

**Duration and exclusiveness of breastfeeding and childhood asthma-related symptoms.**

*Short title: Breastfeeding and asthma-related symptoms*

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## **ABSTRACT**

**Objectives** To examine the associations of breastfeeding duration and exclusiveness with the risks of asthma-related symptoms in preschool children, and to explore whether these associations are explained by atopic or infectious mechanisms.

**Design** This study was embedded in a population-based prospective cohort study among 5,368 children. Information on breastfeeding duration, exclusiveness and asthma-related symptoms, including wheezing, shortness of breath, dry cough and persistent phlegm, was obtained by questionnaires.

**Results** Compared to children who were breastfed for 6 months, those who were never breastfed had overall increased risks of wheezing, shortness of breath, dry cough and persistent phlegm during the first four years (Odds ratios 1.44 (95% Confidence Interval: 1.24, 1.66), 1.26 (1.07, 1.48), 1.25 (1.08, 1.44) and 1.57 (1.29, 1.91), respectively) Similar associations were observed for exclusive breastfeeding. The strongest associations per symptom per year were observed for wheezing at 1 and 2 years. Additionally adjusted analyses showed that the associations of breastfeeding with asthma-related symptoms were not explained by eczema but partly by lower respiratory tract infections.

**Conclusions** Shorter duration and non-exclusivity of breastfeeding were associated with increased risks of asthma-related symptoms in preschool children. These associations seemed at least partly explained by infectious but not by atopic mechanisms.

## **INTRODUCTION**

Asthma-related symptoms are common in early childhood and are a leading cause of morbidity [1]. Known risk factors in early life for asthma-related symptoms include birth weight, gestational age, parental socio-economic status, ethnicity, presence of siblings, day care attendance, family history of asthma or atopy and parental smoking [2]. A substantial body of evidence suggests that breastfeeding is also associated with a reduced risk of childhood asthma and asthma-related symptoms [3-14]. Some studies reported stronger protective effects of breastfeeding on asthma in children with a positive family history of asthma or allergy [8, 15-16] whereas others did not [6, 11-12]. Studies that focused on asthma later in life showed inconsistent results [5, 7-8, 10-11]. Breastfeeding might affect the risk of childhood asthma because of a mediating effect of atopy, infections or both.

Underlying mechanisms might include IgA, cytokines, especially TGF-beta1, and long-chain fatty acids in breast milk that stimulate the infant's immune system [17]. Also, glycans help the innate immune system to inhibit pathogen binding to the host cell target ligand [18], and changes in the delicate balance between pro- and anti-inflammatory compounds [19]. Various methodological issues might have influenced results from previous studies. These include recall bias of feeding habits in retrospective studies, differences in information about duration and exclusiveness of breastfeeding, and adjustment for confounders [2, 5-7, 11, 14].

Therefore, we examined in a population-based prospective cohort study the associations of the duration and exclusiveness of breastfeeding with the risks of asthma-related symptoms during the first 4 years and examined whether any association is explained by atopic or infectious mechanisms.

## **METHODS**

**Design and cohort** This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children from fetal life onwards in Rotterdam, The Netherlands, and has previously been described in detail [20]. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all participants. In total 7,295 children and their parents participated in the postnatal phase of the study. From those children, twins (n = 179) and second or third children of the same mother in the study (n = 539) were excluded from the present analyses to prevent bias due to correlation (Figure S1). Of the remaining children, breastfeeding and asthma-related symptom data were available of 5,368 children.

**Breastfeeding duration and exclusiveness** Information about breastfeeding initiation and continuation was obtained by postal questionnaires at the ages of 2, 6 and 12 months after birth [20]. The duration of breastfeeding was assessed by asking whether they ever breastfed their child (no, yes) and at what age (weeks) they quitted breastfeeding. Subsequently, breastfeeding duration was categorized into four groups: never; younger than 3 months; 3 to 6 months and 6 months or older. Exclusive breastfeeding was defined using information on the introduction of other milk or solids. The information about exclusiveness of breastfeeding was combined and categorized into the following three breastfeeding categories: never; non-exclusive breastfeeding until 4 months and exclusive breastfeeding until 4 months.

**Asthma-related symptoms** Information on asthma-related symptoms was obtained by questionnaires at the ages of 1, 2, 3 and 4 years. Questions about asthma-related symptoms were adapted from the International Study on Asthma and Allergy in Childhood (ISAAC) [21]. Response rates for these questionnaires were 71%, 76%, 72%, 73%, respectively. Information about asthma-related symptoms in the past year included wheezing (never, 1-3 times, >4 times), shortness of breath (never, 1-3 times, >4 times), dry cough at night (no, yes),

and mucus congestion (no, yes). Parents also reported information about doctor-attended eczema and lower respiratory tract infections (pertussis, bronchitis, bronchiolitis, or pneumonia) in the past year which information was used as markers of atopy and infection, respectively.

**Covariates** Information on parental history of asthma or atopy, socio-economical status, ethnicity, parity and pet keeping were obtained by questionnaire, completed by mother at enrollment. Information about active maternal smoking was obtained by postal questionnaires sent in first, second and third trimester of pregnancy and combined into smoking (no, yes) [20]. Socio-economical status was assessed using the highest educational level achieved by the parents. Maternal ethnicity was based on country of birth of her and her parents [20]. We used parity as a proxy for siblings, the correlation between those variables was good ( $\kappa = 0.894$ ). Birth weight, gestational age and sex of the children were obtained from midwife and hospital registries at birth. Home sent questionnaires at the ages of 6 and 12 months provided information about daycare attendance.

**Data analysis** *Longitudinal analyses.* We used generalized estimating equations (GEEs) to examine the longitudinal effects of duration and exclusiveness of breastfeeding with each asthma-related symptom (no, yes) from the age of 1 to 4 years. With GEE analyses, repeatedly measured asthma-related symptoms over time can be analyzed, taking into account that these repeated measurements within the same subject are correlated. Also, breastfeeding and age might be correlated and therefore breastfeeding was used in the model as a time-dependent variable. Covariates were not repeatedly measured over time and were introduced in the models as time-independent. *Additional confounder analyses.* To assess whether the associations of breastfeeding with asthma-related symptoms could be explained by atopic or

infectious mechanisms, we additionally adjusted the analyses for doctor-attended eczema and lower respiratory tract infections measured at the corresponding ages. *Effect modification analyses.* To assess the potential modifying effect of parental history of asthma or atopy we added parental history of asthma or atopy (no, yes) as an interaction term with exclusive breastfeeding in the GEE models with wheezing as the outcome (wheezing = exclusivity of breastfeeding + parental history of asthma or atopy + exclusivity of breastfeeding\*parental history of asthma or atopy + other confounders). Thereafter, we stratified our GEE models for breastfeeding exclusivity by parental history of asthma or atopy. *Survival analysis.* We performed a discrete survival analysis to calculate time to first asthma-related symptom according to breastfeeding duration and exclusiveness. For these analyses, the 4 different asthma-related symptoms were combined into one categorical variable asthma-related symptom (no, yes). *Dose - response analysis.* The associations of breastfeeding duration and exclusivity with frequencies of asthma-related symptoms at the ages of 1, 2, 3 and 4 years were analyzed using multiple logistic regression analysis.

Missing data in the covariates were imputed using the multiple imputation procedure, which is used to select possible values for a missing response. Five imputed data sets were created and analyzed together. All models were adjusted for potential confounders including parental age, education, ethnicity, smoking habits, maternal parity, children's sex, gestational age, birth weight, parental history of asthma or atopy, daycare attendance and pet keeping. Test for trends were performed by including the breastfeeding categories as continuous variables in the regression models. All measures of association are presented with their 95% confidence intervals (CI). The statistical analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, IL, USA) and SAS 9.2 (SAS institute, Cary, NC, USA).

## RESULTS

Of the total group of 5,368 children 92.3% had ever been breastfed. Of those, information about duration and exclusiveness of breastfeeding was available for 79.7% (n = 4,280) and 81.1% (n = 4,353) children, respectively. The median duration of breastfeeding was 3.5 months (95% range 0.5 - 12.0 months) and 21.3% was breastfed exclusively until the age of 4 months. Table 1 shows the parental and child characteristics according to breastfeeding duration. Wheezing was the most frequently reported asthma-related symptom during the first year (Table 2). Child and parental characteristics differed between those with and without available data on asthma-related symptoms except for gender and ever eczema (Table S1). The effect sizes of unadjusted and non-imputed analyses (Tables S2 and S3) of the associations of duration and exclusiveness of breastfeeding with asthma-related symptoms did not materially change after adjustment for confounders or performing multiple imputations of the confounders.

**Duration of breastfeeding** Based on the GEE models, those who were never breastfed had overall increased risks of wheezing, shortness of breath, dry cough and persistent phlegm (Odds ratios 1.44 (95% Confidence Interval: 1.24, 1.66), 1.26 (1.07, 1.48), 1.25 (1.08, 1.44) and 1.57 (1.29, 1.91), respectively) during the first four years, compared to children who were breastfed for more than 6 months (Figures 1a-1d). Analyses focused on these symptoms per year, showed that children who had been breastfed for shorter periods, had increased risks of wheezing at 1, 2 and 3 years (p-values for trend <0.05) (Figure 1a). A non-significant trend in the same direction was observed at the age of 4 years. Prolonged breastfeeding was associated with a lower risk of shortness of breath at 1 year (OR 1.38 (1.05, 1.80)) (Figure 1b) and non-significant trends were observed for the older ages. Breastfeeding duration was also associated with the risk of dry cough at 3 years, but not at other ages (Figure 1c), and with the



risk of persistent phlegm at 1, 3 and 4 years (Figure 1d). Effect estimates for each specific exposure and the asthma-related symptoms (dose-response) are given in the supplement (Table S4). Based on the discrete survival analysis, those who were never breastfed, breastfed for 0-3 or 3-6 months tended to have asthma-related symptoms earlier in life compared to those who were breastfed for more than 6 months (Hazard Ratios (HRs) 1.13 (0.97, 1.32), 1.06 (0.96, 1.17) and 1.03 (0.92, 1.15), respectively) (Figure 3).

**Exclusiveness of breastfeeding** Those who were non-exclusively breastfed for 4 months, had increased risks of wheezing, shortness of breath, dry cough and persistent phlegm during the first 4 years (ORs 1.21 (1.09, 1.34), 1.14 (1.02, 1.28), 1.20 (1.10, 1.31) and 1.21 (1.04, 1.42), respectively), compared to children who were exclusively breastfed for 4 months (Figure 2). Analyses focused on each year separately, showed that compared to children who had been exclusively breastfed for 4 months, those who had been non-exclusively breastfed for 4 months had an increased risk of wheezing at 1,2 and 3 years (p-values for trend <0.05). Non-significant results were observed at 4 years. We observed similar but less consistent tendencies for dry cough (Figure 2c), but not for shortness of breath and persistent phlegm (Figure 2b, d). Based on the discrete survival analysis, those who were never or not exclusively breastfed for 4 months had asthma-related symptoms earlier in life compared to those who were exclusively breastfed (HRs 1.23 (1.05, 1.44), 1.14 (1.03, 1.26), respectively) (Figure 4).

**Atopy and infections** Adjustment for eczema did not materially change the effect estimates of the association between breastfeeding exclusiveness with asthma-related symptoms in the first four years of life, whereas the estimates decreased when lower respiratory tract infections were added as a confounder (Figure 5, table S6). *Effect modification analyses.* Small

differences in risks of wheezing were observed for non-exclusive breastfed children with and without a parental history of asthma or atopy (Figure S2, Table S7). However, no effect modification by parental history of asthma or atopy was observed for the associations of exclusiveness of breastfeeding with wheezing (p-values interaction term  $>0.05$  in the GEE model).

## **DISCUSSION**

Shorter duration and non-exclusivity of breastfeeding were associated with increased risks of asthma-related symptoms in preschool children. The strongest effect estimates were observed for wheezing during the first 3 years.

Previous studies reported consistent results on the associations between duration and exclusive breastfeeding and the risk of asthma in childhood. These suggested an up to 2.22-fold increased risk of recurrent wheezing or asthma at the ages of 2 to 6 years among children who were not breastfed or not exclusively breastfed until the age of 4 months [3-11, 13]. Our effect estimates are in line with these studies and additionally we observed a dose-response relation between breastfeeding and the number of wheezing episodes. We observed similar results for shortness of breath, dry cough at night and persistent phlegm. Also, we found that the first reported asthma-related symptom occurred earlier in life if children were shorter or non-exclusively breastfed. We found evidence for a protective effect of breastfeeding on wheezing until the age of 2 years, but not thereafter. In these first years, wheezing is predominantly associated with respiratory tract infections [22]. Indeed, we observed that the protective effect of breastfeeding on asthma-related symptoms decreased after adjusting for lower respiratory tract infections at the corresponding ages.

The gut microflora is suggested to be different between breastfed and formula fed infants. Compared to breastfed infants, those who receive formula feeding have a more complex

microflora with more facultative anaerobes, bacteroides and clostridia at higher levels and frequencies [23]. We speculate that this might decrease with increasing exclusivity of breastfeeding, leading to lower infection risk and less wheezing by influencing the development of the immune system [22]. Due to this putative effect on the development of the immune system, infections and asthma-related symptoms might occur less frequent even years after stopping breastfeeding. This is in line with the previously reported inconsistent results for the association of breastfeeding with the risk of asthma after the preschool age, as in that period the gut microflora has stabilized, respiratory tract infections are less frequent and atopic mechanisms are more relevant. Also, our results regarding the non-significant associations with asthma-related symptoms at older ages are in line with a previously published randomized clustered trial [24].

Previous studies reported inconclusive or inversed associations of breastfeeding with eczema or atopy. Also, breastfeeding is suggested to have a potential adverse long-term effect on asthma which demonstrates the difficulties of giving breast-feeding advice for atopy-prevention [2, 5, 8]. We did not observe a change in effect estimates for asthma-related symptoms after adjusting for eczema, but did found a small differential effect of breastfeeding on asthma-related symptoms among children with and without a parental history of asthma or atopy. However, the interaction term was not significant probably due to the lack of large statistical power. Our results suggest that the associations of breastfeeding exclusiveness with asthma-related symptoms are at least partly explained by parental asthma or atopy.

Previously, Wright et al. also observed different relationships between breastfeeding and asthma with the presence or absence of maternal asthma and atopy [8]. Breastfed children of asthmatic mothers had an increased risk of asthma from 6 years onwards, compared to breastfed children of non-asthmatic mothers. However, other studies did not report effect

modification of a parental history of asthma or atopy on the association of breastfeeding with wheezing [5, 11-12].

This study was embedded in a population-based prospective design with a large number of subjects being studied from early life onwards, and information about a large number of potential confounders was prospectively collected. We adjusted for a large number of confounders and the results did not differ between non-imputed and imputed analysis.

However, we cannot exclude that other possible (residual) confounders or effect modifiers or the influence of genetic variances might have been present.

Non-response would lead to biased effect estimates if the associations of breastfeeding duration and exclusivity with asthma-related symptoms would be different between those included and not included in the analyses. However, this seems unlikely because biased estimates mainly arose from loss to follow-up rather than from non-response at baseline [25].

Among infants without data on asthma-related symptoms, the frequencies of breastfeeding were lower than among infants with information on symptoms. This might have led to some loss of power and underestimation of the observed protective effects of breastfeeding in our cohort.

The main outcomes in our study were self-reported asthma-related symptoms. This method is widely accepted in epidemiological studies and reliably reflect the incidence of asthma-related symptoms in young children [26]. In preschool children a diagnosis of asthma is based on symptoms [27]. Objective tests, including lung function or bronchial hyperresponsiveness, are difficult to perform in young children or not informative. The most consistent protective effects of breastfeeding over time were observed for wheezing. For the other asthma-related symptoms, more varying and inconsistent patterns from birth to the age of 4 years were found. This might be due to lower prevalences of these symptoms and the possibility that these are related to infections rather than wheeze, representing other diseases more accurate,

such as respiratory tract infections. Reversed causality might be present if the duration and exclusiveness of breastfeeding would have been influenced by early manifestation of asthma-related symptoms and could have lead to underestimation of the effect estimates [5, 7-8, 15, 28]. In our cohort, we assessed only one asthma-related symptom, wheezing (no, yes), before the age of 2 months (n = 4,130). Of children who wheezed in their first year (n = 1,291), 18.8% had had a wheezing episode already in the first 2 months. The frequencies of the duration and exclusiveness of breastfeeding were similar in those who had and had not had a first wheezing episode at the age of 2 months (duration of breastfeeding >6 months 25.1% vs. 26.5%, exclusive breastfeeding 19.8% vs. 18.2%). Furthermore, when we additionally adjusted our presented analyses for wheezing before the age of 2 months, the effect estimates did not materially change. Therefore, it is unlikely that reversed causation was present in our cohort.

In conclusion, our results suggest that a short duration of breastfeeding and non-exclusivity are associated with increased risks of the asthma-related symptoms during the first 4 years of life, with the strongest effect estimates during the first two years. These associations seem to be partly explained by lower respiratory tract infections but not by atopic mechanisms. Further studies are needed to explore the underlying mechanisms and the protective effect of breastfeeding on the various types of asthma in later life.

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## REFERENCES

1. C. Mathers TB, D. Ma Fat. The global burden of disease - 2004 update. Geneva, Switzerland: WHO; 2004.
2. Midodzi WK, Rowe BH, Majaesic CM, Saunders LD, Senthilselvan A. Early life factors associated with incidence of physician-diagnosed asthma in preschool children: results from the Canadian Early Childhood Development cohort study. *J Asthma* 2010; 47(1): 7-13.
3. Fredriksson P, Jaakkola N, Jaakkola JJ. Breastfeeding and childhood asthma: a six-year population-based cohort study. *BMC Pediatr* 2007; 7: 39.
4. Giwercman C, Halkjaer LB, Jensen SM, Bonnelykke K, Lauritzen L, Bisgaard H. Increased risk of eczema but reduced risk of early wheezy disorder from exclusive breastfeeding in high-risk infants. *J Allergy Clin Immunol* 2010; 125(4): 866-871.
5. Elliott L, Henderson J, Northstone K, Chiu GY, Dunson D, London SJ. Prospective study of breast-feeding in relation to wheeze, atopy, and bronchial hyperresponsiveness in the Avon Longitudinal Study of Parents and Children (ALSPAC). *J Allergy Clin Immunol* 2008; 122(1): 49-54, 54 e41-43.
6. Kull I, Almquist C, Lilja G, Pershagen G, Wickman M. Breast-feeding reduces the risk of asthma during the first 4 years of life. *J Allergy Clin Immunol* 2004; 114(4): 755-760.
7. Kull I, Melen E, Alm J, Hallberg J, Svartengren M, van Hage M, Pershagen G, Wickman M, Bergstrom A. Breast-feeding in relation to asthma, lung function, and sensitization in young schoolchildren. *J Allergy Clin Immunol* 2010; 125(5): 1013-1019.
8. Wright AL, Holberg CJ, Taussig LM, Martinez FD. Factors influencing the relation of infant feeding to asthma and recurrent wheeze in childhood. *Thorax* 2001; 56(3): 192-197.
9. Oddy WH, Sly PD, de Klerk NH, Landau LI, Kendall GE, Holt PG, Stanley FJ. Breast feeding and respiratory morbidity in infancy: a birth cohort study. *Arch Dis Child* 2003; 88(3): 224-228.

10. Oddy WH, Holt PG, Sly PD, Read AW, Landau LI, Stanley FJ, Kendall GE, Burton PR. Association between breast feeding and asthma in 6 year old children: findings of a prospective birth cohort study. *BMJ* 1999; 319(7213): 815-819.
11. Scholtens S, Wijga AH, Brunekreef B, Kerkhof M, Hoekstra MO, Gerritsen J, Aalberse R, de Jongste JC, Smit HA. Breast feeding, parental allergy and asthma in children followed for 8 years. The PIAMA birth cohort study. *Thorax* 2009; 64(7): 604-609.
12. Rothenbacher D, Weyermann M, Beermann C, Brenner H. Breastfeeding, soluble CD14 concentration in breast milk and risk of atopic dermatitis and asthma in early childhood: birth cohort study. *Clin Exp Allergy* 2005; 35(8): 1014-1021.
13. Snijders BE, Thijs C, Dagnelie PC, Stelma FF, Mommers M, Kummeling I, Penders J, van Ree R, van den Brandt PA. Breast-feeding duration and infant atopic manifestations, by maternal allergic status, in the first 2 years of life (KOALA study). *J Pediatr* 2007; 151(4): 347-351, 351 e341-342.
14. Karmaus W, Dobai AL, Ogbuanu I, Arshard SH, Matthews S, Ewart S. Long-term effects of breastfeeding, maternal smoking during pregnancy, and recurrent lower respiratory tract infections on asthma in children. *J Asthma* 2008; 45(8): 688-695.
15. Sears MR, Greene JM, Willan AR, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Poulton R. Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. *Lancet* 2002; 360(9337): 901-907.
16. Mandhane PJ, Greene JM, Sears MR. Interactions between breast-feeding, specific parental atopy, and sex on development of asthma and atopy. *J Allergy Clin Immunol* 2007; 119(6): 1359-1366.
17. Friedman NJ, Zeiger RS. The role of breast-feeding in the development of allergies and asthma. *J Allergy Clin Immunol* 2005; 115(6): 1238-1248.



18. Newburg DS. Neonatal protection by an innate immune system of human milk consisting of oligosaccharides and glycans. *J Anim Sci* 2009; 87(13 Suppl): 26-34.
19. Hoppu U, Kalliomaki M, Laiho K, Isolauri E. Breast milk--immunomodulatory signals against allergic diseases. *Allergy* 2001; 56 Suppl 67: 23-26.
20. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A. The Generation R Study: design and cohort update 2010. *Eur J Epidemiol* 2010.
21. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995; 8(3): 483-491.
22. Friedlander SL, Jackson DJ, Gangnon RE, Evans MD, Li Z, Roberg KA, Anderson EL, Carlson-Dakes KT, Adler KJ, Gilbertson-White S, Pappas TE, Dasilva DF, Tisler CJ, Pleiss LE, Mikus LD, Rosenthal LA, Shult PA, Kirk CJ, Reisdorf E, Hoffjan S, Gern JE, Lemanske RF, Jr. Viral infections, cytokine dysregulation and the origins of childhood asthma and allergic diseases. *Pediatr Infect Dis J* 2005; 24(11 Suppl): S170-176, discussion S174-175.
23. Mountzouris KC, McCartney AL, Gibson GR. Intestinal microflora of human infants and current trends for its nutritional modulation. *Br J Nutr* 2002; 87(5): 405-420.
24. Kramer MS, Matush L, Vanilovich I, Platt R, Bogdanovich N, Sevkovskaya Z, Dzikovich I, Shishko G, Mazer B. Effect of prolonged and exclusive breast feeding on risk of allergy and asthma: cluster randomised trial. *BMJ* 2007; 335(7624): 815.
25. Nohr EA, Frydenberg M, Henriksen TB, Olsen J. Does low participation in cohort studies induce bias? *Epidemiology* 2006; 17(4): 413-418.
26. Jenkins MA, Clarke JR, Carlin JB, Robertson CF, Hopper JL, Dalton MF, Holst DP, Choi K, Giles GG. Validation of questionnaire and bronchial hyperresponsiveness against

respiratory physician assessment in the diagnosis of asthma. *Int J Epidemiol* 1996; 25(3): 609-616.

27. Edwards CA, Osman LM, Godden DJ, Douglas JG. Wheezy bronchitis in childhood: a distinct clinical entity with lifelong significance? *Chest* 2003; 124(1): 18-24.

28. Guilbert TW, Stern DA, Morgan WJ, Martinez FD, Wright AL. Effect of breastfeeding on lung function in childhood and modulation by maternal asthma and atopy. *Am J Respir Crit Care Med* 2007; 176(9): 843-848.

**Table 1.** Characteristics of children and their parents according to breastfeeding duration.

	<b>Children (n=4,280)</b>				
	Never n=416	0-3 months n=1,580	3-6 months n=923	≥6 months n=1,361	
<b>Maternal characteristics</b>					
Age (years)	30.7 (4.7)	30.1 (5.0)	31.5 (4.4)	31.8 (4.5)	p<0.001
Education (%)					
Primary, or secondary	64.0 (266)	55.7 (879)	34.9 (322)	32.6 (443)	p<0.001
Higher	28.9 (120)	39.7 (627)	61.2 (565)	63.6 (866)	
Missing	7.2 (30)	4.7 (74)	3.9 (36)	3.8 (52)	
Ethnicity (%)					
European	76.9 (320)	62.8 (993)	70.3 (649)	69.8 (950)	p<0.001
Non - European	17.5 (73)	34.1 (539)	27.5 (254)	27.7 (377)	
Missing	5.5 (23)	3.0 (48)	2.2 (20)	2.5 (34)	
Parity (%)					
0	52.6 (219)	66.5 (1,051)	66.3 (612)	57.5 (783)	p<0.001
≥1	45.0 (187)	32.1 (507)	32.1 (296)	39.8 (542)	
Missing	2.4 (10)	1.4 (22)	1.6 (15)	22.6 (36)	
Smoking during pregnancy (%)					
No	57.9 (241)	69.2 (1,092)	76.5 (706)	78.2 (1,064)	p<0.001
Yes	21.4 (89)	15.9 (251)	8.1 (75)	6.1 (83)	
Missing	20.7 (86)	15.0 (237)	15.4 (142)	15.7 (214)	
<b>Paternal characteristics</b>					
Age (years)	33.3 (5.5)	32.7 (5.7)	34.0 (5.2)	34.4 (5.3)	p<0.001
Education (%)					
Primary, or secondary	42.8 (178)	36.9 (584)	26.6 (245)	22.9 (312)	p<0.001
Higher	23.1 (96)	30.5 (483)	48.1 (444)	45.8 (623)	
Missing	34.1 (142)	32.5 (513)	25.4 (234)	31.3 (426)	
Ethnicity (%)					
European	70.4 (293)	58.4 (923)	67.8 (626)	64.5 (878)	p<0.001
Non - European	19.5 (81)	32.5 (514)	25.7 (237)	27.5 (374)	
Missing	10.1 (42)	9.1 (143)	6.5 (60)	8.0 (109)	
Smoking (%)					
No	39.7 (165)	46.1 (729)	50.9 (470)	53.9 (733)	p<0.001
Yes	40.6 (169)	39.2 (620)	35.1 (324)	30.5 (415)	
Missing	19.7 (82)	14.6 (231)	14.0 (129)	15.7 (213)	
<b>Child characteristics</b>					
Male sex, no (%)	51.2 (213)	50.8 (802)	51.1 (472)	48.5 (660)	p=0.518
Gestational age at birth (%)					
< 37 weeks	3.4 (14)	5.3 (84)	4.6 (42)	2.9 (39)	p=0.008

≥ 37 weeks	96.6 (402)	94.7 (1,496)	95.4 (881)	97.1 (1,322)	
Birth weight (grams)	3,415 (584)	3,398 (556)	3,438 (552)	3,505 (517)	p<0.001
Parental history of asthma or atopy (%)					
No	47.8 (199)	48.9 (773)	50.6 (467)	45.1 (614)	p=0.054
Yes	46.2 (192)	46.5 (734)	45.6 (421)	50.6 (688)	
Missing	6.0 (25)	4.6 (73)	3.8 (35)	4.3 (59)	
Day care attendance 1 <sup>st</sup> year (%)					
No	47.6 (198)	41.5 (655)	37.5 (346)	47.5 (647)	p<0.001
Yes	34.4 (143)	44.2 (699)	54.8 (506)	51.2 (697)	
Missing	18.0 (75)	14.3 (226)	7.7 (71)	1.2 (17)	
Pet keeping (%)					
No	42.8 (178)	53.9 (851)	57.0 (526)	57.0 (776)	p<0.001
Yes	37.0 (154)	30.4 (481)	28.1 (259)	26.6 (362)	
Missing	20.2 (84)	15.7 (248)	15.0 (138)	16.4 (223)	
Ever eczema (%)					
No	74.3 (309)	74.0 (1,169)	73.5 (678)	76.0 (1,034)	p=0.517
Yes	23.8 (99)	24.9 (394)	26.0 (240)	23.4 (318)	
Missing	1.9 (8)	1.1 (17)	0.5 (5)	0.7 (9)	
Ever lower respiratory tract infections (%)					
No	71.4 (297)	74.2 (1,173)	75.3 (695)	79.1 (1,077)	p=0.003
Yes	28.6 (119)	25.4 (402)	24.6 (227)	20.9 (284)	
Missing	0.0 (0)	0.3 (5)	0.1 (1)	0.0 (0)	

Values are shown in % (absolute numbers). Differences between breastfeeding groups were evaluated using chi-squared tests for categorical values and one-way anova for continuous variables (only p-values between the never and > 6 months breastfed groups are given).

**Table 2.** Frequencies of asthma-related symptoms.

	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
<b>Wheezing</b>	<i>n</i> =4,493	<i>n</i> =4,551	<i>n</i> =4,228	<i>n</i> =4,219
No	71.3 (3,202)	79.9 (3,638)	87.3 (3,691)	87.1 (3,675)
Yes	28.7 (1,291)	20.1 (913)	12.7 (537)	12.9 (544)
1 to 3 times per year	22.1 (992)	16.6 (756)	10.2 (432)	10.6 (449)
≥ 4 times per year	6.7 (299)	3.4 (157)	2.5 (105)	2.3 (95)
<b>Shortness of breath</b>	<i>n</i> =4,498	<i>n</i> =4,570	<i>n</i> =4,236	<i>n</i> =4,239
No	77.7 (3,495)	82.1 (3,750)	88.2 (3,738)	89.3 (3,787)
Yes	22.3 (1,003)	17.9 (820)	11.8 (498)	10.7 (452)
1 to 3 times per year	17.4 (781)	14.0 (642)	9.3 (396)	8.2 (346)
≥ 4 times per year	4.9 (222)	3.9 (178)	2.4 (102)	2.5 (106)
<b>Dry cough</b>	<i>n</i> =4,446	<i>n</i> =4,579	<i>n</i> =4,191	<i>n</i> =4,231
No	77.7 (3,453)	76.1 (3,484)	76.4 (3,200)	73.2 (3,099)
Yes	22.3 (993)	23.9 (1,095)	23.6 (991)	26.8 (1,132)
<b>Persistent phlegm</b>	<i>n</i> =4,437	<i>n</i> =4,541	<i>n</i> =4,267	<i>n</i> =4,267
No	86.9 (3,854)	90.2 (4,098)	93.4 (3,986)	92.8 (3,959)
Yes	13.1 (583)	9.8 (443)	6.6 (281)	7.2 (308)

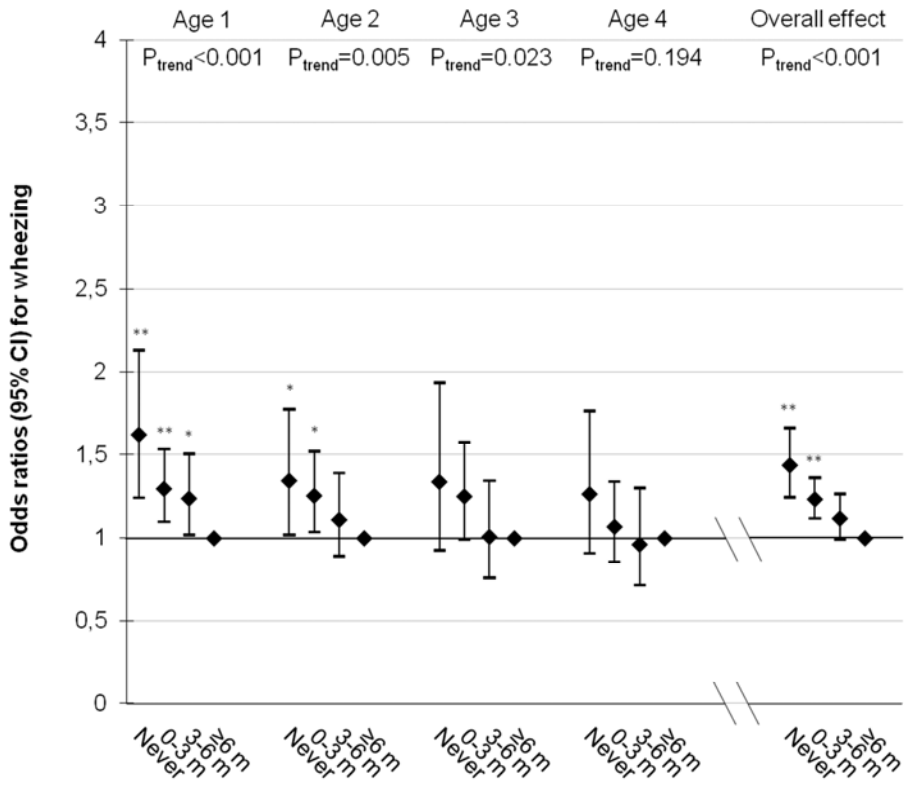
Values are shown in % (absolute numbers).

**Figure 1.** Associations of breastfeeding duration with asthma-related symptoms until the age of 4 years.

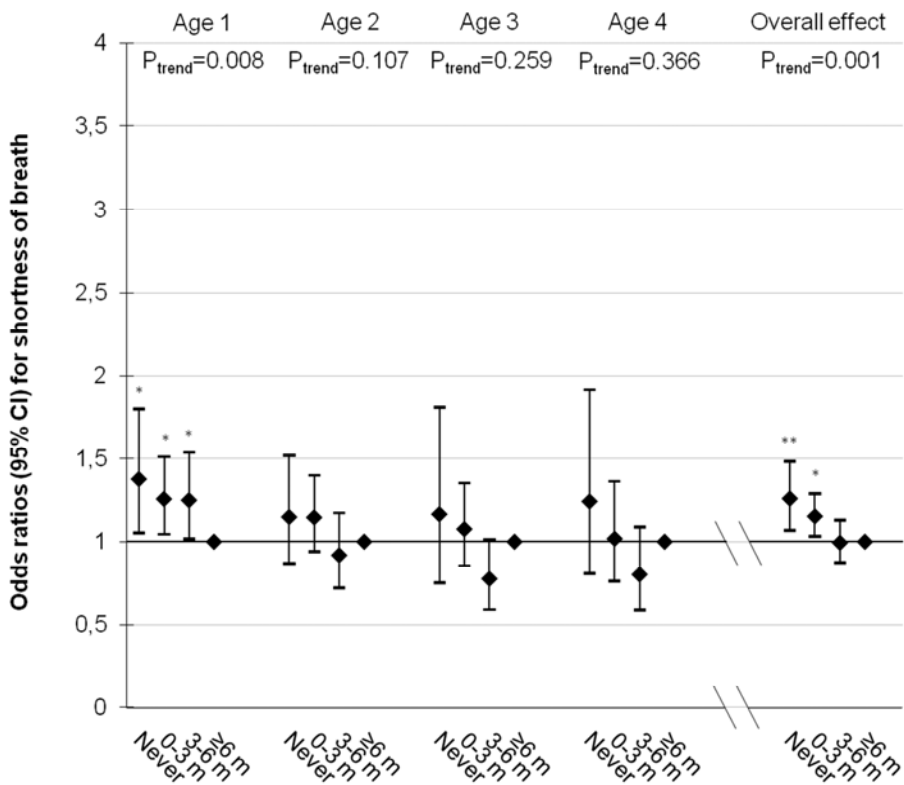
Values are odds ratios (95% confidence interval) from longitudinal generalized estimating equation models. ORs given for the overall effect and (allowing for a time trend) for each year of age separately. Children who were breastfed for > 6 months were used as reference category.

\*P < 0.05 and \*\*p < 0.01. Models are adjusted for parental age, education, ethnicity, smoking habits, maternal parity, children's sex, gestational age, birth weight, parental history of asthma or atopy, daycare attendance and pet keeping

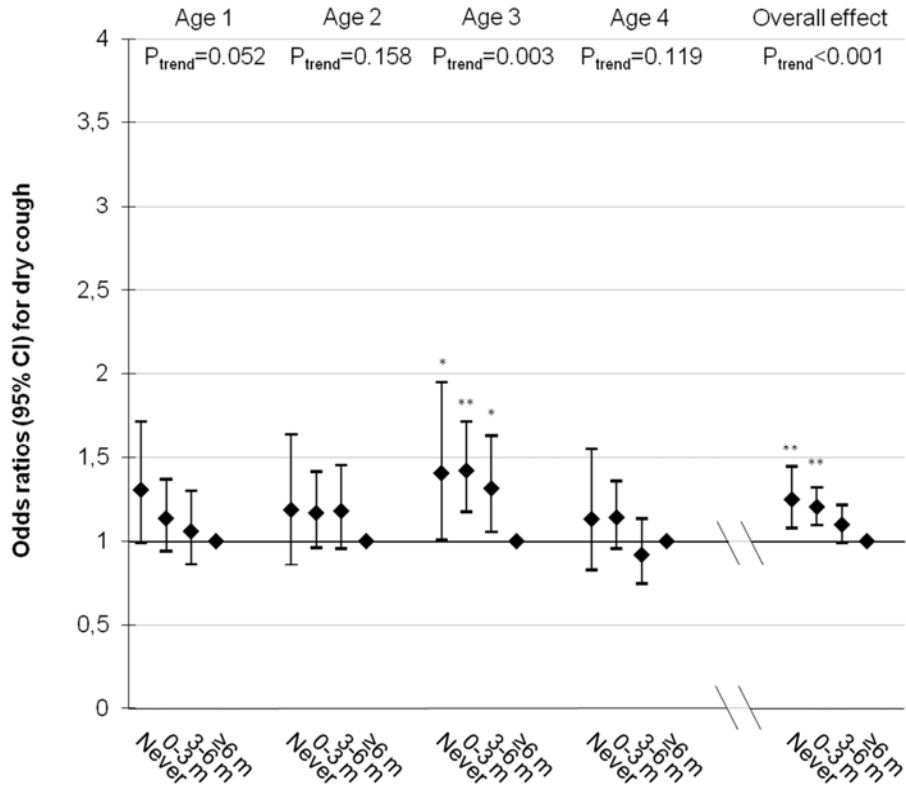
A.



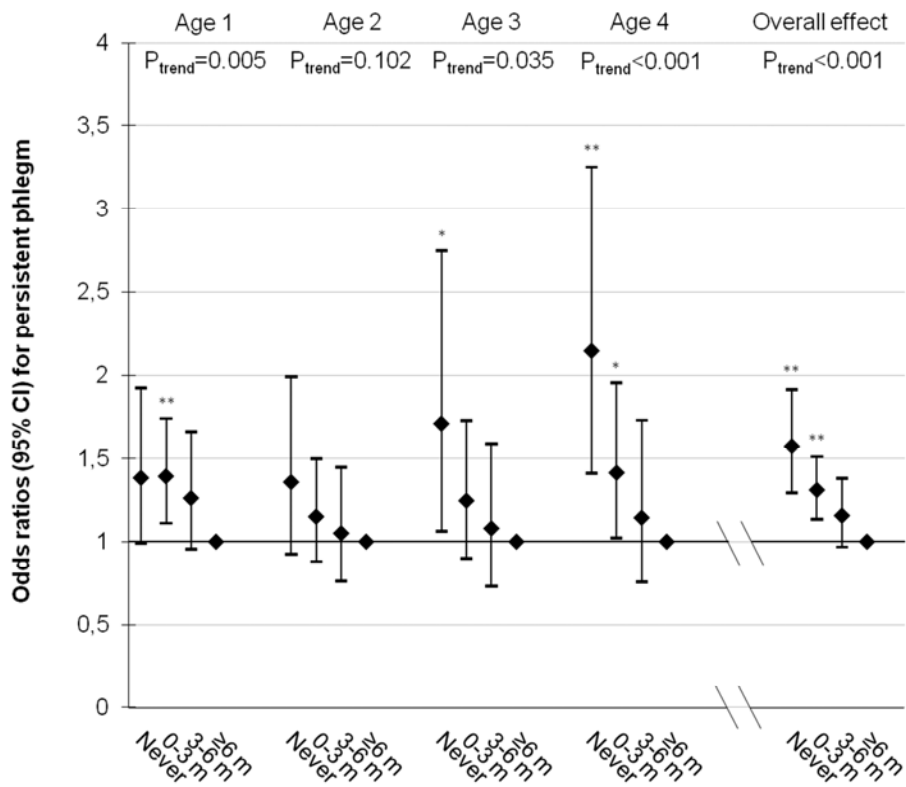
B.



C.



D.



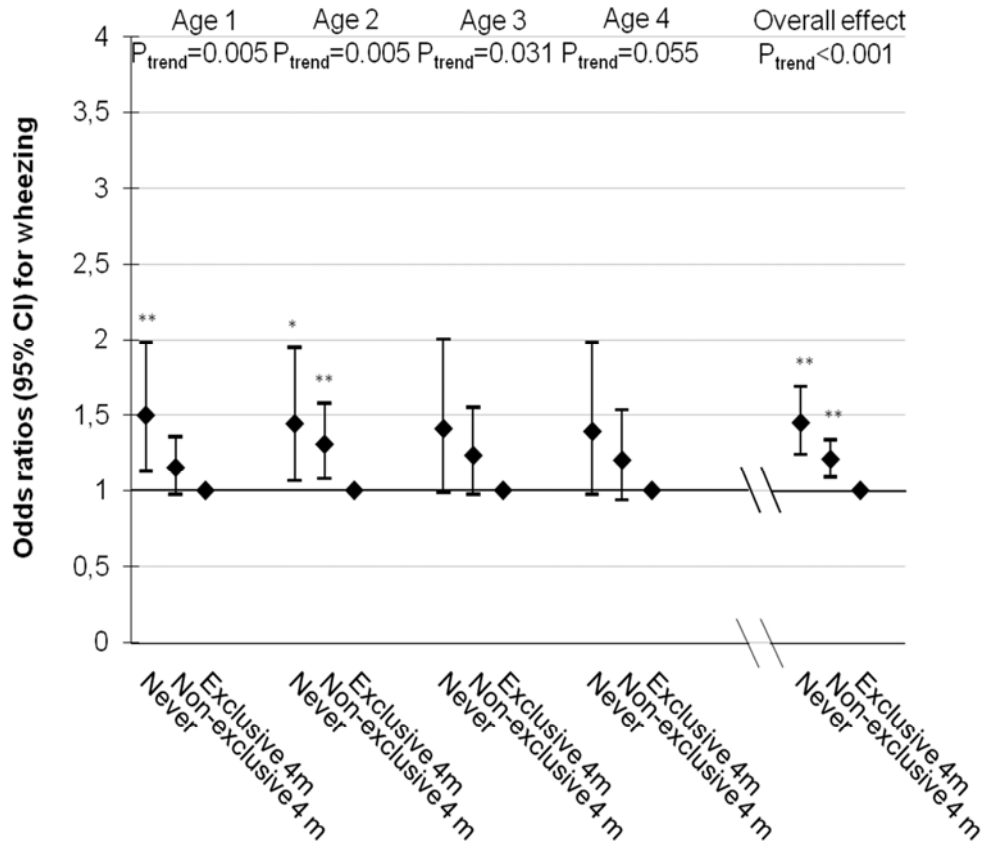


**Figure 2.** Associations of exclusive breastfeeding with asthma-related symptoms until the age of 4 years.

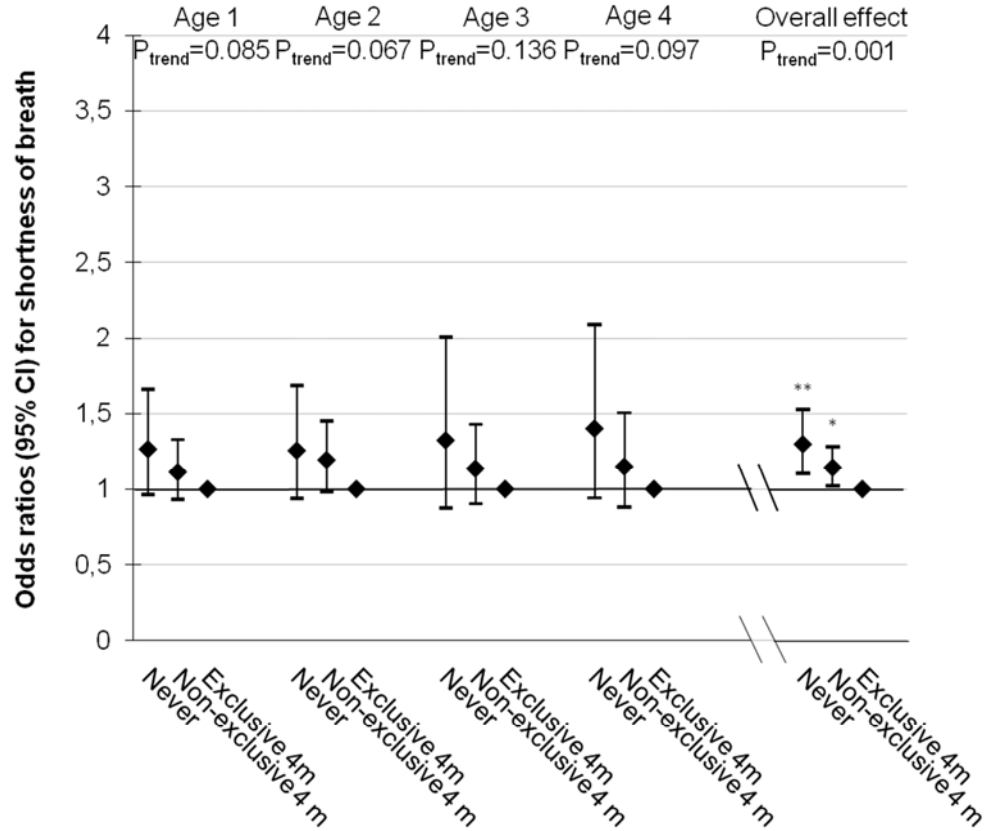
Values are odds ratios (95% confidence interval) from longitudinal generalized estimating equation models. ORs given for the overall effect and (allowing for a time trend) for each year of age separately. Children who were exclusively breastfed for 4 months were used as reference category.

\* $P < 0.05$  and \*\* $p < 0.01$ . Models are adjusted for parental age, education, ethnicity, smoking habits, maternal parity, children's sex, gestational age, birth weight, parental history of asthma or atopy, daycare attendance and pet keeping.

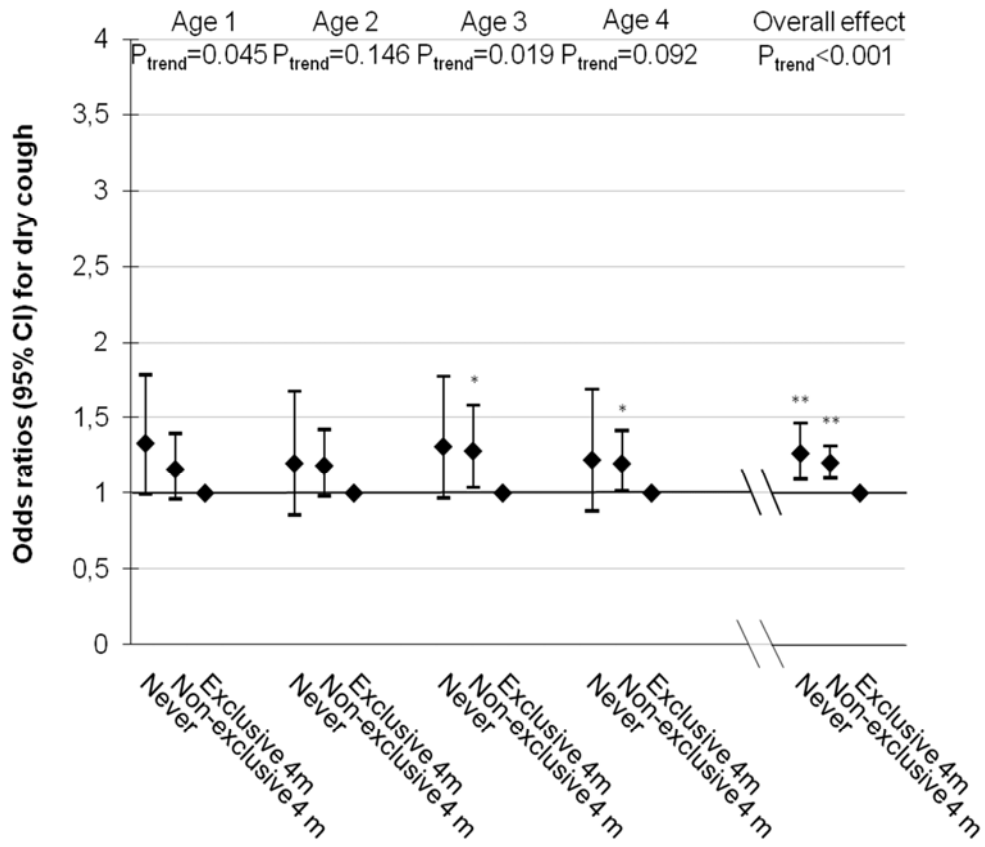
A.



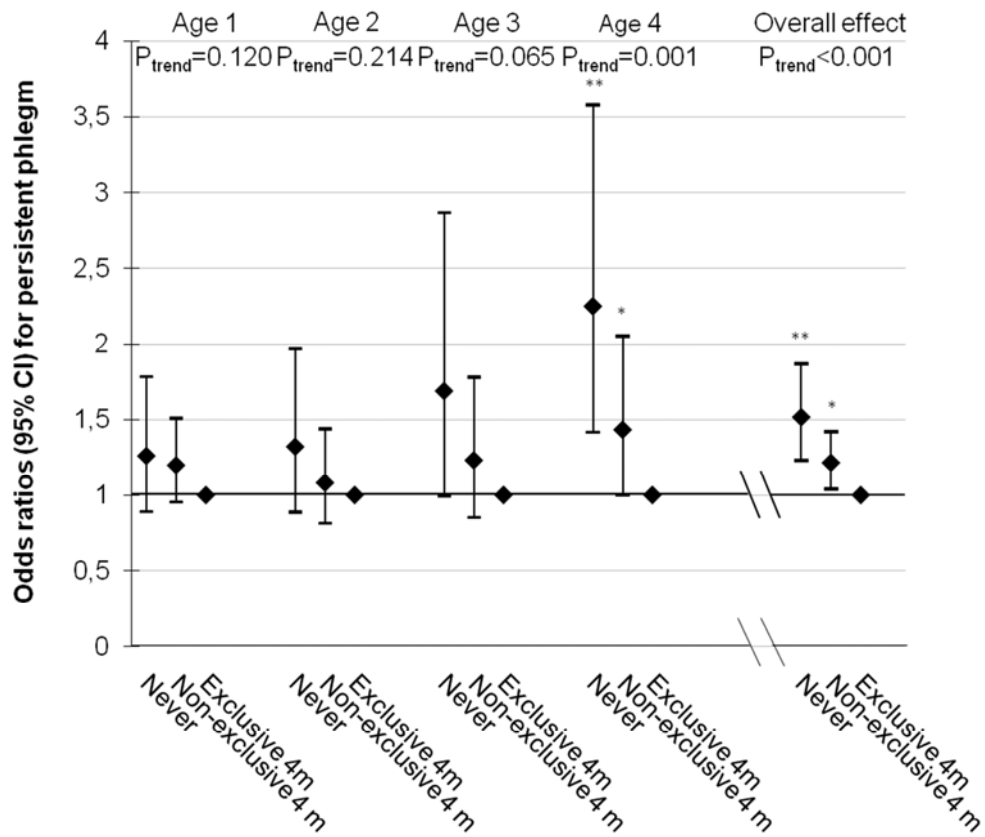
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C.

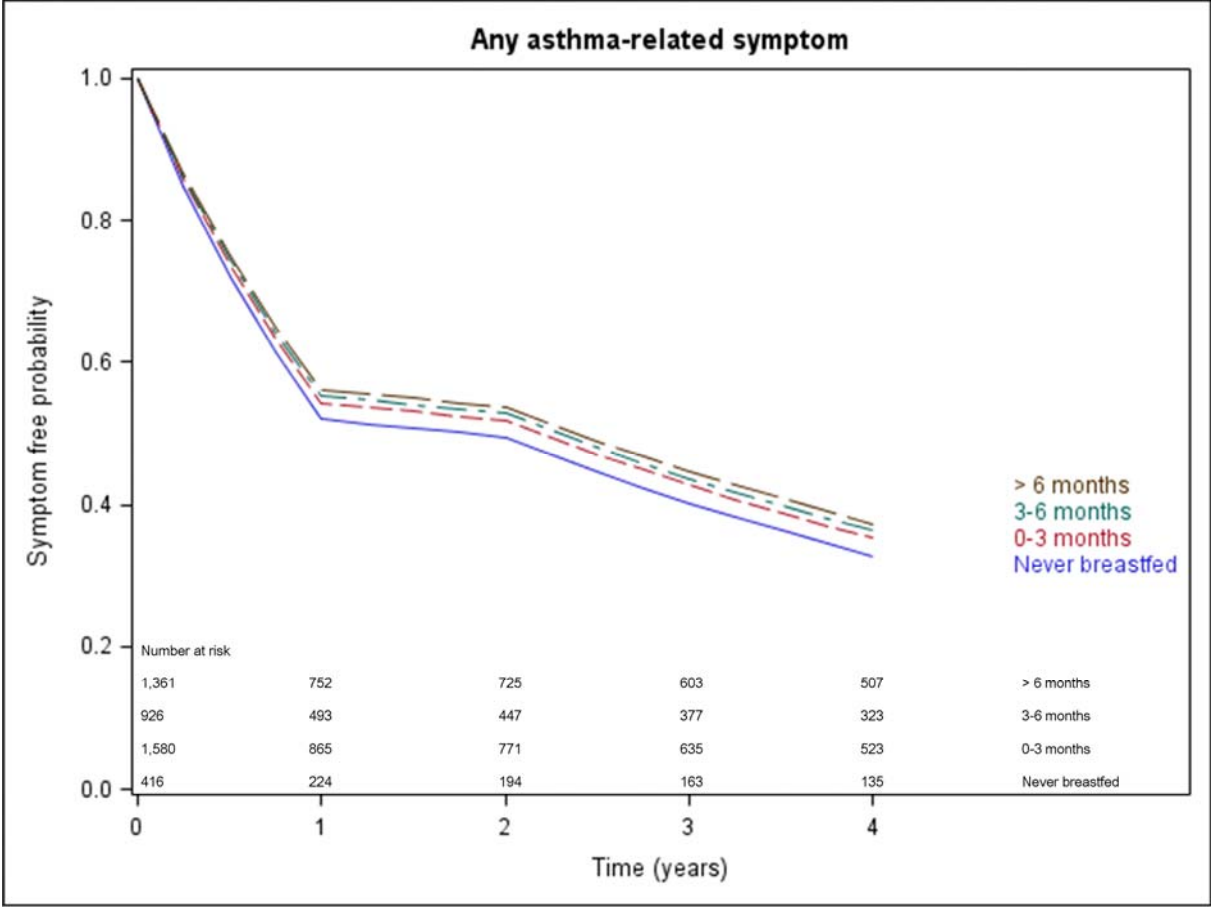


D.



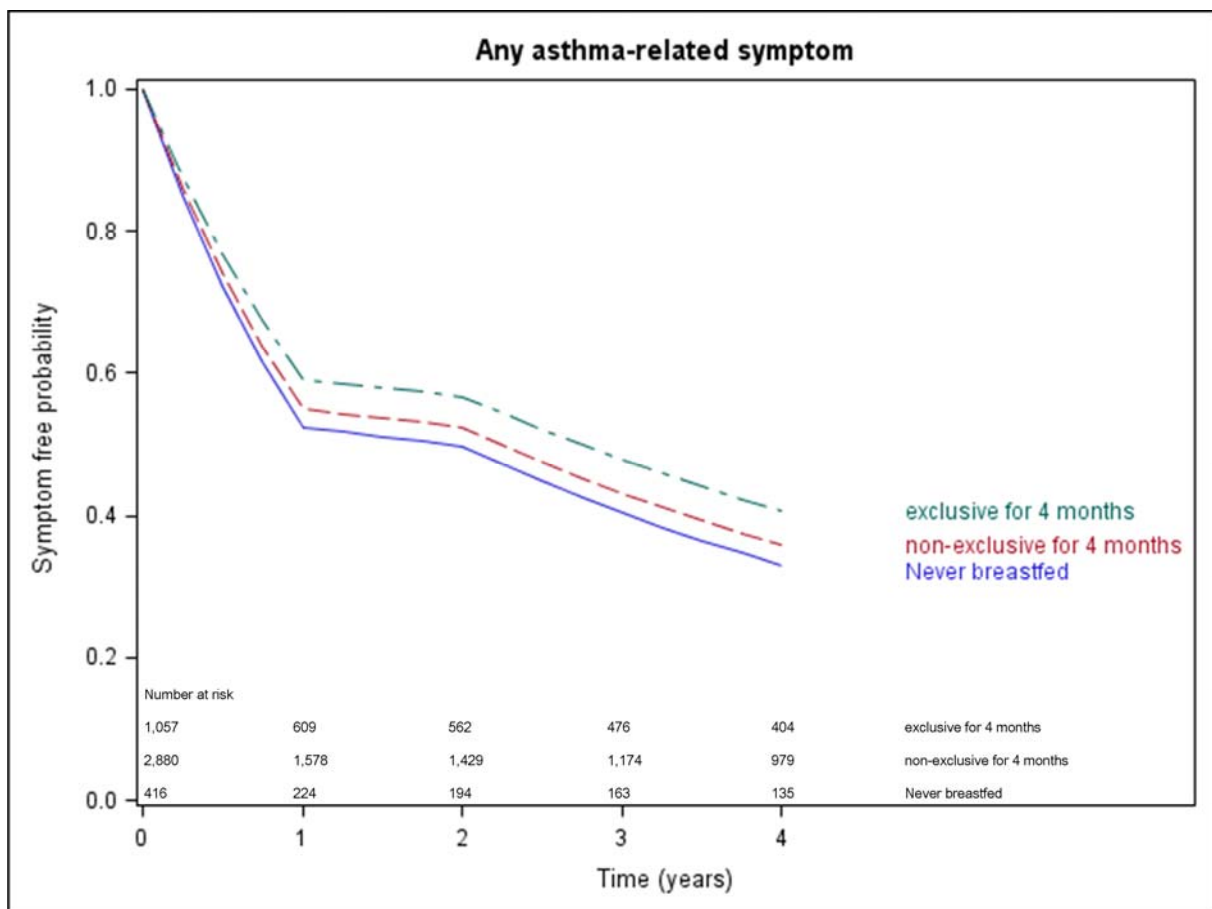
**Figure 3.** Time to any first asthma-related symptom (discrete survival curves) according to duration of breastfeeding.

Models are adjusted for parental age, education, ethnicity, smoking habits, maternal parity, children’s sex, gestational age, birth weight, parental history of asthma or atopy, daycare attendance and pet keeping by taking the mean of the values.



**Figure 4.** Time to any first asthma-related symptom (discrete survival curves) according to exclusivity of breastfeeding.

Models are adjusted by parental age, education, ethnicity, smoking habits, maternal parity, children’s sex, gestational age, birth weight, parental history of asthma or atopy, daycare attendance and pet keeping by taking the mean of the values.

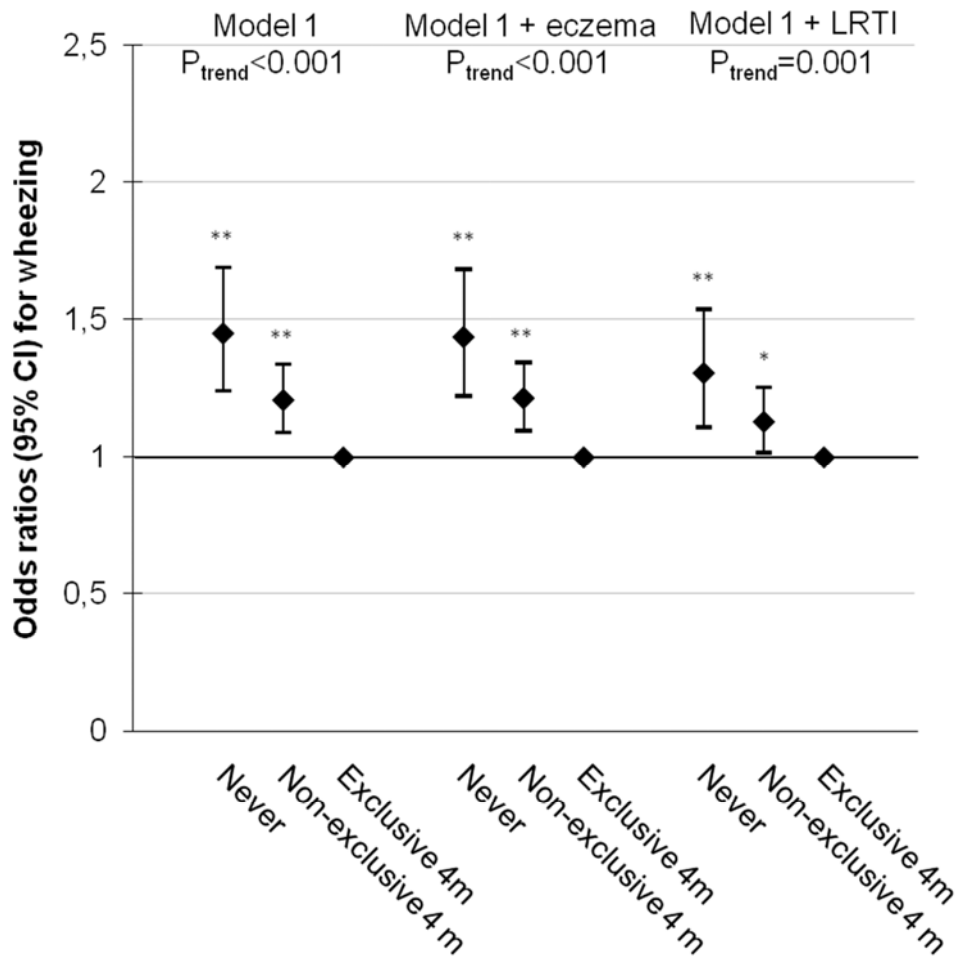


**Figure 5.** Atopic and infectious effects on the associations of breastfeeding exclusivity with overall estimates of asthma-related symptoms.

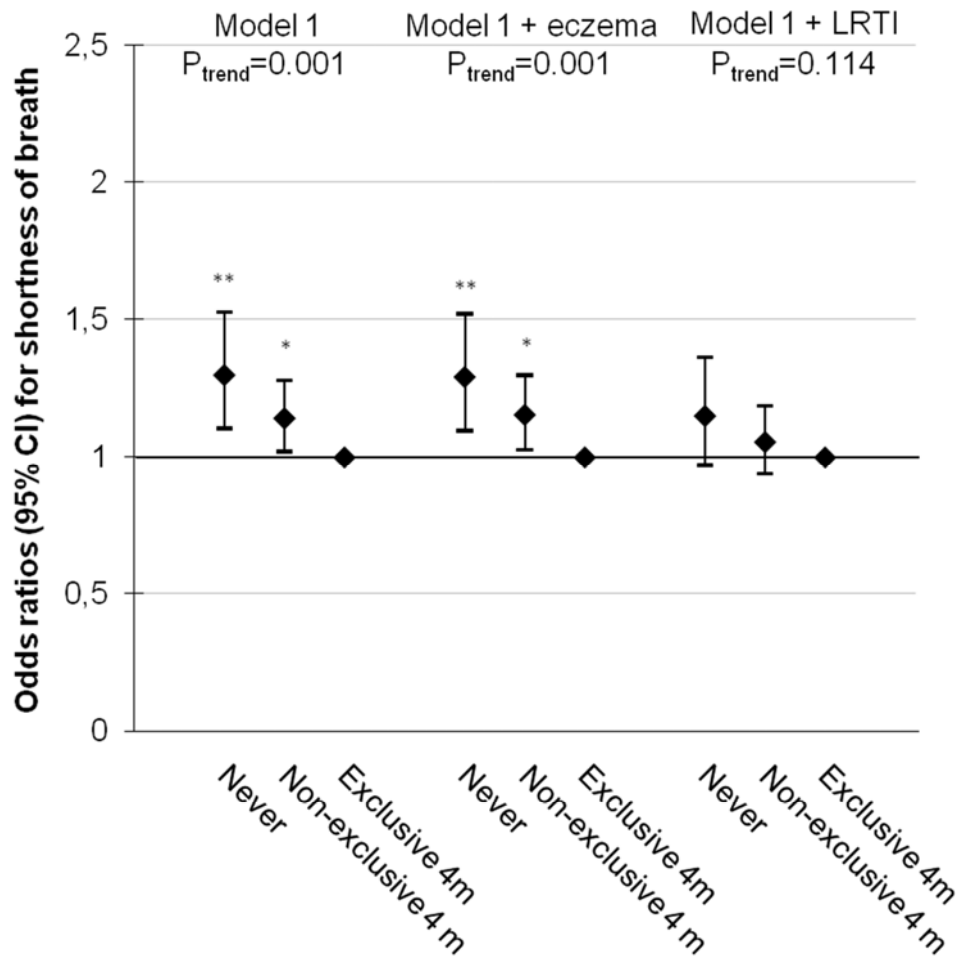
Values are odds ratios with 95% confidence intervals from longitudinal generalized estimating equation models. Children who were exclusively breastfed for 4 months were used as reference category.

\* $P < 0.05$  and \*\* $p < 0.01$ . Model 1 is adjusted for parental age, education, ethnicity, smoking habits, maternal parity, children's sex, gestational age, birth weight, parental history of asthma or atopy, daycare attendance and pet keeping. This model was additionally adjusted for eczema and lower respiratory tract infections (LRTI) which were both not imputed.

A.

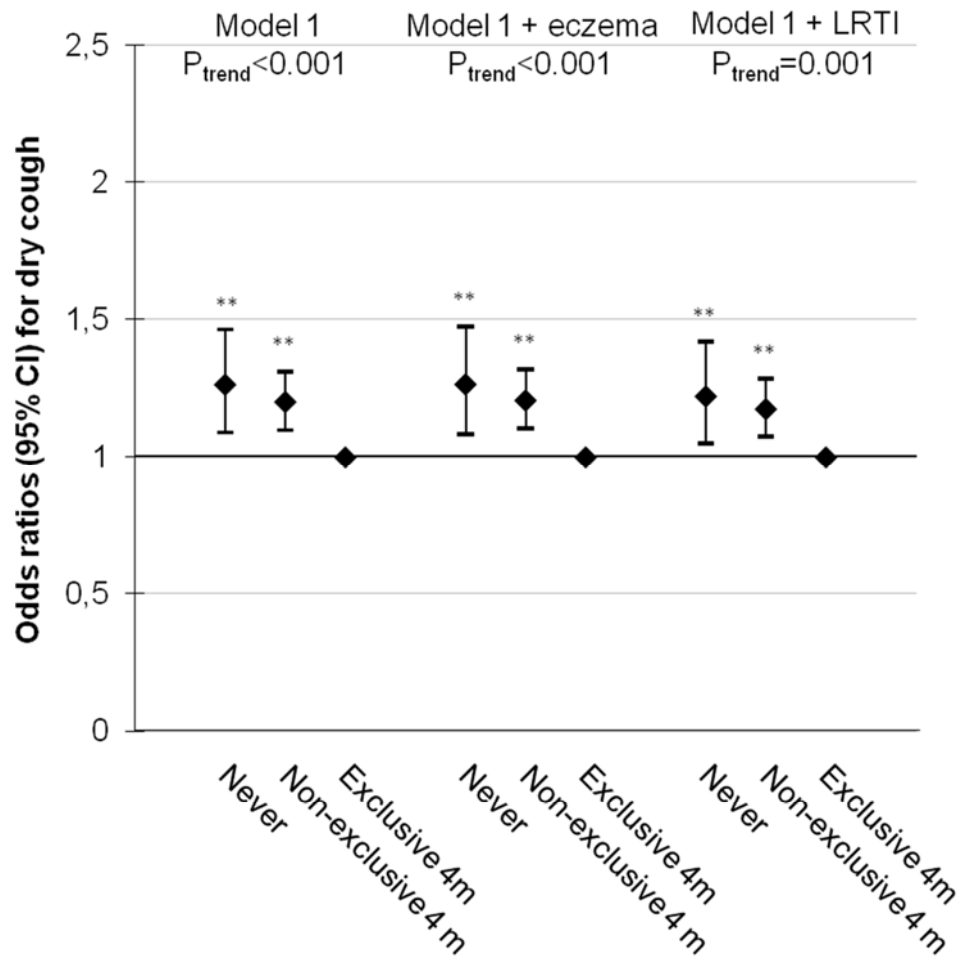


**B.**





C.



D.

