

Original article

Association of an early respiratory syncytial virus infection and atopic allergy

Background: Respiratory syncytial virus (RSV) causes postbronchiolitic wheezing but its role in allergic sensitization is controversial. The purpose of the study was to examine the effect of an early RSV infection on allergic sensitization.

Methods: Seventy-six subjects were examined 6–10 years after hospitalization for RSV infection during the first year of life. Fifty-one subjects (68%) attended clinical studies and 25 filled in a questionnaire. The study protocol included lung function, skin-prick and blood tests. The controls were matched for birth date and sex.

Results: Eight per cent of the subjects and 37% of the controls had at least one positive skin-prick test (SPT) (difference –35%, 95% CI –50 to –19%, $P < 0.0001$). Allergic rhinitis, atopic dermatitis and asthma occurred as often in both groups, but asthma had been diagnosed significantly earlier in the subjects than in the controls [mean age 3.0 years (SD 2.6) and 5.6 years (SD 3.0), difference 2.6 years, 95% CI 0.57–4.65, $P = 0.014$]. In a logistic regression analysis, RSV infection was associated with negative SPTs.

Conclusions: An early RSV infection results in reduction of SPT positivity but not of occurrence of atopic diseases. This finding might explain why there is less atopic sensitization in countries with a greater probability of acquiring RSV infection at an early age.

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Bacteria and viruses are strong immunomodulators that have even been suggested as interfering with the development of atopic diseases (1, 2). This would occur by switching the immunological responses more towards a Th1 balance, which in turn, might prevent allergic sensitization. This hypothesis is based on the observation that infections caused by measles virus and hepatitis A virus protect from atopy as measured by positive skin-prick tests (SPTs) and that there has been an inverse association between tuberculin responses and atopy (3–5). Respiratory syncytial virus (RSV) infection is often the first significant respiratory infection a child acquires and is contracted by nearly all children by the age of two. Usually it has a mild course but in infants it may cause severe bronchiolitis (6). To further characterize the role of RSV, a follow-up study of a historical cohort of children admitted for RSV infection during their first year of life was performed.

Material and methods

The study population was 117 children who had been admitted to the Oulu University Hospital during the years 1991–1994 because of an RSV infection during their first year of life. As RSV epidemics

occur in Finland mainly during winter months in uneven years with minor epidemics during the preceding spring, the study comprises two main epidemics (7). The diagnosis of RSV was based either on antigen detection with enzyme-linked immunosorbent assay or latex agglutination test in nasopharyngeal secretions (8). The mean age at the time of admission for RSV infection was 4.0 months (range 0.7–11.9). The study profile is presented in Fig. 1. Thirty children were excluded on the basis of the following exclusion criteria: premature birth (< 37 gestational weeks, $n = 22$), need for respiratory assistance > 6 h during the neonatal period ($n = 2$) or a concomitant disease such as congenital heart disease ($n = 2$), chromosomal abnormality ($n = 3$), or severe neurological disease ($n = 1$). Seventy-six subjects met the inclusion criteria (47 boys, 29 girls). The subjects were called for a follow-up visit during the fall and winter of 2000–2001 when they were 6–10 years old. Fifty-one (68%) subjects visited the outpatient department and the parents of the remaining 25 completed an interview questionnaire by telephone.

A matched control was chosen from the National Population Registry for each subject by selecting a child of the same sex and date of birth as the index child. Their histories were checked from the history notes of the hospital and those meeting the same exclusion criteria as the subjects ($n = 10$) as well as those who had been treated for a respiratory infection at the hospital during the main RSV seasons ($n = 3$) were not accepted. The Oulu University Hospital is the only hospital treating children with severe respiratory infection in the area. If the first family contacted was not willing to participate, we contacted the second ($n = 12$) or third ($n = 4$) in order. The participation percentage for those contacted first was

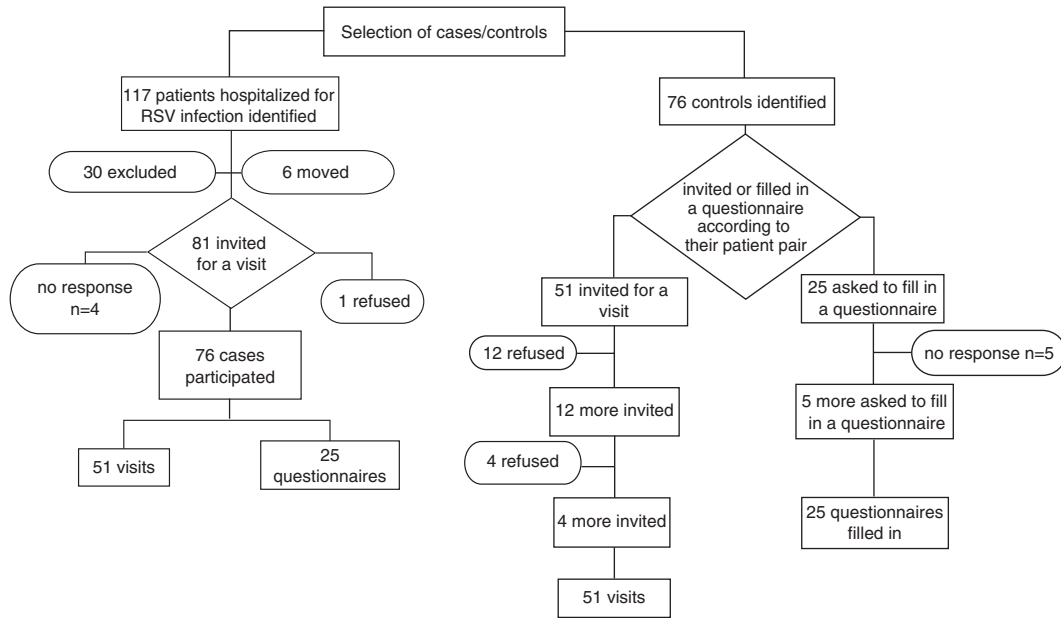


Figure 1. Study profile.

76%. The duration of the selection process caused a 2-month difference in the age at which the controls visited the outpatient department compared with the subjects. However, for the reliability of the results it was considered most important that both the subjects and controls had been at a similar risk of RSV infection because of the same date of birth.

The parents were asked to fill in a questionnaire about asthma and allergic symptoms, and whether a physician had diagnosed any of the atopic disorders. The children were invited for a clinical examination, lung function tests, SPT with 15 common allergens, and blood tests for the determination of serum immunoglobulin E (IgE) concentrations and eosinophil count.

Questionnaire

The same questionnaire as in the International Study of Allergy and Asthma in Childhood (ISAAC) was used along with some additional questions about the type of day-care, breastfeeding, number of siblings, occurrence of otitis media, food allergy, family history of asthma and atopy, pets, and smoking at home (9). The questionnaire was checked during the visit. If the parents were not willing to participate in the clinical examinations, they were interviewed over the telephone to get the same background information.

Definitions

The children were considered to have atopic diseases or symptoms according to the ISAAC questions concerning wheezing at any time, wheezing during the last year, asthma, nose symptoms, allergic rhinitis, and atopic eczema (9). There were also several children without diagnosis of asthma who were reported to have had either more than three attacks of wheezing or dry cough at night for more than 3 weeks without symptoms of infection in the 12 months before the study. They were further examined by an independent paediatric allergologist outside the group. Those diagnosed as having asthma were classified accordingly. The question of allergic rhinitis of some other type was added to the ISAAC questionnaire

because hay fever is often understood as rhinitis merely caused by hay allergy. A family history of asthma was defined as doctor-diagnosed asthma in first-degree relatives. Doctor-diagnosed allergic rhinitis as well as atopic eczema in the family was also recorded.

Lung function tests

A trained nurse performed lung function tests (spirometry and oscillometry with a bronchodilator test, Master Screen IOS, Jaeger, Würzburg, Germany) without knowing which group the child belonged to. The results of spirometry were expressed as percentages of the national reference values (10). For the oscillometry test an impulse oscillometer was used. The best of three measurements of Rrs at 5, 20, and 35 Hz and Xrs at 5 Hz was accepted for the analysis. The results were compared with the national reference values (11).

Skin-prick tests and blood tests

Skin-prick testing was performed on the volar aspect of the forearm with Soluprick® allergens (ALK; Allergologisk Laboratorium A/S, Horsholm, Denmark). The kit included allergens of birch, mugwort, meadow grass, milk, egg, wheat, soy, hazelnut, peanut, banana, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, epithelia of dog and cat, and latex (Stallergenes A.S., Antony cedex, France). A 1-mm, one-peak lancet with a shoulder to prevent deeper penetration was used. Histamine dihydrochloride (10 mg/ml) was used as a positive control and Soluprick® control solution as negative control. The reactions were read at 15 min, and regarded as positive if the mean diameter of the weal minus the diameter of the negative control was 3 mm or greater. Trained nurses analysed the reactions without knowing which group the child belonged to.

The concentrations of IgE were determined by a chemiluminescence method and, according to our laboratory reference values, the concentrations above 130 kU/l for children <8 years of age and above 320 kU/l for children >8 years of age were considered as elevated.

Statistical analysis

The sample size was based on 20% difference in cumulative incidence of asthma between the RSV subjects and controls found in the study of Sigurs et al. (12). To detect a difference of 20% with a power of 80% and alpha error of 0.05, a sample size of 75 subjects and controls was needed. The data were analysed with the SPSS for Windows version 10.0. The Student's *t*-test, chi-squared test, Fisher's exact test, the test for the difference of proportions and the Mann-Whitney *U*-test were used to calculate the significance of differences between the groups. Logistic regression analysis was used to analyse the influence of risk factors on RSV infection while adjusting for confounding factors. The factors were decided by biological plausibility and all variables were entered in the model. Odds ratios and 95% CI were calculated. Paired tests were not used as the subjects and controls were matched only for the date of birth and sex.

Ethical considerations

The Ethical Committee of Oulu University Hospital found the study protocol acceptable. All parents gave their written informed consent.

Results

The subjects had significantly more siblings and their birth order was significantly above that of the controls (Table 1). There were no other significant differences in the background data such as gestational age, number of episodes of acute otitis media, type of day-care, smoking habits in the family, cats or dogs in the family at the time of the study, or living on a farm. Duration of breastfeed-

ing was shorter among the subjects, being in many cases due to the hospitalization for RSV infection according to parental report. There were no significant differences in the background factors between the subjects who came for a visit and those who did not.

The subjects had more asthma and wheezing than the controls, although the differences were not significant (Table 2). Asthma had been diagnosed significantly earlier in the subjects than in the controls, the mean age at the time of diagnosis being 3.0 years (SD 2.6) and 5.6 years (SD 3.0), respectively (difference 2.6 years, 95% CI 0.57–4.65, $P = 0.014$). Of the 20 subjects with asthma, 14 (70%) were reported to have had wheezing only during respiratory infections compared with three (25%) of the 12 controls with asthma. Five children (two subjects and three controls) were diagnosed as having asthma when referred to a paediatric allergologist because of reported recurrent wheezing or chronic cough.

The subjects reported significantly less sneezing and/or running or blocked noses without a concomitant infection than the controls (28% vs 49%, difference –21%, 95% CI –36 to –6%, $P = 0.008$, Table 2). Allergic rhinitis had been diagnosed equally often in both groups. The occurrence of atopic dermatitis was also similar in both groups.

There were no significant differences between the groups in the lung function tests as measured by spirometry and oscillometry (Table 3).

The number of subjects and controls with positive SPTs is presented in Fig. 2. Only 8% of the subjects had at least one positive SPT compared with 43% of

Table 1. Demographic characteristics of subjects and controls

	Subjects ($n = 76$)*	Controls ($n = 76$)*	Difference between the groups	95% CI†
Age at the time of the study [years, mean (range)]	7.8 (6.9–10.3)	8.0 (7.1–10.3)	–0.20	–0.58 to 0.18
No. of siblings [mean (range)]	4.11 (0–16)	2.79 (0–14)	1.32	0.28 to 2.36
Birth order [mean (range)]	3.57 (1–16)	2.72 (1–13)	0.84	0.02 to 1.66
Gestational age [weeks (range)]	39.9 (37.7–42.3)	39.9 (37.1–42.3)	0.09	–0.26 to 0.44
No. of acute otitis media episodes [median (range)]	5.0 (0–20)	4.0 (0–20)		
Duration of breastfeeding, months [mean (range)]	6.92 (0–21)	7.81 (0.2–24)	–0.99	–2.57 to 0.59
Type of day-care [n (%)]				
At 1–2 years of age				
Home	65 (87)	62 (82)	$P = 0.497$	
Family day-care	4 (5)	8 (11)		
Day-care centre	6 (8)	6 (8)		
At 3–4 years of age				
Home	43 (57)	41 (54)	$P = 0.240$	
Family day-care	11 (15)	19 (25)		
Day-care centre	21 (28)	16 (21)		
Mother smoking during pregnancy [n (%)]	8 (10)	14 (19)	–0.08	–0.20 to 0.03‡
Smoking in the household during the last year [n (%)]	32 (42)	25 (33)	0.09	–0.06 to 0.24‡
Pets at home at the time of study [n (%)]	31 (41)	41 (55)	–0.132	–0.28 to 0.03‡

* $n = 51$ for the age of visit; $n = 73$ for gestational age among controls; $n = 72$ among subjects and 75 among controls for the number of acute otitis media episodes and duration of breastfeeding; $n = 75$ for type of day-care in subjects because of missing information.

† By Student's *t*-test.

‡ Difference of proportions.

Table 2. Asthma and atopy among subjects and controls; symptoms and diagnoses [*n* (%)]

	Subjects (<i>n</i> = 76)*	Controls (<i>n</i> = 76)*	<i>P</i> -value†	Odds ratio	95% CI
Wheezing at any time	47 (62)	36 (48)	0.09	1.76	0.92–3.36
Wheezing in the last 12 months	17 (22)	18 (24)	0.85	0.93	0.44–1.98
Asthma ever	20 (26)	12 (16)	0.11	1.91	0.86–4.24
Asthma at age <3 years (of all asthmatics)	11 (55)	1 (8)	0.01	13.44	1.45–124.86
Symptoms of sneezing, or a runny, or blocked nose ever without a cold	21 (28)	37 (49)	0.008	0.4	0.21–0.79
Allergic rhinitis	7 (9)	10 (13)	0.44	0.67	0.24–1.86
Atopic eczema ever	22 (29)	24 (32)	0.72	0.88	0.44–1.76
At least one positive SPT	4 (8)	22 (43)	<0.001	0.11	0.04–0.36
Serum IgE above reference values	11 (22)	22 (43)	0.02	0.36	0.15–0.86

* *n* = 75 among controls for wheezing because of missing information; *n* = 20 among subjects and 12 among controls for age at the time of asthma diagnosis; *n* = 51 among subjects and controls for at least two positive SPTs and serum IgE above reference values.

† By chi-squared test (Fisher's exact test, when *n* < 5).

Table 3. Results of lung function tests on subjects and controls, mean (SD)

	Subjects‡	Controls‡	Difference between the groups§	95% CI
FVC as percentage predicted for height and sex*	101.9 (14.4)	99.0 (14.4)	2.88	–3.46 to 9.21
FEV1 as percentage predicted for height and sex*	102.9 (13.9)	100.5 (11.8)	2.39	–3.26 to 8.05
FEV1/FVC (%)*	91.1 (7.0)	91.5 (6.5)	–0.35	3.32 to 2.62
R5 Hz†	85.0 (19.1)	86.5 (21.2)	–1.47	–9.63 to 6.69
Change in R5 Hz after bronchodilator (%)†	15.3 (12.8)	14.6 (16.6)	0.71	–6.06 to 7.48
R20 Hz†	96.4 (18.2)	96.1 (21.1)	0.34	–7.64 to 8.33
Change in R20 Hz after bronchodilator (%)†	14.7 (10.9)	11.5 (15.0)	3.15	–2.83 to 9.13

* Spirometry was performed successfully on 38 subjects and 44 controls.

† Oscillometry with a bronchodilator test was performed successfully on 38 subjects and 37 controls and oscillometry without a bronchodilator test on 10 subjects and 10 controls.

‡ There were six subjects and six controls receiving continuous medication for asthma at the time of follow-up.

§ By Student's *t*-test.

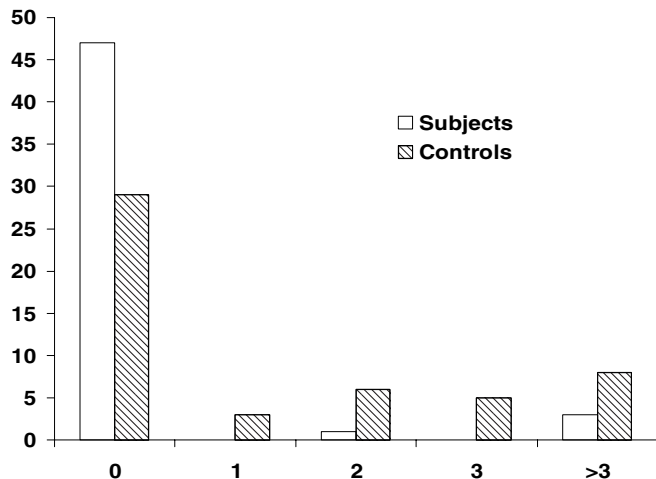


Figure 2. Number of subjects and controls with 0, 1, 2, 3 or > 3 positive skin-prick tests (*P* = 0.001, chi-squared test).

the controls (difference –35%, 95% CI –50 to –19%, *P* < 0.0001). The main allergens causing positive reactions were dander of dog or cat, birch, or meadow grass (Table 4). None of the subjects or controls with asthma diagnosed at an age < 3 years had any positive

Table 4. Number of subjects and controls with positive skin-prick tests to different allergens with percentages in brackets [*n* (%)]

	Subjects (<i>n</i> = 51)	Controls (<i>n</i> = 51)	<i>P</i> -value*	Odds ratio	95% CI
Food allergens	3 (6)	9 (18)	0.065	0.29	0.07–1.15
House-dust mites	0	4 (8)	0.118		
Epithelia of dog or cat	2 (4)	14 (28)	0.001	0.11	0.02 to 0.50
Pollens	3 (6)	15 (30)	0.002	0.14	0.04–0.54

* By Fisher's exact test.

SPTs. There were significantly fewer subjects with serum IgE above the reference values than controls (22% vs 43%, difference –21%, 95% CI –39 to –3%, *P* = 0.02).

There were significantly more subjects with at least one sibling with asthma than the controls (Table 5). The proportion of siblings with asthma of the total number of siblings in each group showed no significant difference. While 8.3% of the siblings of the subjects had asthma (*n* = 26), only 4.7% of the siblings of the controls did (*n* = 10; difference 3.6%, 95% CI –0.9 to 8%, *P* = 0.09). A family history of atopy and/or asthma did not differ between the subjects and controls on the whole. Neither

Table 5. Family history of asthma and atopy [*n* (%)]

	Subjects (<i>n</i> = 76)	Controls (<i>n</i> = 76)	<i>P</i> -value‡	Odds ratio	95% CI
Family history of asthma and/or atopy	59 (80)	60 (80)	0.967	0.98	0.44 to 2.19
Family history of asthma	32 (43)	15 (20)	0.003	2.99	1.45 to 6.21
Maternal history of asthma and/or atopy	35 (46)	29 (38)	0.324	1.38	0.72 to 2.64
Maternal history of asthma	12 (16)	4 (5)	0.062	3.38	1.03 to 10.99
Paternal history of asthma and/or atopy*	17 (23)	24 (32)	0.216	0.63	0.31 to 1.31
Paternal history of asthma*	8 (11)	5 (7)	0.370	1.70	0.53 to 5.46
History of asthma and/or atopy among siblings†	42 (60)	40 (55)	0.529	1.24	0.64 to 2.40
History of asthma in any sibling†	25 (36)	9 (13)	0.001	3.89	1.66 to 9.12
Proportion of siblings with asthma§	26 (8)	10 (5)	0.11	0.03¶	−0.009 to 0.08

* *n* = 73 for subjects and 74 for controls due to single-parent families.

† *n* = 70 for subjects and 72 for controls because of families with only one child.

‡ By chi-squared test.

§ Calculated as proportion of the total number of siblings; *n* = 312 for subjects and 212 for controls.

¶ Difference of proportions.

Table 6. Logistic regression analysis of the association of various risk factors and possible confounding factors and RSV infection

	Odds ratio	95% CI	<i>P</i> -value
No. of positive skin-prick tests at follow-up	0.60	0.42 to 0.87	0.007
Duration of breastfeeding, months	0.89	0.80 to 0.99	0.036
No. of siblings	1.14	0.99 to 1.31	0.066
Family history of asthma and/or atopy, yes/no	0.80	0.27 to 2.32	0.679

was there any difference between the groups in the IgE levels of their parents.

In a logistic regression analysis RSV infection was associated with negative SPTs while adjusting for possible confounding factors such as duration of breastfeeding, number of siblings, and family history of asthma and/or atopy (odds ratio 0.60; 95% CI 0.42–0.87, *P* = 0.007) (Table 6). A statistically significant negative association was also observed between the duration of breastfeeding (in months) and RSV infection (odds ratio 0.89; 95% CI 0.80–0.99, *P* = 0.036). According to parental report, in at least 14 cases, breastfeeding had been discontinued because the child was admitted for RSV infection.

Discussion

The main finding of the study was that children hospitalized for RSV infection early in infancy had significantly fewer positive SPTs than their age-matched controls 6–10 years after the infection. The controls were likely to have had RSV infection either later or as less severe compared with the subjects leading to differences in atopic sensitization. However, there was no significant difference in the occurrence of atopic diseases such as allergic rhinitis and atopic dermatitis, and the occurrence of asthma was even higher among those who had had an early RSV infection. A lower prevalence of atopy in a general population as measured by SPT has been found in countries such as the former East Germany compared

with the former West Germany, and Estonia compared with Sweden (13, 14). Reduction of atopic sensitization caused by an early RSV infection might explain why there are less positive findings in SPTs in countries where people live in more crowded apartments and children have more siblings than in the western countries. Close contact with other children increases the probability of acquiring RSV infection at an early age, as also found in this study.

Early RSV infection may have an immunomodulating effect resulting in reduction in atopic susceptibility as measured by SPTs. The effect of RSV has been shown to depend on the severity of the infection as measured by wheezing and the degree of hypoxia (15). Respiratory syncytial virus infection might induce cytokine cascades that lead to a change in the balance of Th1 and Th2 type cytokine production. The predominant cytokine in respiratory secretions in infants and bronchoalveolar lavage fluids in mice with RSV infection is interferon gamma, which has also been shown to induce obstructive symptoms during infection (16, 17). The rise in interferon gamma is also known to down-regulate the production of Th2-type cytokines, which itself is associated with an atopic phenotype (18). These changes in the cytokine balance might explain both the wheezing symptoms and the reduction in atopy among the subjects. As an alternative explanation there might exist a subgroup of infants with an individual susceptibility to a severe RSV infection. It is difficult to explain why these infants would be less atopic than those with a less severe or later infection, especially as there was no difference in the family history of atopy or asthma.

In a murine model it has been found that if allergen sensitization happens before RSV infection, the infection increases sensitization as measured by airway hyper-responsiveness. If sensitization happens after RSV infection it is reduced compared with sensitization without previous RSV infection (19). Pollen contacts during the first 6 months of life increase the risk of pollen allergy for 20 years or more (20). We suggest that RSV infection

protects infants from sensitization to pollens as measured by positive SPTs if they have acquired the infection before their first contact with pollens. However, no significant differences were found in the occurrence of atopic diseases.

The negative association between RSV infection and SPTs found in this study is similar to that reported by Pullan and Hey (21). In other surveys where no association between RSV and SPTs has been found, the selection of controls or subjects had been biased or the index group had included children with bronchiolitis caused by any virus and not only by RSV (22–24). In the study by Sigurs et al. (25), 20% of the RSV subjects had at least one positive SPT at the age of 7.5 years compared with 6% of the controls. Contrary to Sigurs et al. (25), prematurely born children were excluded in this study. The differences in developing immunological mechanisms between prematurely born and full-term babies could affect both the severity of RSV infection and the development of asthma and allergies, although this can hardly be the only explanation for the different results between these two studies. The study of Sigurs et al. (25) was a well-performed prospective follow-up study and the retrospective nature of this study may have affected the results to some extent.

In most of the previous studies, the prevalence of asthma and wheezing after RSV infection has been higher among the index than the control group, but the differences have been found to decline with time (12, 21, 23–27). Accordingly, a higher prevalence of asthma ever and wheezing at any time was found among the subjects than among the controls, although the differences were not significant. There was no difference between the

subjects and controls in wheezing in the last 12 months. None of the subjects or controls with diagnosis of asthma before the age of 3 years had any positive SPT at the time of the study.

This study was a retrospective cohort study with controls matched for sex and the date of birth. This kind of a study setting could cause a recall bias in the occurrence of atopic symptoms. Skin-prick tests are still an objective measure of atopic sensitization. The occurrence of atopic diseases in the controls is rather high. It is possible that control children with atopic diseases or symptoms are more interested in participating than nonsymptomatic children. However, a similar occurrence of atopic diseases and SPT positivity has been reported among 7–12-year-old children in Finland (28).

In conclusion, it was found that children who had had virologically verified RSV infection during their first year of life had significantly less positive SPTs at the age of 8 years than their controls. Despite this, those who had had RSV infection did not have less clinical or symptomatic atopic diseases. We suggest that an early RSV infection affects sensitization as measured by SPTs but not occurrence of clinical manifestations of atopic diseases.

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