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Prospective study of breastfeeding in relation to wheeze, atopy, and bronchial hyperresponsiveness in the Avon Longitudinal Study of Parents and Children (ALSPAC)

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Methods

ALSPAC Study

Briefly, ALSPAC is a longitudinal birth cohort study recruited in pregnancy and designed to investigate the effects of early life exposures on health and developmental outcomes of childhood. ALSPAC enrolled 14,541 pregnant women residing in Avon, United Kingdom, with expected delivery dates between April 1, 1991, and December 31, 1992. Comprehensive data about the participating women, their partners, and their children were collected from self-completed questionnaires mailed to participants from the time of enrolment (approximately 8 weeks gestation) through their child's approximate age of 91 months. All participating children from age seven years with known addresses were invited to attend annual research clinics for detailed physical tests.

Breastfeeding

Data on breastfeeding were obtained from questions about dietary and feeding habits included in six questionnaires sent to mothers during the first four years of the child's life. The first questionnaire, completed when the child was four weeks of age, included detailed questions about breast and bottle feeding for each week in the first month of life. Questionnaires sent at ages six and 15 months asked about current feeding, age at cessation of breastfeeding and age at introduction of formula, milk, or solids. Questionnaires sent at ages two, three and four years asked about current feeding, including whether the child had received breast milk since the previous questionnaire

We used responses to the six-month questionnaire to categorize early breastfeeding

duration as never, <1 month, 1 to <3 months, 3 to <6 months and 6+ months. For children with missing values for this variable at six months (n = 916 of 13,978), we assigned breastfeeding duration based on responses to the 15-month questionnaire. For children with missing information on both the six month and 15 month questionnaires (n = 1,921), we used the data collected at four weeks, and at two, three, and four years to classify breastfeeding exposure for 566 additional children. The three earliest questionnaires (four weeks, six month and 15 months) also included information about the specific age at breastfeeding cessation, which we used as a continuous variable in some analyses. We were able to assign breastfeeding duration to 90.3% (n = 12,623) of the cohort.

For 251 children missing information on the duration of breastfeeding, we assigned them to categories of ever or never breastfed based on items from the six and 15 month questionnaires. Thus, we were able to assign this dichotomous exposure to 92.1% (n = 12,874) of the cohort. We defined the duration of exclusive breastfeeding as the minimum age at which formulas, milks, or solid foods were introduced. We were able to assign exclusive breastfeeding duration to 90.9% (n = 12,706) of the cohort.

Self-Reported Outcomes

Wheeze

Our primary self-reported outcome was wheeze. We defined wheeze in the first three years of life as a positive response to either of the following questions asked at 6, 18, 30 and 42 months: “Has your child wheezed?” and “Has your child had wheezing or

whistling in his chest when he breathed?"

For children with reported wheeze, we assigned age at onset using information from the 6 and 18 month questionnaires ("How old was (s)he at first wheeze attack?"). For the 1,198 children with missing information about age at onset of wheeze, we assigned a value midway between the age at first reported wheeze and the previous questionnaire. For example, children with missing data for age of onset who reported wheeze at 6 months. were assigned age at onset of 3 months; those with first reported wheeze at 18 months were assigned age at onset of 12 months. Questions about age at onset were not included on the 30- and 42-month questionnaires; thus, the 925 children with first reported wheeze at these later dates were assigned ages at onset of 24 and 36 months, respectively. We were able to classify 78.9% of the cohort with respect to wheezing status in the first 3 years.

Missing data on wheeze are primarily due to missing questionnaires rather than failure to answer questions regarding wheeze. For example, information on wheeze was missing for less than 1% of participants in the first two years, and for approximately 3% on the 30-month questionnaire. For a large cohort study, response rates were relatively high in the first three years (e.g., from 87% at 4 weeks to 74% at 30 months), and the attrition rate was gradual.

We also considered wheezing at the questionnaire administered at 7½ years, between the measurements of the two objective outcomes (atopy and bronchial hyperresponsiveness). Wheeze at age 7½ years was defined by a positive response to the question "Has your child had wheezing in the past 12 months?". We had data on wheeze at 7½ years on

8,200 children.

Objective Outcome Measures

Atopy

Drops of each allergen, positive control (histamine, 10 mg/mL), and negative control (saline) were placed on the forearm and pricked with separate sterile lancets. After 5 minutes, the drops were blotted off to avoid contamination. After a further 10 minutes, the maximum diameters of wheals and flares were measured and recorded.

Children were considered non-atopic if they had negative tests for all three core aero-allergens. We excluded the 559 children missing data on one of the three core skin tests, children who reacted to the negative control (n = 15) or did not react to the positive control (n = 143).

Parents were asked to withhold antihistamines from children for 48 hours before the visit. Of 70 children with reported antihistamine use, 55 had taken them within 48 hours of their visit. We repeated analyses excluding the 70 children having antihistamine use.

Lung function and bronchial hyper-responsiveness

Children were instructed to blow at least three times to produce a maximal expiratory maneuver, for a total of three reliable and reproducible maneuvers. A reliable maneuver was a maximal exhalation for >1 second, with a clear plateau of flow. Reproducibility criteria were set to three maneuvers within 200 ml for forced vital capacity (FVC). A respiratory pediatrician inspected all flow-volume curves to ensure that reproducibility criteria had been met and that the best of three curves was selected for analysis.

Each participant inhaled three breaths of normal saline from a hand-operated bulb nebulizer (DeVilbiss Co., Pennsylvania), and the FEV_1 measurement repeated 1 minute later was taken as the baseline for the challenge. This was followed at one-minute intervals by inhalation of eight doubling doses of methacholine from 0.03 to 6.1 micromoles (μmol). FEV_1 was measured one minute after each dose and followed immediately by the next dose. The challenge was stopped when the FEV_1 fell by more than 20% from the post-saline value (defined as bronchial hyperresponsiveness = yes) or when the maximum dose (6.1 μmol) was reached (defined as bronchial hyperresponsiveness = no).

Children were not tested if, within the previous three weeks, they had been treated with medications for acute asthma or chest infections, or had had an upper respiratory

infection or cold.

Statistical Analysis

Using a change-in-estimate method, we assessed the following potential confounders: sex, preterm birth, daycare in the first 2 years of life, maternal age, older siblings (defined as maternal parity >0 during this pregnancy), pet exposure in the first two years of life, maternal smoking in last 2 months of pregnancy, environmental tobacco smoke exposure in the first 4 years of life, maternal asthma, maternal allergy, and crowding (defined as $\geq$1 resident per room of the family home).

Subjects with missing data on any of the covariates were excluded, resulting in the exclusion of the following numbers of subjects with data on breastfeeding: 1,062 (10.4%) for wheeze by three years, 704 (8.7%) for wheeze by 7½ years, 638 (9.9%) for atopy and 397 (9.2%) for bronchial hyperresponsiveness. Thus the numbers of subjects included in the adjusted analyses were 9,100 for wheeze by three years, 7,432 for wheeze at 7½ years, 5,779 for atopy and 3,920 for bronchial hyperresponsiveness (Table 2). Crude logistic regression results did not differ materially between the entire population versus the subset with nonmissing data on any covariates (data not shown).

To examine the potential for reverse causation, we used life table analysis and Kaplan Meyer survival curves [24] to describe the temporal relationship between wheezing and cessation of breastfeeding. We examined breastfeeding duration stratified by early wheezing status, including children who never wheezed and those who wheezed before

cessation of breastfeeding. There were 9,166 children with information on both early wheezing and breastfeeding. Approximately 84% (n = 7,672) of these children were breastfed, of whom 47% (n = 3,605) never wheezed and 21% (n = 1,630) wheezed before breastfeeding was stopped. The remaining breastfed children (n = 2,437) first wheezed after cessation of breastfeeding and were not included in the life table or Kaplan Meier analysis.

Bayesian Analysis

The joint model comprised six separate mixed-effect logistic models, each including a shared random effect variable measuring each child's susceptibility to wheeze and the risk of developing each allergic outcome (asthma, atopy, and bronchial hyperresponsiveness). The first component modeled the relationship between breastfeeding duration and wheeze; the second component modeled the probability of continuing to breastfeed with increasing age; the next four components modeled the probability of having wheeze, asthma, atopy, and bronchial hyperresponsiveness in later childhood given breastfeeding duration; and the final component modeled whether the child missed the atopy examination. Joint models with shared random effects have been increasingly used in investigating causal effects, accounting for reverse causation, informative missingness that can lead to selection bias, and other complications[25-26]. The Bayesian approach implemented in WinBUGS provides a convenient approach for fitting joint models, which allows prior distributions for the random effect and other parameters to be updated with all the data available for a child. The updating of prior values resulted in a posterior

distribution for the random effect which was dependent on the child's wheeze history and whether the child missed the atopy examination, hence automatically adjusting the models for the dependence between wheeze and breastfeeding duration and for informative missingness.

Table E1. Characteristics of the study population at baseline and with outcome data at ages 7-8 years[1]

	Alive at 1 year (N = 13,978)	**Skin Prick (N = 6,512)**	**BHR (N = 4,364)**	**Wheeze at 7½ yrs (N=8,200)**
Breastfed ever	9,364 (67.0)	5,133 (78.8)	3,501 (80.2)	6,339 (77.3)
Missing	1,104 (7.9)	95 (1.5)	47 (1.1)	64 (0.8)
Breastfed				
Never	3,510 (25.1)	1,284 (19.7)	816 (18.7)	1,797 (21.9)
< 3 months	4,062 (29.1)	1,986 (30.5)	1,327 (30.4)	2,554 (31.2)
3 - < 6 months	1,554 (11.1)	901 (13.8)	641 (14.7)	1,097 (13.4)
6+ months	3,497 (25.0)	2,193 (33.7)	1,503 (34.4)	2,647 (32.3)
missing	1, 355 (9.7)	148 (2.3)	77 (1.8)	105 (1.3)
Exclusive breastfeeding				
Never	3,510 (25.1)	1,284 (19.7)	816 (18.7)	1,797 (21.9)
< 4 months	8,003 (57.3)	4,377 (67.2)	2,994 (68.6)	5,453 (66.5)
≥ 4 months	1,193 (8.5)	723 (11.1)	481 (11.0)	854 (10.4)
missing	1,272 (9.1)	128 (2.0)	73 (1.7)	96 (1.2)
Sex				
Female	6,756 (48.3)	3,240 (49.8)	2,179 (49.9)	3,982 (48.6)
Male	7,220 (51.7)	3,272 (50.2)	2,185 (50.1)	4,218 (51.4)
Missing	517 (0.01)	(0.0)	(0.0)	(0.0)
Ethnic background				
White	11,474 (82.1)	5,915 (90.8)	4,013 (92.0)	7,533 (91.9)
Non-white	609 (4.4)	255 (3.9)	145 (3.3)	299 (3.7)
Missing	1,895 (13.6)	342 (5.3)	206 (4.7)	368 (4.5)
Older siblings:				
Yes	7,212 (51.6)	3,474 (53.4)	2,289 (52.5)	4,288 (52.3)
Missing	1,038 (7.4)	212 (3.3)	130 (3.0)	247 (3.0)
Maternal asthma				
Yes	1,423 (10.2)	727 (11.2)	464 (10.6)	879 (10.7)
Missing	1,519 (10.9)	239 (3.7)	143 (3.3)	231 (2.8)
Maternal allergy				
Yes	2,802 (20.1)	1,539 (23.6)	991 (22.7)	1,856 (22.6)
Missing	1,679 (12.0)	311 (4.8)	201 (4.6)	324 (4.0)
Maternal smoking in pregnancy[1]				
Yes	2,791 (20.0)	1,118 (17.2)	706 (16.2)	1,486 (18.1)

[1] Smoking in last 2 months of pregnancy

	Alive at 1 year (N = 13,978)	Skin Prick (N = 6,512)	BHR (N = 4,364)	Wheeze at 7½ yrs (N=8,200)
Missing	2,143 (15.3)	383 (5.9)	212 (4.9)	365 (4.5)
Environmental tobacco smoke[2]				
Not at all	5,283 (37.8)	3,023 (46.4)	2,047 (46.9)	3,762 (45.9)
Low	2,270 (16.2)	1,224 (18.8)	843 (19.3)	1,555 (19.0)
Moderate-high	4,879 (34.9)	2,111 (32.4)	1,395 (32.0)	2,810 (34.3)
Missing	1,546 (11.1)	154 (2.4)	79 (1.8)	73 (0.9)
Mean maternal age (years) at child's birth (SD)	27.7 (5.0)	28.7 (4.6)	28.7 (4.5)	28.6 (4.6)
Wheeze in first six months of life				
Yes	2,471(17.7)	1,252 (19.2)	834(19.1)	1,586(19.3)
Missing	2,505(17.9)	420 (6.4)	235(5.4)	366(4.5)

[1] Counts and percentages

[2] Exposure to environmental tobacco smoke in the first 4 years of life, defined as time in a smoky room each week: not at all, low (<1 hour per week), moderate (1-2 hours per week), high (>2 hours per week)