, such as host genome, transcriptome, and epigenome)¹⁰ should help improve disease management and clarify the relationship of infant bronchiolitis with the development of asthma.

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Key messages

- In a large cohort of infants hospitalized for bronchiolitis, the authors used a clustering approach to identify 3 distinct clinical profiles of severe bronchiolitis.
- Significant differences across the profiles for allergy and inflammatory markers and nasopharyngeal microbiota were found, suggesting a biological basis for the observed heterogeneity.
- The severe bronchiolitis profiles were differentially associated with development of recurrent wheezing by age 3 years.

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METHODS

Data collection

Nasopharyngeal aspirate collection and virology testing at baseline hospitalization.

Nasopharyngeal aspirates were collected by trained site personnel using the standardized protocol used in a previous cohort study of children with bronchiolitis.^{E1} All sites used the same collection equipment (Medline Industries, Mundelein, Ill) and collected the samples within 24 hours of hospitalization. The nasopharyngeal sample was added to transport medium, immediately placed on ice, and then stored at -80°C . Frozen samples were shipped in batches on dry ice to Baylor College of Medicine (Houston, Tex), where they were tested for 17 respiratory viruses by using real-time PCR assays, as previously described.^{E2} Tested viruses included RSV types A and B; human rhinovirus; parainfluenza virus types 1, 2, and 3; influenza virus types A and B; 2009 novel H1N1; human metapneumovirus; coronaviruses NL-65, HKU1, OC43, and 229E; adenovirus; human bocavirus type 1; and enterovirus. These tests are routinely conducted in the laboratory of one of the investigators (P.A.P.).

Blood specimens at baseline hospitalization: Allergen-specific IgE, total IgE, 25(OH)D, and LL-37 levels.

Trained study personnel collected blood specimens during hospitalization; 79% of the time, the collection occurred within 24 hours of hospitalization. Total IgE and allergen-specific IgE tests were performed by Phadia Immunology Reference Laboratory (Portage, Mich) with ImmunoCAP Total IgE and 2 different assays (ImmunoCAP Specific IgE and ImmunoCAP ISAC) for specific IgE. The specific IgE allergen assays conducted were for milk, egg white, peanut, cashew nut, and walnut; a positive test result was defined as 0.35 kU/L or greater. The ImmunoCAP ISAC microarray immunoassay measures IgE antibodies to 112 components from 51 allergen sources including foods (eg, eggs, cow's milk, fish, shellfish, tree nuts, peanuts, soybeans, wheat, and some fruits) and aeroallergens (eg, grasses, weeds, trees, animals [cat, dog, horse, and mouse], molds, dust mites, and cockroach). A positive result was defined as 0.30 ISAC standardized units or greater; ISAC standardized units provide an indication of IgE levels and are standardized to ImmunoCAP Specific IgE units. Serum 25(OH)D concentrations were measured by using liquid chromatography–tandem mass spectrometry. LL-37 is the main active form of human cathelicidin, and serum LL-37 concentration was quantified by using a commercially available ELISA (Hycult Biotech, Uden, The Netherlands), as described previously.^{E3}

16S rRNA gene sequencing and compositional analysis.

16S rRNA gene sequencing methods were adapted from the methods developed for the National Institutes of Health Human Microbiome Project. The details of the methods have been published elsewhere.^{E4} Briefly, bacterial genomic DNA was extracted with a MO BIO PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, Calif). The 16S rDNA V4 region was amplified by using PCR and sequenced in the MiSeq platform (Illumina, San Diego, Calif) by using a 2×250 -bp paired-end protocol that yields paired-end reads that overlap almost completely. Primers used for amplification contained adapters for MiSeq sequencing and single-end barcodes, allowing pooling and direct sequencing of PCR products.

Sequencing read pairs were demultiplexed based on the unique molecular barcodes, and reads were merged with USEARCH v7.0.1090.^{E5} Rarefaction curves of bacterial operational taxonomic units (OTUs) were constructed by using sequence data for each sample to ensure coverage of the bacterial diversity present. Samples with suboptimal amounts of sequencing reads were resequenced to ensure that the majority of bacterial taxa were encompassed in our analyses.

16S rRNA gene sequences were clustered into OTUs at a similarity cutoff value of 97% by using the UPARSE algorithm.^{E6} OTUs were determined by mapping the centroids to the SILVA database containing only the 16S V4 region to determine taxonomies.^{E7} A custom script constructed a rarefied OTU table (rarefaction was performed at only one sequence depth) from output files generated in the previous 2 steps for downstream analyses of α -diversity (ie, Shannon index) and β -diversity (ie, weighted UniFrac distance). We used multiple quality control measures, including use of

non-template controls in microbial DNA extraction, 16S rRNA gene amplification, and amplicon sequencing processes.

The relative abundance of each OTU was calculated for each nasopharyngeal sample. Analyses were conducted at the genus level because each genus was dominated by 1 OTU. All OTUs assigned to the same genus were collapsed into a single group for reporting. As described earlier,^{E4} to identify the nasopharyngeal microbiota profiles, we performed unbiased clustering by partitioning around medoids using weighted UniFrac distance. Each partitioning around medoids cluster is defined by a point designated as the center (the “medoid”) and minimizes the distance between samples in a cluster.

Respiratory outcomes by age 3 years. Respiratory outcomes were assessed from follow-up interviews with children eligible for long-term follow-up (ie, who completed the run-in procedure: contact at 1 week after hospital discharge and 3 weeks after hospitalization; 91% of infants initially enrolled). Trained interviewers began interviewing parents/legal guardians by telephone when the children were age 6 months and then in 6-month intervals (ie, at age 12, 18, 24, 30, and 36 months). For children older than 6 months at hospital admission (baseline), the 6-month intervals commenced at age 12 months. At each follow-up interview, data were collected about the child's prescribed medication (ICSs or montelukast to help prevent or treat breathing problems) and “breathing problem” episodes since study enrollment (6-month follow-up) or last follow-up interview (12- and 18-month follow-up). Breathing problems were defined to the parents as follows: “You may recognize breathing problems when [child] is coughing a lot, or breathing faster or breathing harder than normal. [Child]’s health care provider may even hear wheezing (high-pitched whistling sounds when breathing out) and have said that [child] has bronchiolitis, wheezy bronchitis, reactive airways, asthma, or pneumonia. When we say ‘breathing problems’ we are talking about more than a stuffy nose. We are talking about episodes where the coughing wakes [child] at night (or may even cause vomiting), or when [child] has wheezing, or shortness of breath.” For each reported breathing problem, parents were also asked about the date when the breathing problem started, irrespective of whether it affected the child's sleep, and about medication use the days before, during, or after the breathing problem.

These data were compiled to assess recurrent wheezing by age 3 years, which was defined in close accordance with the 2007 National Institutes of Health asthma guidelines^{E8} as having at least 2 corticosteroid-requiring breathing problems within 6 months or having at least 4 breathing problems episodes within 12 months that lasted at least 1 day and affected sleep. Treatments with either inhaled, oral, or intravenous corticosteroids were considered. In the survival analysis time to event was calculated in days from hospital discharge to the date when the recurrent wheezing outcome definition was met. Participants were censored at the date of the 36-month follow-up or at their last follow-up interview.

Finally, asthma by age 3 years was defined based on parental report of doctor-diagnosed asthma in the 30- or 36-month surveys (“Has child ever been told by a doctor or other health professional that [he/she] has asthma?”).

Severe bronchiolitis profiles at baseline: LCA

Severe bronchiolitis profiles were defined by using LCA, a clustering approach that is used to identify more homogeneous subgroups of patients from a large set of observed clinical characteristics. We used the same methodology as previously described in similar US and Finnish cohorts^{E9}: we first selected a large set of 15 variables (Table E1). This set of variables was reduced by using a multiple correspondence analysis (MCA) to select the most relevant variables for cluster analysis, as described below. LCA was then performed based on selected variables.

MCA. The purpose of the MCA was to reduce the number of variables to introduce in the LCA model by selecting variables with the highest contributions to the first axes and avoiding selecting several variables representing similar dimensions. Indeed, because the LCA is based on an assumption of conditional independence (ie, within each latent class, all variables are statistically independent), it is recommended to avoid introducing several highly dependent variables in the LCA model.

Variables included in the MCA were as follows: (1) medical history (history of breathing problems, history of eczema, and parental history of asthma); (2) clinical characteristics at time of ED presentation (fever, respiratory rate, retraction severity, oxygen saturation, respiratory symptoms [cough and wheeze], and inadequate oral intake); (3) characteristics of the hospital course (length of stay, intensive care unit [ICU] admission with or without mechanical ventilation; ie, intubation or continuous positive airway pressure [CPAP]) or highest respiratory rate; and (4) viral etiology, with a focus on RSV and rhinovirus. Results of the MCA are presented in Table E1. The first dimension was mostly characterized by indicators of the severity of the disease (ICU admission and intubation or CPAP, length of stay, severity of retractions, and respiratory rate). The second dimension was characterized by history of breathing problems and eczema, wheezing and cough at ED presentation, and some severity indicators (retractions). The third dimension was mainly characterized by the type of viral infection.

Only variables contributing the most to each dimension were selected for the LCA to limit the number of parameters in the LCA model.^{E10,E11} For instance, among the variables indicating a more severe clinical course (ICU with or without mechanical ventilation or length of stay), only length of stay was included in the LCA. A similar classification was observed when length of stay was replaced by ICU and intubation or CPAP in the LCA (sensitivity analysis, data not shown).

LCA. All subjects were included in the LCA models because missing data for the variables are handled in the procedure. A high proportion of observations (92%) were “fully observed” (ie, did not have any missing value for the variables entered in the LCA model). For each given number of classes, the model was estimated 10 times, and the model with the highest likelihood was selected. To select the number of class, models with 2 to 5 classes were examined with regard to the Bayesian information criterion (BIC; Fig E1),^{E10,E12,E13} and we retained the solution with the lowest BIC. The mean class membership probability (highest posterior probability) range was 81% to 84%, indicating that participants were assigned to classes with a fairly high probability.

LCA results are traditionally presented as probability for a subject belonging to a given class to present with each characteristic (variables used for classification), as estimated by using the model. These results are presented in Table E2. In Table I we presented LCA results as the actual proportion in the study population of children presenting the characteristic according to the class to which they were assigned by using LCA. This presentation was chosen for consistency with Table II, in which additional characteristics (variables not used in the classification) are presented by profile. Table I (actual proportion) and Table E2 (probabilities estimated by the LCA model) differ slightly but lead to similar class description and interpretation.

Statistical software. All analyses were performed with R version 3.0.2 software (R Project for Statistical Computing, www.r-project.org). The R poLCA package (a package for polytomous variable LCA) was used for the LCA model.

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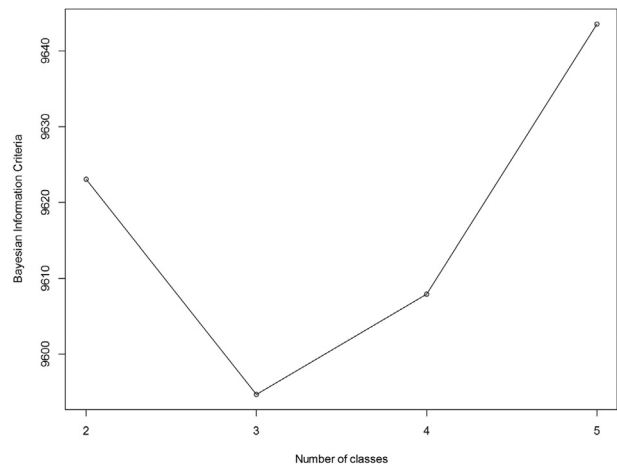


FIG E1. Model fit information for 2- to 5-class LCA models.

TABLE E1. Contribution of the variables to the 3 first dimensions in the MCA

	Dimension 1	Dimension 2	Dimension 3
Medical history			
History of breathing problems: No	0.03	4.80	0.89
History of breathing problems: Yes	0.10	18.0	3.33
History of eczema: No	0.02	1.46	0.00
History of eczema: Yes	0.10	8.31	0.02
Parental history of asthma: No	0.20	1.02	0.05
Parental history of asthma: Yes	0.42	2.09	0.11
ED presentation			
Fever: No	0.03	0.77	0.30
Fever: Yes	0.09	2.21	0.86
Respiratory rate <60	1.36	1.13	2.28
Respiratory rate ≥60	3.01	2.49	5.02
Retractions: None	2.37	6.74	9.23
Retractions: Mild	2.38	0.03	3.37
Retractions: Moderate to severe	7.56	3.85	0.01
O ₂ saturation by pulse oximetry: <90	7.64	0.54	1.77
O ₂ saturation by pulse oximetry: 90-93.9	1.32	0.89	0.90
O ₂ saturation by pulse oximetry: ≥94	2.06	0.03	0.00
Cough: No	0.58	2.68	5.28
Cough: Yes	0.02	0.10	0.19
Wheeze: No	0.02	9.28	7.20
Wheeze: Yes	0.01	5.48	4.25
Inadequate oral intake: No	3.67	0.27	0.09
Inadequate oral intake: Yes	2.42	0.18	0.06
Hospitalization course			
No ICU admission	5.28	0.02	0.64
ICU admission without intubation or PPV	0.90	1.43	0.22
ICU admission with intubation or PPV	16.8	0.07	1.86
Length of stay <3 d	7.62	0.00	0.23
Length of stay 3-6 d	4.81	0.30	3.79
Length of stay ≥7 d	10.7	1.80	8.80
Highest respiratory rate <60 breaths/min	8.91	0.12	0.43
Highest respiratory rate ≥60 breaths/min	8.65	0.12	0.42
Virus			
RSV: No	0.50	10.9	21.4
RSV: Yes	0.10	2.28	4.49
Rhinovirus: No	0.06	2.12	2.49
Rhinovirus: Yes	0.26	8.51	10.0

Variables in boldface are the 9 variables selected to be included in the LCA.

PPV, Positive pressure ventilation (CPAP or high-flow oxygen therapy).

TABLE E2. LCA results presented as probability of infant presenting with characteristics according to the profiles (A-C; n = 921)

	Profiles		
	A (15%)	B (49%)	C (36%)
History of breathing problems	44	12	22
History of eczema	24	14	13
Wheeze at ED presentation	69	59	66
Cough at ED presentation	92	99	94
Retractions at ED presentation			
None	23	23	12
Mild	43	54	31
Moderate to severe	34	24	56
Hospital length-of-stay (d)			
<3	87	82	20
3-6	12	18	60
≥7	2	0	20
Highest respiratory rate during entire hospital stay ≥60 breath per min	24	29	88
Viral etiology			
RSV	21	96	89
Rhinovirus	55	12	16

Results are presented as the probability of infants presenting characteristic within profiles A to C expressed as percentages.

TABLE E3. Association of severe bronchiolitis profiles with food allergen or aeroallergen sensitization profiles (n = 914)

	All (%)	Severe bronchiolitis profiles					
		Profile A		Profile B (reference)		Profile C	
		Percentage	OR* (95% CI)	Percentage	OR*	Percentage	OR* (95% CI)
Nonsensitized	81	82	1	81	1	80	1
Monosensitization	15	11	0.56 (0.30-1.03)	16	1	16	1.06 (0.71-1.57)
Multisensitization	4	7	1.34 (0.54-3.30)	3	1	3	1.07 (0.47-2.40)

Monosensitization, Only 1 positive value for allergen-specific IgE; *multisensitization*, 2 or more positive values for allergen-specific IgE.

OR, Odds ratio.

*Multinomial logistic regression model adjusted for age, sex, and race/ethnicity.

TABLE E4. Sensitivity analyses for associations between severe bronchiolitis profile at baseline and respiratory outcomes at age 3 years

	No.	Recurrent wheezing*		ICS or montelukast use†	
		No. of events	HR (95% CI)	No. of events	HR (95% CI)
Model further adjusted for day care attendance, number of siblings at home, exposure to environmental tobacco smoke, maternal smoking during pregnancy, gestational age, delivery mode (vaginal vs caesarian section), breast-feeding (from birth to age 3 mo), and annual household income.					
Profile A	136	59	2.58 (1.87-3.55)	64	2.76 (1.99-3.85)
Profile B	452	97	1 (reference)	109	1 (reference)
Profile C	333	95	1.51 (1.16-1.95)	123	1.80 (1.37-2.35)
Model‡ accounting for a potential center effect (patient clustering at hospital level)					
Profile A	136	59	2.43 (1.77-3.34)	64	2.55 (1.71-3.80)
Profile B	452	97	1 (reference)	109	1 (reference)
Profile C	333	95	1.47 (1.20-1.79)	123	1.85 (1.52-2.24)

All results are adjusted for age at baseline, sex, and race/ethnicity. The proportional hazard assumption was tested for all covariates; when not valid, stratification was used instead of adjustment.

HR, Hazard ratio.

*Having at least 2 corticosteroid-requiring exacerbations in 6 months or having at least 4 wheezing episodes in 1 year that last at least 1 day and affect sleep.

†Parent-reported use of ICSs or montelukast.

‡Generalized estimating equations.