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Neonatal bronchial hyperresponsiveness precedes acute severe viral bronchiolitis in infants

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Background: Respiratory syncytial virus and other respiratory tract viruses lead to common colds in most infants, whereas a minority develop acute severe bronchiolitis often requiring hospitalization. We hypothesized that such an excessive response to respiratory tract viral infection is caused by host factors reflected in pre-existing increased bronchial responsiveness.

Objective: We sought to compare bronchial responsiveness and lung function in 1-month-old neonates who later develop acute severe bronchiolitis with those who do not.

Methods: We measured infant lung function (n = 402) and bronchial responsiveness to methacholine (n = 363) using the raised-volume rapid thoracoabdominal compression technique before any respiratory symptoms in 1-month-old neonates from the Copenhagen Prospective Study of Asthma in Childhood birth cohort born to mothers with asthma. The children were prospectively monitored for respiratory symptoms and given a diagnosis of acute severe bronchiolitis according to a fixed algorithm.

Results: Thirty-four (8.5%) infants had acute severe bronchiolitis before 2 years of age, 21 (62%) were hospitalized, and 23 (67%) of the cases were associated with respiratory syncytial virus. Children who later had acute severe bronchiolitis irrespective of viral species had a 2.5-fold increased responsiveness to methacholine (provocative dose of methacholine producing a 15% decrease in transcutaneous oxygen pressure [PD_{15}]) at age 1 month compared with control subjects (median PD_{15} in cases vs control subjects, 0.13 vs 0.33 μmol; $P = .01$), whereas differences in baseline airflow were not significant for forced expiratory volume at 0.5 seconds (mean z score for cases vs control subjects, −0.18 vs −0.01; $P = .36$) and forced expiratory flow at 50% of forced vital capacity (mean z score for cases vs control subjects, −0.37 vs −0.09; $P = .13$).

Conclusion: Bronchial hyperresponsiveness in at-risk neonates precedes acute severe bronchiolitis in response to infections with respiratory tract virus. (J Allergy Clin Immunol 2012;130:354-61.)

Key words: *Bronchial responsiveness, infants, lung function measurements, viral bronchiolitis*

Children with acute severe bronchiolitis in relation to airway virus infections, such as respiratory syncytial virus (RSV) and rhinovirus, have an increased risk of asthma and allergy at school age.[1-4] This has led to the suggestion of a causal role of RSV and rhinovirus on the risk of asthma.[5,6] On the other hand, asthma heredity and troublesome asthma-like symptoms in early infancy are significant risk factors for subsequent RSV-related hospitalization during infancy,[7,8] suggesting that host factors might determine a shared predisposition to viral bronchiolitis and childhood asthma. We hypothesized that pre-existing abnormal infant spirometry and bronchial hyperresponsiveness in neonates precedes later development of acute severe bronchiolitis.

We recently demonstrated that neonatal pulmonary function was associated with asthma by age 7 years in the Copenhagen Prospective Studies on Asthma in Childhood ($COPSAC_{2000}$).[9] The present study investigates whether neonatal pulmonary function is also associated with acute severe bronchiolitis in infants (ie, whether neonatal lung function is a shared host factor for both asthma and acute severe bronchiolitis).

This study was nested in $COPSAC_{2000}$, a prospective clinical study of a birth cohort of 411 neonates born of mothers with a history of asthma.[10] The study included measurements of infant spirometry and bronchial responsiveness to methacholine at 1 month of age in 402 of the 411 cohort participants. The deep phenotyping with close clinical, single-center follow-up of the birth cohort by the COPSAC physicians allowed prospective identification of infants who developed acute severe bronchiolitis.

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The Copenhagen Studies on Asthma in Childhood (COPSAC) is funded by private and public research funds listed on www.copsac.com. The Lundbeck Foundation, the Danish Strategic Research Council, the Pharmacy Foundation of 1991, the Augustinus Foundation, the Danish Medical Research Council, and the Danish Pediatric Asthma Centre provided the core support for the COPSAC research center. S.L.J. is supported by a Chair from Asthma UK (CH11SJ), MRC Centre Grant G1000758, ERC FP7 Advanced grant 233015, and Predicta FP7 Collaborative Project grant 260895.

Disclosure of potential conflict of interest: S. L. Johnston has consultant arrangements with AstraZeneca, Centocor, Sanofi-Pasteur, Synairgen, GlaxoSmithKline, and Chiesi and has a share option with Synairgen. H. Bisgaard has consulted for Merck and Chiesi; has received lecture fees from Merck; receives research support from the Lundbeck Foundation, the Pharmacy Foundation of 1991, the Augustinus Foundation, the Danish Medical Research Council, and the Danish Pediatric Asthma Centre; and has provided legal consultation or expert witness testimony for the European Medicines Agency (EMEA) on guidelines on pediatric studies for documenting asthma drugs. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication November 17, 2011; revised April 25, 2012; accepted for publication April 26, 2012.

Available online June 17, 2012.

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0091-6749/$36.00

doi:10.1016/j.jaci.2012.04.045

Abbreviations used
COPSAC: Copenhagen Prospective Studies on Asthma in Childhood
FEF_{50}: Forced expiratory flow at 50% of forced vital capacity
$FEV_{0.5}$: Forced expiratory volume at 0.5 seconds
FVC: Forced vital capacity
IQR: Interquartile range
PD_{15}: Provocative dose of methacholine producing a 15% decrease in transcutaneous oxygen pressure
$PtcO_2$: Transcutaneous oxygen pressure
RSV: Respiratory syncytial virus

METHODS

Study population

Four hundred eleven infants born at term of mothers with a history of physician-diagnosed asthma were enrolled at 1 month of age in the $COPSAC_{2000}$ prospective birth cohort study.[11-13] Key exclusion criteria were symptoms of lower airway infection or neonatal mechanical ventilation before inclusion, gestational age of less than 36 weeks, and any congenital abnormality or systemic illness.

Ethics

The study was conducted in accordance with the guiding principles of the Declaration of Helsinki and was approved by the local ethics committee ([KF] 01-227/97) and the Danish Data Protection Agency (2008-41-1754). Both parents provided written informed consent before enrollment.

Neonatal lung function measurement

Infant spirometry was assessed during sedation with an oral dose of 90 mg/kg chloral hydrate at age 1 month by applying the raised-volume rapid thoracoabdominal compression technique in agreement with the American Thoracic Society and European Respiratory Society standards.[14] An inflatable "squeeze" jacket was wrapped around the sedated infant's chest and abdomen. Repeated ventilations to predefined mouth pressures ensured expansion of lung volume before an instant inflation of the jacket caused a full exhalation during which the flow was measured by using a pneumotachograph with an air-cushion facemask.[10,15]

The software identified forced vital capacity (FVC) as the first plateau on the volume-time curve; only measurements with an FVC appearing after 0.5 seconds and with the forced expiratory volume at 0.5 seconds ($FEV_{0.5}$) being less than or equal to the FVC were accepted. Three to 5 acceptable curves were obtained at each measurement; the curve containing the median value of $FEV_{0.5}$ was used for the analyses of both volume ($FEV_{0.5}$) and flow (forced expiratory flow at 50% of forced vital capacity [FEF_{50}]) parameters.

Baseline lung function measurement was repeated after a saline inhalation. Subsequently, methacholine was administered in quadrupling dose steps administered through a dosimeter attached to a nebulizer (SPIRA 08 TSM 133; Respiratory Care Center, Hämeenlinna, Finland), as previously detailed.[15] Bronchial responsiveness was assessed by means of repeated measurements of infant spirometry and continuous assessment of transcutaneous oxygen pressure ($PtcO_2$; TCM3; Radiometer, Copenhagen, Denmark). The provocative dose of methacholine producing a 15% decrease in transcutaneous oxygen pressure (PD_{15}) was estimated from the dose-response curves fitted with a logistic function. Our previous sensitivity analyses showed $PtcO_2$ determined bronchial responsiveness with greater sensitivity than any of the forced flow indices of infant spirometry[15,16]; therefore $PtcO_2$ (PD_{15}) was used to assess bronchial responsiveness in the present analyses.

Acute severe bronchiolitis cases

The birth cohort was prospectively monitored closely for respiratory symptoms with daily diary cards from 1 month of age and clinical examinations performed by the COPSAC physicians at the research clinic every 6 months and in cases of acute respiratory symptoms. Acute severe bronchiolitis was defined as an acute respiratory tract illness with onset before the age of 2 years based on symptoms of coryza progressing over a few days to cough, tachypnea, chest retractions, and auscultative widespread crepitation and/or rhonchi.[17-19]

In cases in which the infants had been brought directly to the local pediatric emergency department for admission, hospital records were retrieved and carefully reviewed to verify respiratory symptoms compatible with the abovementioned criteria for diagnosing acute severe bronchiolitis.

Viruses were detected by means of PCR analysis of nasopharyngeal aspirate samples for RSV; rhinoviruses; influenza viruses AH1, AH3, and B; parainfluenza viruses 1 to 3; coronaviruses 229E and OC43; picornaviruses; bocavirus; adenoviruses; and human metapneumovirus, as previously detailed.[20]

Bronchiolitis occurring before age 2 years irrespective of the viral trigger (ie, any bronchiolitis) was used as the primary end point, excluding children with acute asthma-like exacerbations. Secondary end points were (1) RSV-induced bronchiolitis, (2) non–RSV-induced bronchiolitis, (3) acute asthma-like exacerbations, (4) bronchiolitis before age 1 year, and (5) any bronchiolitis, including acute asthma-like exacerbations.

Atopic comorbidity

Recurrent episodes of troublesome lung symptoms were defined as 3 consecutive days recorded with significant cough and/or wheeze and/or dyspnea.[21] The number of episodes of troublesome lung symptoms before development of acute severe bronchiolitis among cases was compared with the number of episodes in the control group occurring before the median age at diagnosis of acute severe bronchiolitis in the cases.

Allergic sensitization was determined at age 18 months by means of measurement of serum specific IgE levels against 15 common inhalant and food allergens (cat, dog, horse, birch, timothy grass, mugwort, house dust mite, molds [*Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, and *Alternaria tenuis*], hen's egg, cow's milk, cod, wheat, peanut, soybean, or shrimp) using the ImmunoCAP assay (Pharmacia Diagnostics AB, Uppsala, Sweden). Sensitization was defined as a specific IgE level of 0.35 kU/L or greater[22,23] for any of the tested allergens and was analyzed as a dichotomized measurement.

Total IgE levels were measured at age 18 months by using ImmunoCAP (Pharmacia Diagnostics AB), with a detection limit of 2 kU/L.[22]

Blood eosinophil counts (10^9 cells per liter) were assessed at age 18 months.

Eczema was diagnosed by using the Hanifin-Rajka criteria, as previously detailed.[24] Skin lesions were described at both scheduled and acute visits according to predefined morphology and localization.

Genetics

The study population was genotyped for filaggrin-null mutations and *ORMDL3* and *DENND1B* variants, as described in detail in the Methods section in this article's Online Repository at www.jacionline.org.

Statistical analysis

Lung function data ($FEV_{0.5}$, FEF_{50}, and PD_{15} [$PtcO_2$]) were adjusted for lifespan and birth length by using a generalized linear model, as previously detailed.[16] Lifespan at examination date was calculated as the sum of estimated gestational age in weeks at birth and weeks since birth. The lung function measurements were log transformed before analysis to obtain normal distribution of data.

Generalized linear models were used to compare lung function data in subjects with and without acute severe bronchiolitis, assuming Gaussian distribution of the data and using the identity link function as in multiple regression analyses. Adjustment for significant and borderline-significant

TABLE I. Baseline characteristics of the study group

		Primary end point	Secondary end points			*P* value
	Control subjects (n = 366)	Bronchiolitis cases* (n = 34)	RSV-induced bronchiolitis cases (n = 23)	Non–RSV-induced bronchiolitis cases† (n = 9)	Asthma-like cases (n = 11)	Bronchiolitis cases vs control subjects
Baseline characteristics						
Sex						
Male (no.)	64.7% (22)	48.4% (177)	—	—	—	.07¶
Anthropometrics						
BMI at birth (cm/kg^2), median (IQR)	12.5 (11.6-13.7)	12.8 (12.0-13.7)	—	—	—	.25#
Environment						
Smoking, 3rd trimester (no.)	35.3% (12)	13.1% (48)	—	—	—	.005¶
Alcohol, 3rd trimester (no.)	14.7% (5)	18.6% (68)	—	—	—	.58¶
Cat at birth (no.)	17.7% (6)	14.8% (52)	—	—	—	.65¶
Dog at birth (no.)	14.7% (5)	13.6% (48)	—	—	—	.86¶
Older siblings at birth (no.)	52.9% (18)	42.4% (155)	—	—	—	.23¶
Household income at birth (no.)						.50‡‡
Low, <53,000€	30.3% (10)	29.6% (101)	—	—	—	
Medium, 53,000€-80,000€	54.6% (18)	46.6% (159)	—	—	—	
High, >80,000€	15.2% (5)	23.8% (81)	—	—	—	
Paternal history of asthma (no.)	24.2% (8)	16.5% (59)	—	—	—	.26¶
Age at start of day care (d), median (IQR)	296 (230-349)	341 (240-417)	—	—	—	.08#
Solely breast-fed (d), median (IQR)	120 (72-179)	122 (89-155)	—	—	—	.47#
Lung function at age 1 mo						
$FEV_{0.5}$ (z score), mean (SD)	−0.18 (0.91)	−0.01 (0.99)	—	—	—	.36#
FEF_{50} (z score), mean (SD)	−0.37 (1.05)	−0.09 (1.00)	—	—	—	.13#
PD_{15} ($PtcO_2$ [μmol]), median (IQR)	0.13 (0.07-0.60)	0.33 (0.12-0.88)	—	—	—	.01#
Genetics						
Filaggrin mutation	18.2% (6)	9.7% (34)	—	—	—	.13¶
ORMDL3 (TT)	48.5% (16)	26.7% (92)	—	—	—	.008¶
DENND1B genotype (rs2786098)			—	—	—	.17**
AA	3.5% (1)	4.2% (14)	—	—	—	
AB	13.8% (4)	27.5% (91)	—	—	—	
BB	82.7% (24)	68.3% (226)	—	—	—	
Atopic intermediary markers						
Allergic sensitization‡ (18 mo [no.])	9.1% (3)	12.0% (38)	0% (0)	22.2% (2)	20.0% (2)	.78††
Blood eosinophil count (18 mo [10^9/L]), median (IQR)	0.27 (0.18-0.43)	0.21 (0.13-0.32)	0.26 (0.18-0.43)	0.21 (0.15-0.38)	0.41 (0.22-0.48)	.03#
Total IgE (18 mo [kU/L]), median (IQR)	15.3 (6.3-29.5)	8.2 (3.9-17.1)	15.5 (6.3-28.0)	10.2 (4.7-30.2)	7.0 (5.2-30.9)	.03#
Episodes of troublesome lung symptoms,§ median (IQR)	2 (1-6)	1 (1-2)	1 (1-5)	6 (3-7)	2 (1-4)	.0005§§
Eczema (0-2 y [no.])	38.2% (13)	38.4% (131)	30.4% (7)	66.7% (6)	40.0% (4)	.98¶
Episode characteristics						
Fever at diagnosis‖ (no.)	—	59% (20)	57% (13)	56% (5)	36% (4)	—
Auscultation						
Crepitations (no.)	—	41% (14)	44% (10)	33% (3)	27% (3)	—
Rhonchi (no.)	—	85% (29)	78% (18)	100% (9)	91% (10)	—
Hospitalization (no.)	—	62% (21)	83% (19)	22% (2)	36% (4)	—
Days hospitalized, median (range)	—	3 (1-15)	4 (1-15)	2 (2-2)	2 (1-4)	—

(Continued)

baseline characteristics and intermediary atopic markers was done by adding the variables as covariates in the model.

The comparison of baseline characteristics and the development of atopic stigmata in cases versus control subjects were done by using univariate analyses (ie, t tests and χ^2 tests). The number of episodes of troublesome lung symptoms in cases versus control subjects was analyzed by using Poisson regression.

Analyses were done with SAS version 9.1 software for Windows (SAS Institute, Inc, Cary, NC). A P value of .05 or less was considered significant.

TABLE I. *(Continued)*

		Primary end point	Secondary end points			*P* value
	Control subjects (n = 366)	Bronchiolitis cases* (n = 34)	RSV-induced bronchiolitis cases (n = 23)	Non–RSV-induced bronchiolitis cases† (n = 9)	Asthma-like cases (n = 11)	Bronchiolitis cases vs control subjects
Treatment	—					—
Inhaled β_2-agonist (no.)	—	91% (31)	87% (20)	100% (9)	100% (11)	—
Inhaled corticosteroid (no.)	—	47% (16)	48% (11)	44% (4)	81% (9)	—
Oral corticosteroid (no.)	—	24% (8)	13% (3)	56% (5)	36% (4)	—
Intravenous corticosteroid (no.)	—	9% (3)	13% (3)	0	10% (1)	—
Nasal CPAP (no.)	—	9% (3)	13% (3)	0	0	—
Antibiotics (no.)	—	62% (21)	61% (14)	67% (6)	18% (2)	—
Age at diagnosis (d), median (IQR)	—	327 (128-446)	199 (88-359)	462 (430-551)	383 (324-576)	—
Time from lung function to diagnosis (d), median (range)	—	267 (8-617)	160 (8-500)	425 (91-617)	349 (191-677)	—

Statistical tests: ¶χ^2 test; #*t* test; **log-additive model; ††Fisher exact test; ‡‡1-way ANOVA; and §§Poisson regression

CPAP, Continuous positive airway pressure.

*Two bronchiolitis cases are without available nasopharyngeal suction.

†Two cases with picornavirus; 1 case with bocavirus; 1 case with rhinovirus; 1 case with bocavirus and rhinovirus; 1 case with human metapneumovirus, picornavirus, influenza virus, parainfluenza virus, coronavirus, and bocavirus; and 3 cases with negative nasopharyngeal suctions.

‡Any allergic sensitization (specific IgE >0.35 kU/L) against 15 common inhalant and food allergens (cat, dog, horse, birch, timothy grass, mugwort, house dust mite, molds, hen's egg, cow's milk, fish, wheat, peanut, soybean, or shrimp).

§Comparison of number of episodes in cases before development of acute bronchiolitis with number of episodes in control subjects occurring before median age at diagnosis of acute bronchiolitis (327 days) in the cases.

||Rectal temperature ≥38.0°C.

RESULTS

Study group selection

Infant spirometry was completed in 402 children, and bronchial responsiveness to methacholine was assessed in 363 of the 411 children in the cohort at age 1 month before the development of any respiratory symptoms.

Eleven children had an episode of acute asthma-like symptoms not fulfilling all the diagnostic criteria for acute severe bronchiolitis. These children were excluded because of a lack of objective signs of respiratory distress, such as tachypnea, chest retractions, or both (see Table I and Table E1 in this article's Online Repository at www.jacionline.org for baseline characteristics of the excluded children). These 11 children were excluded from both the case and control groups, leaving 400 evaluable children (see Fig E1 in this article's Online Repository at www.jacionline.org for the study group flow chart).

Bronchiolitis cases

Thirty-four (8.5%) of the 400 children in the selected cohort had acute severe bronchiolitis before age 2 years (median age at diagnosis, 327 days; interquartile range [IQR], 128-446 days). Of these 34 cases, 65% (n = 22) were boys, 59% (n = 20) had fever at the time of diagnosis (rectal temperature, >38.0°C), 41% (n = 14) had crepitations, and 85% (n = 29) had rhonchi at auscultation. Median time from infant spirometry to diagnosis of acute severe bronchiolitis was 267 days (range, 8-617 days). In 2 of the 34 bronchiolitis cases, there were no available nasopharyngeal aspirates. RSV was identified in 23 of the 32 bronchiolitis cases, whereas the remaining 9 cases were classified as having non–RSV-induced bronchiolitis (2 with picornavirus; 1 with bocavirus; 1 with rhinovirus; 1 with bocavirus and rhinovirus; 1 with human metapneumovirus, picornavirus, influenza virus, parainfluenza virus, coronavirus, and bocavirus; and 3 with negative nasopharyngeal suctions).

Hospitalization was required for 21 (62%) of the 34 bronchiolitis cases, with a median duration of hospitalization of 10 days (IQR, 6-21 days). Half of the admitted children needed treatment with nasal continuous positive airway pressure, one third were prescribed systemic corticosteroid treatment, and 90% received nebulized β_2-agonist. Children with acute severe bronchiolitis who were not hospitalized received outpatient treatment with inhaled β_2-agonists (92%), inhaled corticosteroids (62%), systemic corticosteroids (31%), and antibiotics (77%).

Baseline characteristics of children with acute severe bronchiolitis versus control subjects

Comparison of baseline characteristics of the bronchiolitis cases (n = 34) and control subjects (n = 366, Table I) showed that maternal smoking during the third trimester of pregnancy was significantly more common among infants with acute severe bronchiolitis compared with control subjects (35% vs 13%, $P = .005$). The *ORMDL3* TT genotype was also significantly associated with the development of acute severe bronchiolitis during the first 2 years of life (cases vs control subjects, 49% vs 27%; $P = .008$), whereas male sex (cases vs control subjects, 65% vs 49%; $P = .07$) and young age at the start of day care (median age at start for cases vs control subjects, 296 vs 341 days; $P = .08$) were borderline significant. We found no association between cases and control subjects with respect to anthropometrics; maternal alcohol consumption during the third trimester of pregnancy; the presence of a cat, a dog, or an older sibling in the home at birth; household income; length of sole breast-feeding; paternal history of asthma; filaggrin-null mutations; and *DENND1B* genetic variants (Table I).

Children with acute severe bronchiolitis had significantly ($P = .0005$) more episodes of troublesome lung symptoms before bronchiolitis diagnosis (median, 2; IQR, 1-6) compared with control subjects before the median age at bronchiolitis diagnosis

TABLE II. Comparison of lung function at age 1 month in children who later have acute severe bronchiolitis versus healthy control subjects

Control subjects (n = 366) vs:	Acute severe bronchiolitis (n = 34)			
Lung function, age 1 mo	**Mean difference (95% CI)**	***P* value**	**Adjusted mean difference* (95% CI)**	***P* value**
$FEV_{0.5}$ (z score)	−0.17 (−0.52 to 0.19)	.36	−0.12 (−0.55 to 0.31)	.59
FEF_{50} (z score)	−0.28 (−0.65 to 0.08)	.13	−0.33 (−0.76 to 0.10)	.14
$logPD_{15}$ ($PtcO_2$ [μmol])	−0.69 (−1.24 to −0.15)	.01	−0.79 (−1.44 to −0.13)	.02

*Adjusted for sex, mother's smoking during the third trimester of pregnancy (yes/no), age at start of day care, *ORMDL3* genotype, blood eosinophil count and total IgE level at age 18 months, and episodes of troublesome lung symptoms before diagnosis.

among the cases (median, 1; IQR, 0-2). Blood eosinophil counts and total IgE levels at age 18 months were significantly higher in cases versus control subjects (blood eosinophil count: median, 0.27×10^9 cells/L [IQR, 0.18-0.43 $\times$ 10^9 cells/L] vs 0.21×10^9 cells/L [IQR, 0.13-0.32 $\times$ 10^9 cells/L], $P = .03$; total IgE level: median, 15.3 kU/L [IQR, 6.3-29.5 kU/L] vs 8.2 kU/L [IQR, 3.9-17.1 kU/L], $P = .03$). Eczema and allergic sensitization were equally distributed among cases and control subjects.

Children with RSV-induced bronchiolitis compared with those with non–RSV-induced bronchiolitis were younger at diagnosis (199 vs 462 days, $P < .01$) and were more often admitted to the hospital (83% vs 22%, $P < .01$). There were no significant differences in the development of atopic stigmata during the first 2 years of life between cases with RSV-induced bronchiolitis and those with non–RSV-induced bronchiolitis (all $P > .05$).

A detailed description of the 34 bronchiolitis cases and the control group is outlined in Table I.

Neonatal lung function in bronchiolitis cases versus control subjects

Distributions of PD_{15} ($PtcO_2$), $FEV_{0.5}$, and FEF_{50} measured at age 1 month before any respiratory symptoms in children who subsequently had acute severe bronchiolitis versus control subjects are shown in Fig 1. Children with acute severe bronchiolitis reacted to lower doses of methacholine compared with the control group (median PD_{15} [$PtcO_2$] dose in cases vs control subjects, 0.13 vs 0.33 μmol; $P = .01$; Fig 1, *A*). This phenomenon remained significant ($P = .02$) after adjustment for significant and borderline-significant baseline characteristics, including atopic intermediary markers (sex, maternal smoking during the third trimester, age at the start of day care, *ORMDL3* genotype, episodes of troublesome lung symptoms, and total IgE levels and blood eosinophil counts). Increased bronchial responsiveness to methacholine in cases versus control subjects was confirmed in a sensitivity analysis, including the excluded cases of acute asthma-like symptoms not fulfilling all the diagnostic criteria for acute bronchiolitis ($P = .01$). The distribution of $FEV_{0.5}$ and FEF_{50} in Fig 1, *B* and *C*, suggested lower airflows in infants with acute severe bronchiolitis compared with control subjects, but these trends were not significant (mean z score for $FEV_{0.5}$ in cases vs control subjects, −0.18 vs −0.01 [$P = .36$]; mean z score for FEF_{50} in cases vs control subjects, −0.37 vs −0.09 [$P = .13$]).

Subgroup analysis of PD_{15} ($PtcO_2$) in cases with RSV-induced bronchiolitis and those with non–RSV-induced bronchiolitis excluding cases with acute asthma-like exacerbations and bronchiolitis occurring before age 1 year suggested pre-existing hyperresponsive airways independent of these case definitions, although the statistical power was lost in some of these small

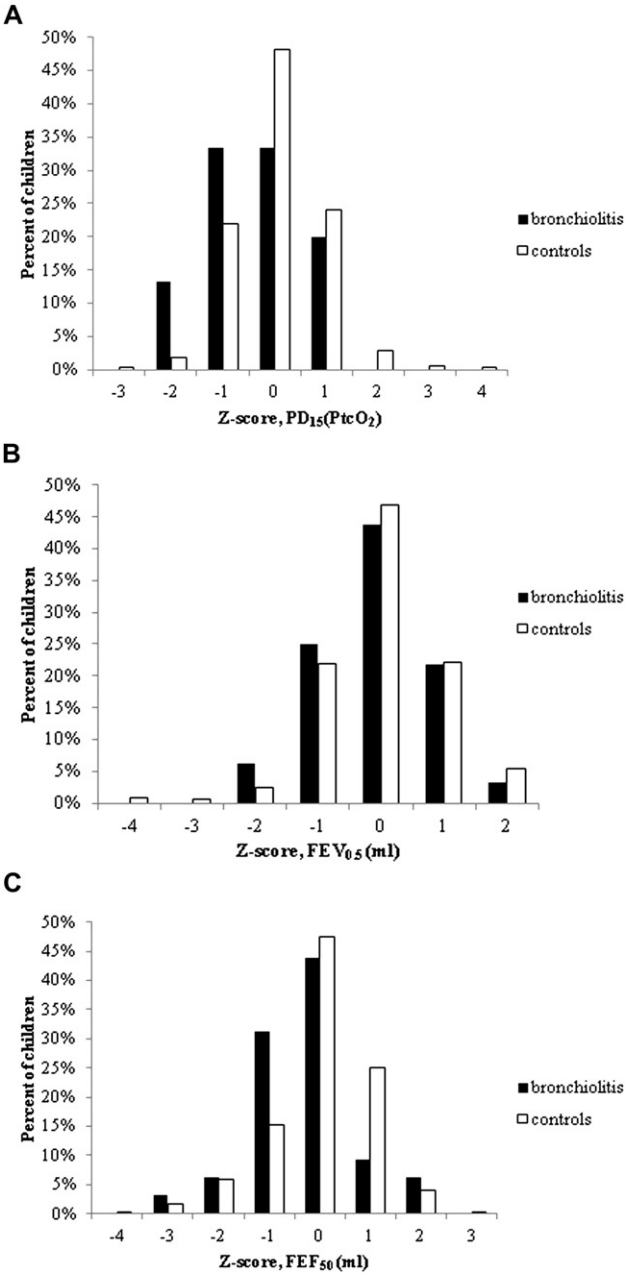

FIG 1. z Scores of neonatal bronchial responsiveness to methacholine **(A)**, baseline $FEV_{0.5}$ **(B)**, and baseline FEF_{50} **(C)** in children with acute severe bronchiolitis in the first 2 years of life compared with children who do not have acute severe bronchiolitis.

TABLE II. *(Continued)*

RSV-induced bronchiolitis (n = 23)		Non–RSV-induced bronchiolitis (n = 9)		Asthma-like cases (n = 11)		Bronchiolitis cases <1 y (n = 20)	
Mean difference (95% CI)	*P* value	Mean difference (95% CI)	*P* value	Mean difference (95% CI)	*P* value	Mean difference (95% CI)	*P* value
−0.10 (−0.52 to 0.33)	.66	−0.31 (−1.01 to 0.38)	.37	−0.15 (−0.75 to 0.45)	.62	0.02 (−0.46 to 0.52)	.92
−0.12 (−0.56 to 0.31)	.58	−0.71 (−1.42 to −0.01)	.05	−0.28 (−0.91 to 0.35)	.39	−0.19 (−0.67 to 0.29)	.43
−0.42 (−1.06 to 0.13)	.12	−1.37 (−2.38 to −0.35)	.01	−0.76 (−1.82 to 0.30)	.16	−0.98 (−1.71 to −0.24)	.01

subgroups, including the specific RSV-induced bronchiolitis subgroup ($P = .12$). Subgroup analyses of baseline $FEV_{0.5}$ and FEF_{50} values suggested similar trends of impaired neonatal airflow related to the subsequent development of acute severe bronchiolitis (see Table II).

DISCUSSION

Principal findings

Neonatal bronchial hyperresponsiveness increases the risk of acute severe bronchiolitis in early life in the $COPSAC_{2000}$ at-risk birth cohort. This demonstrates that neonatal airway hyperresponsiveness is a pre-existing host factor common to later development of both acute severe viral bronchiolitis and childhood asthma.[9]

Strengths and limitations of the study

This is the first assessment of premorbid neonatal lung function and bronchial responsiveness in a birth cohort of healthy at-risk neonates combined with a close prospective clinical follow-up for subsequent development of acute severe bronchiolitis.

The risk of misclassification of the clinical end point of acute severe bronchiolitis was minimized in this prospective birth cohort with close clinical monitoring, including daily diary card recordings of respiratory symptoms, 6-month visits to the clinic for clinical assessments by pediatricians, and visits to the clinic at the time of acute respiratory symptoms. The clinical research unit was acting as the primary health care resource for the cohort in relation to all respiratory symptoms, ensuring that diagnosis and treatment followed a predefined algorithm, including a standard clinical definition of acute severe bronchiolitis.

In particular, our clinical definition of acute severe bronchiolitis was based on objective assessment of tachypnea, chest retractions, and auscultative wide-spread fine crepitation and/or expiratory rhonchi[17-19] and not solely based on parental reporting. This approach enabled us to exclude cases of acute severe asthma-like exacerbations from the study group, which is important because impaired pulmonary function early in life has been described in association with subsequent recurrent asthma-like symptoms,[25-29] and asthma.[9] This selection process is essential because the inclusion of children with acute severe asthma-like exacerbations in the acute severe bronchiolitis group could have driven our findings. Still, sensitivity analyses, including such asthma-like exacerbations, provide similar conclusions, suggesting that the clinical distinction between acute bronchiolitis and acute asthma symptoms is arbitrary and the underlying pathophysiology is similar.

Bronchiolitis is difficult to differentiate from asthma-like symptoms, and indeed, we suspect it to be the same entity. However, the literature suggests that these subtle clinical differences represent different phenotypes. This is precisely the issue we are questioning in our study. We choose a conservative definition of acute bronchiolitis using a strict predefined algorithm, including objective signs of respiratory distress, which is in line with recently published reviews.[17-19]

RSV-induced bronchiolitis has been associated with asthma later in life, but it was our research hypothesis that bronchial hyperresponsiveness is the common cause of both bronchiolitis and asthma symptoms independently of viral species. In line with this, it has been reported that also rhinovirus-induced bronchiolitis increases the risk of asthma later in life.[1,2] Children with RSV in our study were younger than the non-RSV cases but showed no differences in atopic stigmata. Furthermore, adjusting the analyses for atopic intermediary end points did not alter the associations.

In reverence to the long tradition of emphasizing the unique quality of RSV, we analyzed a number of secondary bronchiolitis end points: (1) RSV-induced bronchiolitis, (2) non–RSV-induced bronchiolitis, (3) acute asthma-like exacerbations, (4) bronchiolitis before age 1 year, and (5) any bronchiolitis, including acute asthma-like exacerbations. All these secondary analyses show similar patterns of pre-existing increased reactivity to methacholine in cases versus control subjects. Some of these subgroup analyses (eg, the RSV group) did not reach statistical significance, probably because of the lack of power from dividing the main study group into smaller subgroups. The similarities of increased bronchial responsiveness in all groups of infants presenting with symptoms of acute severe airway obstruction independently of trigger or age at onset suggest a common deficiency rather than particular trigger attributes. It is our interpretation that bronchial hyperresponsiveness is the common underlying phenotypic trait in infants presenting with acute severe airway obstruction in response to viral infections.

It is our clinical practice only to admit infants if they need support with feeding tubes; nasal continuous positive airway pressure; suction of the upper airways; nebulized inhalations, such as isotonic saline; or β_2-agonist or systemic steroids, depending on symptom severity. We are aware of the poor evidence of this, but presumably, our clinical tradition builds from a concern over the "bronchiolitis phenotype" might indeed be an asthmatic reaction in which such treatment might be meaningful and in which no alternatives exist.

It is a significant strength of this study that lung function was assessed within a narrow age range around 1 month after birth based on results of infant spirometry with the state-of-the-art raised-volume rapid thoracoabdominal compression technique in agreement with recognized international guidelines.[14] This is the

largest published sample of lung function assessments in neonates performed under standardized conditions comprising measurements in 402 asymptomatic infants.

The relatively rare prevalence of acute severe bronchiolitis is a limitation of the study. Interestingly, we did not find significantly lower airflow in the infants later having bronchiolitis symptoms. This might suggest that bronchial responsiveness is the primary pathology, leading to acute obstruction in response to virus later in life, and that airflow limitation might not be the primary pathology. Alternatively, we might be unable to see differences in baseline airflow because of the low number of cases, which hampers the statistical power.

The high-risk nature of the birth cohort might diminish the generalizability of our findings because the absolute levels of lung function and bronchial responsiveness might not be representative of the general population, although this would not affect the comparison of lung function within the cohort. A recent study of preterm infants reported that palivizumab prophylaxis significantly reduced the risk of recurrent wheeze in children without atopic predisposition, whereas there was no effect in children with an atopic family history,[30] suggesting a differential effect of viral infection depending on atopic predisposition. Therefore our findings cannot be extrapolated beyond a high-risk population.

Interpretation

The significant association between bronchial hyperresponsiveness at 1 month of age in at-risk children and the later development of acute severe bronchiolitis demonstrates a preexisting propensity for an exaggerated airway response to common respiratory tract viral infections.

An older study reported trends of reduced airflow but no increase in the bronchial responsiveness to histamine in infants subsequently having bronchiolitis (n = 17) compared with control subjects (n = 236). This study was limited from the use of infant spirometry, which was not volume anchored, testing the infants at a wider age range up to 10 weeks, and from a retrospective questionnaire–defined end point of "doctor-diagnosed bronchiolitis" recalled by the parents at follow-up by 2 years of age.[31]

The association between airway hyperresponsiveness and the subsequent development of acute severe bronchiolitis demonstrated in this study and our recent observation of an increased risk of asthma at age 7 years in children with neonatal bronchial hyperresponsiveness,[9] together with observational evidence of an association between acute severe bronchiolitis early in life and the development of asthma,[1-4] suggests neonatal bronchial hyperresponsiveness as a shared host factor for bronchiolitis and asthma rather than a causal effect of bronchiolitis on asthma development.

Notably, the incidence of acute severe bronchiolitis in our study (8.5%) was higher than reported in some studies (1-3%)[32-35] but comparable with the incidence of 7% reported in a cohort of 253 infants with a high prevalence (71%) of atopic predisposition.[31] This association between asthma predisposition and increased incidence of acute bronchiolitis in the infants supports our research hypothesis that a host factor is responsible for acute bronchiolitis.

Children with acute severe bronchiolitis had a high prevalence of early troublesome lung symptoms before bronchiolitis, had increased total IgE levels and blood eosinophil counts, were more often exposed to tobacco smoke *in utero*, were of the male sex, were carrying the *ORMDL3* risk variant, and started day care at an early age compared with children not having such acute severe bronchiolitis. We previously demonstrated the *ORMDL3* risk allele is associated with neonatal increased bronchial reactivity and increased risk of asthma in early childhood in our cohort.[36] Together these characteristics are all typical of children at risk of asthma, supporting that children with acute severe bronchiolitis have a premorbid constitution, including bronchial hyperresponsiveness, which predisposes them to both an exaggerated response to respiratory tract viral infection and later development of asthma.

Therefore it might be speculated that acute severe bronchiolitis during infancy is not a specific disease entity but simply a severe early debut of asthma persisting to school age.[3,4]

Conclusion

Bronchial hyperresponsiveness in asymptomatic at-risk neonates precedes the later development of acute severe bronchiolitis, suggesting a pre-existing common propensity of the airways to develop asthma and to react adversely to common respiratory tract viruses.

We express our gratitude to the children and families of the COPSAC cohort study for all their support and commitment. We acknowledge and appreciate the unique efforts of the COPSAC research team. We thank S. Jensen for statistical support.

Key messages

- **RSV and other respiratory tract viruses lead to common colds in most infants, whereas a minority have acute severe bronchiolitis.**
- **Bronchial hyperresponsiveness in at-risk neonates precedes acute severe bronchiolitis in response to respiratory tract viral infections.**

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METHODS

Bronchial responsiveness

Baseline lung function was repeated after a saline inhalation. Subsequently, methacholine was administered in quadrupling dose steps administered through a dosimeter attached to a nebulizer (SPIRA 08 TSM 133, Respiratory Care Center), as previously detailed.[E1] Bronchial responsiveness was assessed by using repeated measurements of infant spirometry and continuous assessment of $PtcO_2$ (TCM3, Radiometer). The PD_{15} was estimated from the dose-response curves fitted with a logistic function. Our previous sensitivity analyses showed $PtcO_2$ to be more sensitive than any of the forced flow indices of infant spirometry.[E1,E2] Therefore $PtcO_2$ was used in the analyses.

Genetics

Blood was sampled at age 6 months, centrifuged and separated into serum and serum cells, and immediately stored at −80°C until analysis. After thawing, DNA was purified from the serum cells by using the QIAamp DNA Blood Maxi Kit (Qiagen, Valencia, Calif).[E3]

Filaggrin genotyping for the 2 common independent null mutations R501X and 2282del4 was performed, as previously described.[E4] Children were defined as having a filaggrin mutation if they carried at least 1 of the mutations.

For *ORMDL3* genotyping, allelic discrimination at the rs7216389 single nucleotide polymorphism on chromosome 17q12-21 was performed, as previously detailed.[E5]

For *DENND1B* genotyping, allelic discrimination at the rs2786098 on chromosome 1q31.3 was performed, as described by Sleiman et al.[E6]

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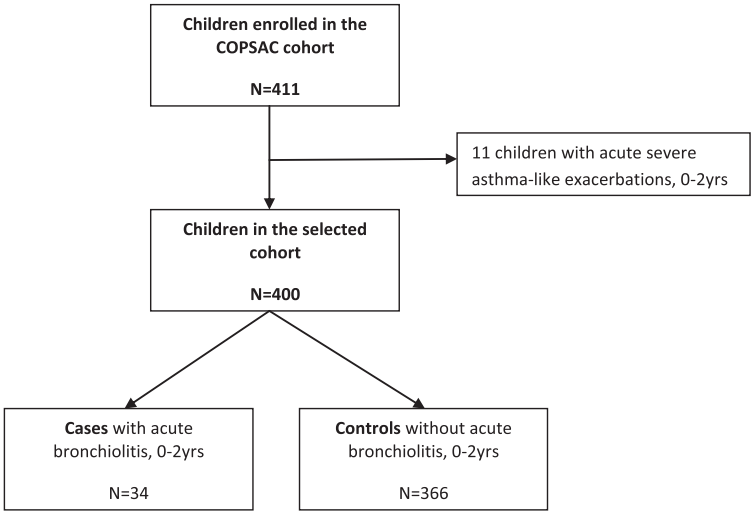

FIG E1. Study group flow chart.

TABLE E1. Baseline characteristics and lung function results of children with acute severe bronchiolitis during the first 2 years of life, excluding cases with acute severe asthma-like exacerbations but not fulfilling all diagnostic criteria for acute severe bronchiolitis, and control subjects

Study group (n = 411)	Acute asthma-like exacerbations (n = 11)	Bronchiolitis (n = 34)	Control subjects (n = 366)
Sex			
Male sex (no.)	36.4% (4)	64.7% (22)	48.4% (177)
Anthropometrics			
BMI at birth (cm/kg^2), median (IQR)	12.3 (11.3-14.8)	12.5 (11.6-13.7)	12.8 (12.0-13.7)
Environment			
Smoking, 3rd trimester (no.)	18.2% (2)	35.3% (12)†	13.1% (48)
Alcohol, 3rd trimester (no.)	0% (0)	14.7% (5)	18.6% (68)
Cat at birth (no.)	20.0% (2)	17.7% (6)	14.8% (52)
Dog at birth (no.)	10.0% (1)	14.7% (5)	13.6% (48)
Older siblings at birth (no.)	45.5% (5)	52.9% (18)	42.4% (155)
Household income at birth (no.)			
Low, <53,000€	9.1% (1)	30.3% (10)	29.6% (101)
Medium, 53,000€-80,000€	45.5% (5)	54.6% (18)	46.6% (159)
High, >80,000€	36.4% (4)	15.2% (5)	23.8% (81)
Paternal history of asthma (no.)	11.1% (1)	24.2% (8)	16.5% (59)
Age at start of day care (d), median (IQR)	252 (194-376)	296 (230-349)	341 (240-417)
Solely breast-fed (d), median (IQR)	122 (27-123)	120 (72-179)	122 (89-155)
Lung function at age 1 mo			
$FEV_{0.5}$ (z score), mean (SD)	−0.16 (1.38)	−0.18 (0.91)	−0.01 (0.99)
FEF_{50} (z score), mean (SD)	−0.37 (0.96)	−0.37 (1.05)	−0.09 (1.00)
PD_{15} ($PtcO_2$ [μmol]), median (IQR)	0.09 (0.04-0.25)	0.13 (0.07-0.60)†	0.33 (0.12-0.88)
Genetics			
Filaggrin mutation	20.0% (2)	18.2% (6)	9.7% (34)
ORMDL3 (TT)	40.0% (4)*	48.5% (16)†	26.7% (92)
DENND1B genotype (rs2786098)			
AA	11.1% (1)	3.5% (1)	4.2% (14)
AB	33.3% (3)	13.8% (4)	27.5% (91)
BB	55.6% (5)	82.7% (24)	68.3% (226)

*Significant difference, children with acute asthma-like exacerbations versus control subjects.
†Significant difference, bronchiolitis cases versus control subjects.