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Maria Nieto contributed to acquisition of data, revision of content. Isabel Lafuente contributed to acquisition of data, revision of content. Rafael Calderon contributed to conception and design, revision of content. Sonia Uixera contributed to conception and design, revision of content. Raquel Pina contributed to acquisition of data, revision of content. Susana Calaforra contributed to acquisition of data, revision of content. Isidoro Cortell contributed to conception and design, revision of content. Antonio Nieto contributed to conception and design, drafting and revision of the

text. Angel Mazon contributed to conception and design, analysis and interpretation of data, drafting and revision of the text. All the authors have approved the final version.

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Vitamin D, virus etiology, and atopy in first-time wheezing children in Finland

To the Editor,

Vitamin D may be protective against acute respiratory infections and atopic illnesses, but studies have been conflicting (1, 2). One likely reason is the vast variability in measurement of vitamin D (based on interview about dietary intake vs. actual measurement of 25-hydroxyvitamin D [25OHD] concentrations) and major differences in study populations (age, confirmation of atopy status, use of vitamin D supplements, and latitude of study region). The recommended minimum level of serum 25OHD varies from 50 to 75 nmol/l (2, 3). In Finland, all young children are instructed to have vitamin D

supplement of 10 µg (400 IU) per day. Despite the recommendations, the 25OHD levels in serum have been deficient (<50 nmol/l) in up to 21–31% of Finnish children (1, 2, 4). In other Westernized populations, 10–17% of the children have been reported to have 25OHD levels <50 nmol/l (2). However, data remain scarce on the 25OHD levels in high-risk children susceptible to viral infections and asthma development, such as young wheezing children (1). To investigate this issue, we analyzed serum 25OHD levels in a cohort of first-time wheezing children that were well characterized for virus etiology and atopy status.

Serum samples and nasopharyngeal aspirates were collected from 107 first-time wheezing children in the Turku University Hospital (Turku, Finland) from June, 2007 to March, 2010. The children participated in a randomized controlled trial, which studied the effectiveness of oral corticosteroid (5). The inclusion criteria consisted of age 3–23 months, delivery at ≥36 gestational weeks, first wheezy episode (parental report and confirmed from

Abbreviations:

B-Eos, Blood eosinophil count; CI, 95% confidence intervals; IgE, Immunoglobulin E; IQR, Interquartile range; PCR, Polymerase chain reaction; RSV, Respiratory syncytial virus; s.d., Standard deviation; 25OHD, 25-hydroxyvitamin D; 25OHD2, 25-hydroxyvitamin D2; 25OHD3, 25-hydroxyvitamin D3.

medical records), and written informed consent from a parent or guardian. Exclusion criteria consisted of chronic non-atopic illness, previous systemic or inhaled corticosteroid treatment, or need for intensive care unit treatment. The study was commenced only after obtaining written informed consent from the parent. The study protocol was approved by the Ethics Committee of the Turku University Hospital.

At study entry, the guardian was asked to fill in a standard health questionnaire (Online Supplementary). The child was then physically examined by a study physician, a nasopharyngeal aspirate sample was obtained for viral diagnostics using a standardized procedure, (5) and a baseline blood sample was drawn. Wheezing refers to expiratory breathing difficulty with high pitch sound during expiration. Eczema was defined as atopic eczema when IgE sensitization was present. The need for hospitalization was decided by an on-duty physician outside of the study team. The illness severity score is a summed score for the degree of dyspnea (0 = none, 1 = mild, 2 = moderate, 3 = severe), type of breathing (0 = normal, 1 = use of stomach muscles, 2 = use of intercostal muscles, 3 = nasal flaring), severity of auscultatory findings (0 = none, 1 = expiratory, 2 = inspiratory and expiratory, 3 = audible without stethoscope) an assessment of expiratory:inspiratory time (0 = 1:2, 1 = 1:1, 2 = 2:1, 3 = 3:1). The children were discharged from the hospital when the respiratory symptom score was <3, and there was no more wheezing. Parents filled a diary of respiratory symptoms (cough, breathing difficulty, noisy breathing, symptoms during sleep, use of bronchodilators) for 2 weeks after hospital discharge. See more details on Online Supplementary.

Serum 25OHD measurements were taken by liquid chromatography-tandem mass spectrometry at Massachusetts General Hospital (Boston, USA). Blood eosinophil count and serum levels of allergen-specific IgE for common allergens (cut-off >0.35 kU/l, Phadiatop Combi®; Phadia, Uppsala, Sweden) were analyzed by the routine diagnostics of the Central Laboratory of Turku University Hospital.

Rhinovirus species A, B, and C, enteroviruses, and respiratory syncytial virus (RSV) A and B were detected within 3 days from refrigerated nasopharyngeal aspirates using 'in house' reverse-transcriptase PCR at the Virus Diagnostic Laboratory, Department of Virology, University of Turku. In addition, a multiplex PCR test (Seeplex RV12 ACE Detection; Seegene, Seoul, Korea) was used for detection of rhinovirus A and B, adenovirus, coronavirus (229E, NL63, OC43 and HKU1), human metapneumovirus, influenza A and B virus, parainfluenza virus types 1–3, and RSV A and B virus after long-term storage in -80°C . Human bocavirus-1 was analyzed using PCR and serology, as previously described (5). To analyze the virus load (i.e., copy number), a sterile flocked swab (catalog number 502CS01; Copan, Brescia, Italy) was first dipped in the aspirate and then diluted with 1 ml of phosphate-buffered saline.

Basic statistical analyses two sample *t*-test, one-way ANOVA, Spearman correlation, and regression analysis were used to analyze the association between 25OHD concentration and different variables. Multivariable linear regression analysis was used for age-adjusted analyses. Rhinovirus load was log-transformed before analysis. Parametric variables are expressed

as mean and standard deviation (s.d.) and nonparametric variables as median and interquartile range (IQR). Linear regression results are expressed as mean difference and 95% confidence intervals (CI). A two-sided $p < 0.05$ was considered statistically significant. All analyses were performed using SAS Enterprise Guide 4.3 (SAS Institute, Cary, NC, USA).

Of the 125 children eligible for the study, 12 declined participation. After exclusion of another six patients, because of the lack of vitamin D measurements, 107 children were included in the analytic cohort (Fig. 1). Mean age was 12 months (s.d. 6.0) and 72 (67%) were boys. Fifty-nine (55%) children had atopy or another allergy-related characteristic (i.e., sensitization, blood eosinophil count $>0.4 \times 10^9/\text{l}$, or atopic eczema). At least one virus was detected in 100% of the samples, rhinovirus in 82 (77%) children, respiratory syncytial virus in 31 (29%), and bocavirus in 19 (18%) children. Seventeen (16%) children had serodiagnosed acute bocavirus infection, and PCR-diagnosed bocavirus was found in 18 (17%) children. Virus coinfection was detected in 41 (38%) children. Mean 25OHD concentration was 86 nmol/l (s.d. 21, range 35–150). Five (5%) children had serum 25OHD concentration <50 nmol/l and 34 (33%) <75 nmol/l. Serum 25OHD level of 100 nmol/l or higher was detected in 28 (26%) of the children (Table 1, Table S1).

In unadjusted analysis, serum 25OHD concentration was inversely associated with age ($p < 0.0001$), female sex ($p = 0.03$), bocavirus etiology ($p = 0.007$), and blood eosinophil count over $0.4 \times 10^9/\text{l}$ ($p = 0.01$), but not with sensitization, atopic eczema, rhinovirus etiology, or RSV etiology (all $p > 0.2$). In age-adjusted analysis, 25OHD concentration was inversely associated with female sex, but not with atopic characteristics or virus etiology. Atopic characteristics did not modify the association of 25OHD concentration and virus etiology. No association was found between 25OHD and hospital admission, season of

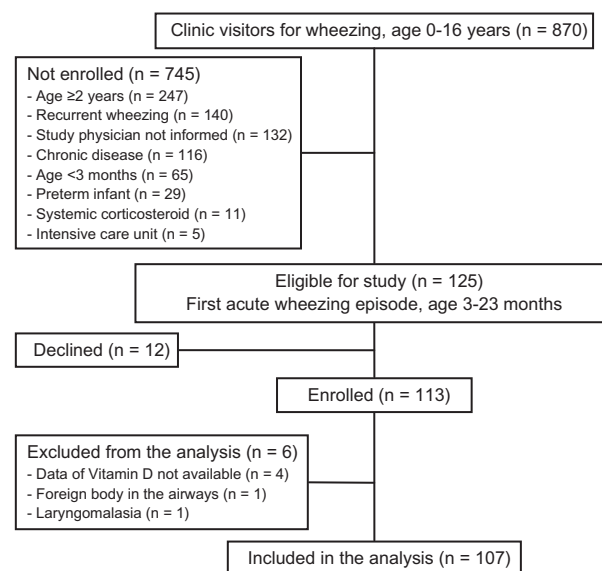


Figure 1 Study flow chart.

Table 1 Patient characteristics by quartile of serum 25-hydroxyvitamin D concentration

	Quartile of Serum 25OHD Concentration			
	1	2	3	4
No. of subjects	27	27	27	26
25OHD (median, range)	62.2 (35.2–71.9)	78.6 (72.1–85.6)	90.1 (85.9–101.3)	113.1 (102.1–146.0)
Age (months)	15 (5.5)	13 (6.2)	11 (6.1)	9.0 (4.2)
Male sex	12 (44%)	20 (74%)	21 (78%)	19 (73%)
Atopic illness*	19 (70%)	14 (52%)	18 (67%)	8 (31%)
Eczema	9 (33%)	9 (33%)	6 (22%)	6 (23%)
B-eos $>0.4 \times 10^9/l$	16 (59%)	10 (37%)	15 (63%)	3 (12%)
Sensitization	7 (26%)	8 (30%)	6 (23%)	4 (15%)
Virus etiology				
Rhinovirus	21 (78%)	20 (74%)	23 (85%)	18 (69%)
RSV	6 (22%)	8 (30%)	7 (26%)	10 (38%)
Bocavirus-1	10 (37%)	3 (11%)	5 (19%)	1 (4%)
Coinfection	10 (37%)	9 (33%)	10 (37%)	12 (46%)

25OHD, serum 25-hydroxyvitamin D; B-eos, blood eosinophil count; RSV, respiratory syncytial virus.

Data are expressed as mean (standard deviation), median (interquartile range), or number (%) unless otherwise noted.

Data were analyzed by two sample *t*-test, one-way ANOVA, Spearman correlation, or linear regression analysis.

*Included IgE sensitization, eczema, or elevated blood eosinophil count.

recruitment, indoor pets, family history of asthma, virus coinfection, or illness severity, including illness duration before admission to hospital, heart rate, blood oxygen saturation, temperature and illness severity score at study entry, duration of hospitalization, and duration of respiratory symptoms within 2 weeks after discharge (Table 2, Table S2).

In our study, age was a major determinant of 25OHD concentration in first-time wheezing children. Despite that all participating families, at the 4-year follow-up visit ($n = 77$), reported having given vitamin D supplement during early life, a strong inverse association was seen in our study as well as in earlier studies (6, 7). We speculate that the supplement use becomes more irregular after early childhood. On the contrary, the incidence of atopic sensitization increased with age. One might expect an association between 25OHD and atopy (or other atopic characteristics) in these kinds of circumstances (2), but we did not find it in age-adjusted analysis. A recent case-control study by Stenberg et al. (6) reported an association between vitamin D insufficiency and wheezing in young children, but they did not find an association between vitamin D status and atopy-related factors. A few studies of young children without wheezing, or of older children, have reported an inverse association between the 25OHD level and atopic dermatitis or severity of atopic dermatitis (2, 7, 8). The differences between our finding and these other studies may be explained by the adequate mean 25OHD concentration in our study population (86 nmol/l). In the other studies, the mean levels of serum 25OHD were much lower than in our study – for example, 29 nmol/l (8) and 48 nmol/l (7).

Besides atopy, a low 25OHD concentration has been reported to be associated with increased susceptibility to acute respiratory infection (9). We did not find a connection between 25OHD and specific respiratory virus infection, not even for sensitive markers of rhinovirus load, virus coinfection or prolonged shedding of bocavirus (based on combined PCR and

Table 2 The association between patient characteristics and serum 25-hydroxyvitamin D concentration

	Unadjusted	Age-adjusted
Age (months)	<i>−1.4 (−2.0 to −0.75)</i> <i>p < 0.0001</i>	
Male sex	<i>9.6 (1.1 to 18)</i> <i>p = 0.03</i>	<i>8.3 (0.45 to 16)</i> <i>p = 0.04</i>
Any atopic illness*	−6.2 (−14 to 1.9) <i>p</i> = 0.13	−0.20 (−8.3 to 7.9) <i>p</i> = 0.96
Eczema	−2.9 (−12 to 6.2) <i>p</i> = 0.53	1.5 (−7.1 to 10) <i>p</i> = 0.74
B-eos $>0.4 \times 10^9/l$	<i>−10 (−19 to −2.2)</i> <i>p = 0.01</i>	−5.4 (−14 to 2.8) <i>p</i> = 0.20
Any sensitization	−3.9 (−14 to 5.9) <i>p</i> = 0.42	3.3 (−6.2 to 13) <i>p</i> = 0.49
Virus etiology		
Rhinovirus	−1.8 (−11 to 7.8) <i>p</i> = 0.71	4.5 (−4.8 to 14) <i>p</i> = 0.34
RSV	2.6 (−6.4 to 12) <i>p</i> = 0.57	−2.7 (−11 to 5.9) <i>p</i> = 0.63
Bocavirus-1	<i>−14 (−24 to −3.9)</i> <i>p = 0.007</i>	−9.3 (−19 to 0.67) <i>p</i> = 0.07

B-eos, blood eosinophil count RSV, respiratory syncytial virus.

Data are expressed as mean difference in 25OHD serum concentration (95% confidence interval). Significant associations are shown with bold and italic. Total 25 OHD was used as dependent factor.

Data were analyzed by two sample *t*-test, one-way ANOVA, Spearman correlation or linear regression analysis.

*Included any IgE sensitization, eczema, or elevated blood eosinophil count.

serology diagnostics) in wheezing children. Urashima et al. (10) studied the effect of vitamin D intake on susceptibility to influenza A infections in Japanese school children and reported

an inverse effect. However, they did not measure serum 25OHD levels. A population-based study of Science et al. (9) reported that low serum 25OHD levels are associated with increased risk of acute respiratory infections. Compared to our study, the median levels of serum 25OHD were again lower in this study (62 nmol/l), which may explain the significant associations.

Our study has some limitations. Our sample size was rather small. Moreover, as few children had 25OHD levels <50 nmol/l, vitamin D deficiency could not be investigated. Our results may not be applicable for children in other countries because Finnish children are instructed to take a daily vitamin D supplement. Although the intake of vitamin D supplement was not closely recorded, we did assess the best measure of vitamin D status, serum 25OHD level.

In conclusion, in wheezing children receiving vitamin D supplement of 400 IU per day with mean 25OHD level of 86 nmol/l (IQR 71–101 nmol/l), the 25OHD level was not associated with rhinovirus, RSV or bocavirus infection, virus coinfection, atopy, or severity of illness. These results generate the hypothesis that serum 25OHD levels thought to be adequate for healthy children are sufficient also for high-risk children susceptible to asthma.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Additional patient characteristics by quartile of serum 25-hydroxyvitamin D concentration

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Conflict of interest

In relation to this paper, the authors have no conflict of interest.

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Table S2. Additional associations between patient characteristics and serum 25OHD concentration

Appendix S1. Parental questionnaire.

Appendix S2. Clinical score chart at the emergency room or hospital ward.

Appendix S3. Symptom and medication diary for 2 weeks after discharge.

Online Supplementary**Vitamin D, virus etiology, and atopy in first-time wheezing children in Finland**

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13 Online Supplementary Table 1. Additional patient characteristics by quartile of serum 25-
 14 hydroxyvitamin D concentration

	Quartile of Serum 25OHD Concentration			
	1	2	3	4
No. of subjects	27	27	27	27
25OHD2	19 (0, 27)	14 (0, 33)	29 (16, 49)	17 (0, 43)
25OHD3	43 (17)	57 (22)	61 (24)	87 (31)
Atopic eczema	5 (19%)	6 (22%)	3 (12%)	3 (12%)
B-Eos ($\times 10^9/L$)	0.45 (0.20, 0.69)	0.37 (0.08, 0.68)	0.48 (0.15, 0.75)	0.14 (0.048, 0.36)
Food sensitization	7 (26%)	8 (30%)	5 (19%)	4 (15%)
Aeroallergen sensitization	2 (7%)	4 (15%)	5 (19%)	1 (4%)
Perennial sensitization	2 (7%)	4 (15%)	4 (16%)	1 (4%)
Illness duration before admission to hospital	4 (2, 7)	3 (2, 6)	5 (3, 6)	5 (2.8, 7)
Illness severity				
Inpatientcy	19 (70%)	25 (93%)	23 (85%)	19 (73%)
Severity score	5 (3, 7)	7 (4, 9)	4,5 (3, 7)	5 (4, 6)
Heart rate at study entry	140 (21)	140 (22)	140 (18)	150 (16)
Saturation rate at study entry	97 (96, 99)	97 (95, 98)	97 (95, 98)	97 (95, 99)
Temperature rate at study entry	37 (37, 38)	37 (37, 38)	37 (37, 38)	37 (37, 38)
Duration of rhinitis	14 (8, 22)	12 (7, 28)	16 (8, 20)	15 (7, 24)
Duration of noisy breathing	7 (4, 12)	10 (5, 18)	13 (6, 20)	14 (8, 17)
Duration of breathing difficulty	2 (0, 7)	3 (0, 6)	3 (1, 7)	3 (0, 8)
Rhinovirus load/ml	560 (0.81, 5500)	4800 (160, 77000)	10000 (170, 25000)	2300 (79, 16000)
Bocavirus-1 serodiagnosed	9 (33%)	3 (11%)	4 (15%)	1 (4%)

Bocavirus-1 PCR-	10 (37%)	3 (11%)	4 (15%)	1 (4%)
diagnosed				

- 15 Abbreviations: 25OHD, serum 25-hydroxyvitamin D; B-eos, blood eosinophil count.
- 16 Data are expressed as mean (standard deviation), median (interquartile range), or number (%) unless otherwise
- 17 noted.
- 18 Data were analysed by two sample t-test, one-way ANOVA, Spearman correlation or linear regression analysis.

19 Online Supplementary Table 2. Additional associations between patient characteristics and
 20 serum 25OHD concentration

	Unadjusted	Age-adjusted
Atopic eczema	-6.6 (-18 to 4.5) p = 0.24	1.6 (-9.5 to 13) p = 0.78
LogB-eos	-2.1 (-5.4 to 1.09) p = 0.19	-0.61 (-3.7 to 2.5) p = 0.70
Food sensitization	-3.8 (-14 to 5.9) p = 0.44	2.2 (-7.3 to 12) p = 0.65
Aeroallergen sensitization	-3.6 (-17 to 9.4) p = 0.58	7.1 (-5.7 to 20) p = 0.27
Perennial sensitization	-3.9 (-17 to 9.6) p = 0.57	6.9 (-6.6 to 20) p = 0.31
Illness duration before admission to hospital	-0.018 (-0.22 to 0.19) p = 0.86	-0.038 (-0.23 to 0.15) p = 0.69
Illness severity		
Inpatientcy	0.44 (-9.8 to 11) p = 0.93	0.18 (-9.3 to 9.6) p = 0.97
Severity score	0.12 (-1.6 to 1.8) p = 0.89	0.46 (-1.1 to 2.1) p = 0.57
Heart rate at study entry	0.15 (-0.059 to 0.36) p = 0.15	0.089 (-0.11 to 0.28) p = 0.38
Oxygen saturation at study entry	-0.070 (-1.7 to 1.5) p = 0.93	-0.58 (-2.1 to 0.90) p = 0.44
Temperature at study entry	-0.085 (-6.3 to 6.1) p = 0.98	0.88 (-4.9 to 6.7) p = 0.76
Duration of rhinitis	-0.15 (-0.48 to 0.19) p = 0.39	-0.064 (-0.38 to 0.25) p = 0.69
Duration of noisy breathing	0.19 (-0.14 to 0.51) p = 0.25	0.20 (-0.10 to 0.50) p = 0.20
Duration of breathing difficulty	0.072 (-0.69 to 0.83) p = 0.85	0.16 (-0.54 to 0.87) p = 0.65

>3000 rhinovirus copies/ml	0.34 (-9.8 to 10) p = 0.95	1.3 (-8.1 to 11) p = 0.78
Bocavirus-1, serodiagnosed	-15 (-26 to 4.6) p = 0.006	-10 (-21 to 0.080) p = 0.05
Bocavirus-1, PCR- diagnosed	-15 (-25 to 4.6) p = 0.005	-10 (-20 to 0.14) p = 0.05
Interaction		
Atopy x Rhinovirus	p = 0.25	p = 0.20
Atopy x RSV	p = 0.68	p = 0.66
Atopy x Bocavirus	p = 0.28	p = 0.37
Atopy x coinfection	p = 0.42	p = 0.61

-
- 21 Abbreviations: B-eos, Blood eosinophil count.
- 22 Data are expressed as mean difference in 25OHD serum concentration (95% confidence interval). Total 25 OHD
- 23 was used as dependent factor.
- 24 Data were analysed by two sample t-test, one-way ANOVA, Spearman correlation or linear regression analysis.

25 **Appendix 1. Parental questionnaire.***

26 **The key questions**

27 **To be filled by study physician at parental interview**

28 Name: _____

29 Social security number: _____

30 Names of the parents / guardians: _____

31 _____

32 Address: _____

33 Phone: _____

34 Email: _____

35

36 **Does the child fulfill inclusion criteria of the study:** age 3-23 months, $\geq$ h37+0, first episode
37 of breathing difficulty and written informed consent from the parents?

38 Yes

39 **Does the child fulfill inclusion criteria of the intervention trial:** rhinovirus PCR positive
40 and still signs of lower respiratory infection (breathing difficulty, noisy breathing or cough)?

41

42 Yes No

43 **Randomized to receive the study drug:** Yes No

44 **If yes, when (day, time)** _____

45 **Any exclusion criteria:** chronic other than atopy related illness, previous systemic or inhaled
46 corticosteroid treatment, participation to another study (excluding long-term follow-up studies
47 in childhood), varicella contact if previously intact, need for intensive care unit treatment, or
48 poor understanding of Finnish No

- 49 Parents / guardians have received routine hospital wheezy questionnaires (2 forms) and
 50 symptom diaries (3 forms): Yes
- 51 Height _____ cm and weight _____ kg
- 52 Still breastfeeding Yes No
- 53 Duration of breastfeeding _____ months
- 54 Duration of exclusive breastfeeding _____ months
- 55 Does the child have doctor-diagnosed atopic eczema: Yes No
- 56 Mother Father
- 57 Doctor-diagnosed asthma: Yes No Yes No
- 58 Allergic rhinitis: Yes No Yes No
- 59 Smoking: Yes No Yes No
- 60 Furry pets: Yes No
- 61 Number of children in the family: _____ children
- 62 Daycare: Home Small group Kindergarden
- 63

64 **Wheezy questionnaire**65 **To be filled by a parent/guardian**

66 1. Does your child have a family doctor?

67 No Yes , Dr _____ practicing in _____

68 2. Type of daycare?

69 1) Home 2) Family day care 3) Day care center 4) Other , what? _____

70 3. Type of home?

71 1) Apartment building 2) House 3) Row house 4) Farm

72 5) Other , what? _____

73 4. Number of children in the family? _____

74 5. Parental smoking? No Yes , if yes, smoking:

75 1) inside No Yes

76 2) in the car No Yes

77 6. Pets at home? dog No Yes

78 cat No Yes

79 other animals No Yes , what? _____

80 7. Other allergen sources at home?

81 feather pillows/blankets No Yes

82 fitted carpet No Yes

83 8. At day care pets/animals? No Yes , what? _____

84 smoking? No Yes

85 9. At other places, weekly exposure to

86 animals? No Yes

87 smoking? No Yes

88 10. Are there allergic symptoms in the family?

89 eczema No Yes , underline: mother / father / sibling

90 rhinitis No Yes , underline: mother / father / sibling

91 asthma No Yes , underline: mother / father / sibling

92 11. Does the child have allergic symptoms? Please, mark the suspected source on the reverse

93 side. eczema No Yes

94 rhinitis No Yes

95 asthma No Yes

96 12. Does your child have an “allergy diet”?

97 No Yes Please, specify the diet to the study nurse.

98 13. Has your child ever undergone skin prick tests?

99 No Yes , when ____/____(month/year), where _____

100 14. Information about **allergies** (please circle the suspected sources):

101 1) Dietary: chocolate, cocoa, citrus, egg, fish, tomato, strawberry, pea, apple,

102 carrot, nuts, pear, peach, cow’s milk, breast milk substitute, rye, barley, oats,

103 wheat, other _____

104 2) Animals: dog, cat, horse, cow, guinea pig, feather, other _____

105 3) Pollen: birch, alder, conifer, hay, mugworth, other _____

106 4) Other causes: room dust, fungal spore, other _____

107 15. Information about the child’s **respiratory infections**:

108 During the last 12 months:

109 1) “common cold” _____ times

110 2) antibiotic prescription _____ times

111 3) pneumonias _____ times

112 4) bronchitis _____ times

113 5) otitis _____ times

114 6) parasentesis _____ times

115 7) other, what? _____

116 16. Adenoidectomy

117 No Yes , when ____/____ (month/year), where _____

118 17. Maxillary sinus puncture

119 No Yes , when ____/____ (month/year), where _____

120 18. Information about breathing difficulty symptoms:

121 Were there “common cold” symptoms during the current difficulty in breathing?

122 No Yes I can’t say

123 If you suspect other causes, please name them: _____

124 19. The duration of respiratory symptoms before study entry?

125 1) rhinitis_____ days

126 2) cough _____ days

127 3) rhinitis_____ days

128 20. Have other family members had “common cold” symptoms? No Yes

129 21. Is this your child’s first episode of breathing difficulties? No Yes

130 22. Does your child have any regular medication? No Yes , what? _____

131 *The key questions are directly translated from Finnish study form. The wheezy questionnaire
132 contains selected questions from 2 page standard wheezy questionnaire and 7 page standard
133 allergy questionnaire used at Turku University Hospital.

134

135 **Appendix 2. Clinical score chart at the emergency room or hospital ward.**

136 **Name** _____ **Social security number** _____

137 (After study entry, to be filled in the mornings 2 hours after bronchodilator).

138 An example

Date, time	7.9.2007 8.00				
Heart rate/min	112				
Breathing frequency/min	55				
Oxygen saturation	98				
Body temperature	37.6				
Inspiratory:expiratory time: 0 (2:1), 1 (1:1), 2 (1:2), 3 (1:3)	1:2				
Degree of dyspnea: 0 (normal), 1 (mild), 2 (moderate), 3 (severe)	2				
Auscultatory findings on wheezing: 0 (none), 1 (expiratory), 2 (inspiratory and expiratory), 3 (audible without stethoscope)	2				
Type of breathing: 0 (normal), 1 (use of stomach muscles), 2 (use of intercostal muscles), 3 (nasal flaring)	1				
Pneumonic crackles: 0 (no), 1 (mild), 2 (moderate), 3 (severe)	0				
Otitis media: 0 (no), 1 (yes)	0				
Antibiotic: 0 (no), 1 (yes), what and when initiated	0				
Other symptoms +/-/+ and their connection to study medication: 1 (no), 2 (maybe), 3 (yes)					
Cough	++/1				
Rhinitis	+/1				
Eye symptoms	-				
Eczema	-				

Any other symptoms/signs (report everything)	Restlessness ++/1				
Other notes, e.g. the cause of symptoms	flu				
Study drug given (tally)	Ill				
Medication (drug name, dosage) before physical examination (tally)	ER: albuterol 1.2 mg x2, ward: albuterol 1.5 mg x2				
...and before blood draw	+ albuterol 1.5 mg x2				
Oxygen (hour) or i.v. or nasogastric hydration (volume/time)	Oxygen 5 h i.v. fluid 700 ml				
Exact times: Study entry at ER/ward (1), study drug initiation (2), ready for discharge considering breathing difficulties (3), actual discharge (4).	(1) 15.30				

139 **Appendix 3. Symptom diary 1, Vinku2 study.**140 **Name** _____ **Social security number** _____

141 Daily symptom and medications until 2-week visit.

Date (fill in one column per day)	An example 1.1.07								
Hospitalization for expiratory breathing difficulty, yes or no	Yes								
Cough, 0 (no) - 3 (severe)	2								
Expiratory breathing difficulty, 0 (no) - 3 (severe)	1								
Noisy breathing, 0 (no) - 3 (loud)	0								
Rhinitis, 0 (no) - 3 (severe)	0								
Night waking for breathing difficulties, 0 (no), 1 (once), 2 (often), 3 (continuously)	1								
Temperature, exact or on scale 0 (no) - 3 (high)	Fever 37.9, or 1								
Any other symptom (report any deviation from normal)	Fell in stairs, tearful								
Other notes (e.g. cause of symptom)	scute otitis media, playing with a cat								
Study drug taken (tally)	111								

Bronchodilator (name, dose, number of doses; tally)	Ventoline 0.1 mg, puffs 1111								
Other medication	Naprosyn mixt. 5 mg/ml 3 ml/dose, 111 Amorion mixt 80 mg/ml 3.7 ml x2								
Doctor's appointment: where, why, name of the doctor, and treatment	Healthcenter Mäntymäki 1, fever, cough, tearful, Naprosyn and Amorion for acute otitis media								

142 If any questions, do not hesitate to contact study physician by phone.