

Maternal employment in child-care institutions and the risk of infant wheeze and atopic dermatitis in the offspring

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It has been proposed that exposure to infections and microbes protects against atopic diseases, but epidemiological data has so far been conflicting. We hypothesized that maternal exposure to infections and microbes before or during pregnancy would be of particular importance. To test this hypothesis, we studied the incidence of wheezing and atopic dermatitis (AD) in infants of mothers employed in child-care institutions – and thus presumably being highly exposed to infections and microbes – compared with infants of mothers not so employed. A total of 31471 mother-child pairs enrolled in the Danish National Birth Cohort were followed prospectively. Information on wheezing episodes, AD, maternal employment, and other variables were collected by interview at 12 and 30 wk of gestation, and 6 and 18 months of age, and by linkage to the Danish Medical Birth Register and the Child-care Database. The relative risk was estimated in Cox proportional hazard models. Analyses were stratified by sibling status (first born or not), as older siblings are likely to be a significant source of infectious agents. The adjusted relative risks of wheeze, recurrent wheeze and AD was 1.14 (95% CI: 0.96–1.37), 1.37 (95% CI: 1.05–1.77), and 1.03 (95% CI: 0.81–1.31), respectively, for first-born infants of mothers employed in child-care institutions compared with infants of mothers not so employed. There was no effect of maternal employment in child-care institutions among infants with older siblings. In conclusion, the results did not support the hypothesis that maternal microbial exposure before or during pregnancy as reflected by maternal employment in child-care institutions protects the offspring against infant wheeze and AD.

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Epidemiological studies have shown that the risk of atopic diseases, such as atopic dermatitis (AD), asthma, and allergic rhinitis decreases with increasing number of older siblings (1–5). It has been hypothesized that this sibling effect is because of transmission of infections between siblings leading to increased stimulation of the immune system resulting in immune deviation towards the non-atopic phenotype (6). Accordingly, early day care and being raised on a farm, which are plausible markers of increased

exposure to infections, have been associated with a lower risk of atopic diseases (7–14). Nevertheless, subsequent studies have not convincingly been able to document that increased frequency of infections represents the link between siblings and lower risk of atopic diseases (15–17). Furthermore, some studies have indicated that the sibling effect may originate *in utero* as it was found present at birth (18–22). Thus, pregnancy may be an important time period with regard to exposures influencing the development of the

immune system of the off-spring and there is evidence that the maternal immune system during pregnancy influences the fetal immune system. Pre-natal maternal cytokines, antibodies, and antigens may pass the fetoplacental barrier and thereby be transmitted to the fetus. Recently, a German study showed that maternal exposure to farm environment during pregnancy is particularly protective against the development of atopic sensitization in the offspring (10).

We hypothesized that a possible protective effect of exposure to infections and microbes against the development of atopic diseases may be particularly important, and more evident, when such exposure takes place *in utero*, indirectly through exposure of the pregnant mother. Our study hypothesis is that maternal employment in child-care institutions before and during the pregnancy as a surrogate marker of high maternal exposure to common infections and pathogens is inversely related to lower incidence of infant wheeze and AD. It is not easy to distinguish between direct microbial exposure from older siblings and indirect exposure *in utero* through the mother. Therefore, we performed separate analyses for first-born children and children with older siblings.

Methods

Participants

The study was based on mother-child pairs enrolled in The Danish National Birth Cohort (DNBC) (23, 24). The DNBC has consecutively recruited 100000 pregnant Danish women in the period from 1997 to 2002. Pregnant women were invited to participate in DNBC the first time they consulted their general practitioner about their pregnancy. Participation involved four computer-assisted telephone interviews at gestational weeks 12 and 30 (first and second interview) and when the child was 6 and 18 month old (third and fourth interview). From April 2000 onwards, the fourth interview included questions about AD. When the present study was initiated in 2002, 51900 women had answered questions about AD in the fourth interview. However, 12994 missed one or more of the interviews. Women were called up four times for each interview. If not found home and available for interview, the interview was cancelled. Almost no women actively denied participation. We enrolled the 38906 remaining pairs; of these a total of 7435 had missing information on one or more of the variables included in the analyses.

Thus, 31471 pairs were included in the study. Information on maternal occupation was obtained in the first interview at 12 wk of gestation. A total of 2215 mothers were employed in child-care institutions. Mothers employed in family day care ($n = 326$) assumed to be in an intermediate exposure situation was excluded from the analysis.

Information on residence and siblings was obtained from the Danish Civil Registration System (DCRS). The population density at the children's residence was calculated. Information regarding enrolment into child-care institutions was obtained from the Child-care Database (25). During the study period the database covered all attendance to child care in 0- to 5-year-old children in 266 of 271 municipalities in Denmark, corresponding to approximately 90% of all Danish children aged 0–5 yr. Each record in the databank includes data of enrollment and withdrawal. Children living in municipalities covered by the Child-care Database but who did not have a record of attendance to child care were considered in home care. Age at first day care outside the home was calculated as date of first attendance minus date of birth, and was categorized as follows: < 3 months, 3–9 months, > 9–15 months or > 15 months. Children born in a municipality not covered by the Child-care Database were excluded from the analyses ($n = 3292$). DNBC was approved by The Ethical Committees in Denmark and by the Data Protection Board.

Definitions

Definitions of wheeze and AD was defined based on answers to interviews within the national birth cohort have been described elsewhere (23). Definition of wheeze: in the third and fourth interview, the mother was asked whether the child had ever had episodes of wheezing. If yes, age at first episode and the number of episodes were recorded. 'Wheeze ever' was defined as at least one episode of wheezing during the period 0–6 month or 6–18 months. Furthermore, the mother was asked how many episodes of wheezing the child had had since the last interview. 'Recurrent wheeze' was defined as episode of wheezing at least for three periods. Because the definition of AD and 'recurrent wheeze' was based on repeated episodes of symptoms, subjects were only considered as cases of AD, 'wheeze ever', and 'recurrent wheeze' if the first episode of symptoms occurred before 15 months of age. Definition of AD: a diagnosis of AD was based on data obtained in the fourth interview

when the child was 18 months of age. The mother was asked whether the child had ever had an itchy rash, and if so, localization and age when affected (recorded as half-month periods) was obtained. She was also asked whether the child had AD, and if so whether a doctor had verified the diagnosis. As described elsewhere (26), a dermatologist examined a subgroup of the participating infants and the combination of answers that resulted in the highest sum of sensitivity and specificity was determined. Thus, cases of AD were defined as children whose mothers reported that the child had or had had i) an itchy rash and/or doctor-verified AD, ii) recurrent rash and/or rash for at least four consecutive half-month periods, and iii) localization of the rash in elbow creases, behind the knees, face, wrist/hands, or generalized (four or more localizations) (26). Definition of fever: fever was defined in the second interview at gestational week 30 as a positive answer to the question 'Have you had fever during your pregnancy'? Definition of urinary tract infections: urinary tract infections were defined in the second interview as positive answers to the questions 'Have you had cystitis during pregnancy?' or 'Have you had pyelonephritis during pregnancy?' and additionally in the third interview as positive answers to the questions 'Did you have cystitis after the gestational week 30?' or 'Did you have pyelonephritis after the gestational week?'

Statistical analysis

Data from the DNBC was merged with data from the DCRS. Relative risk of AD or wheeze in infants of mothers employed in a child-care institution compared with other infants was calculated in a Cox proportional hazard model. Subjects were followed from birth to 15 months. In these survival analyses, the children were censored (excluded from being at risk of disease) from the estimated date of onset of wheeze/AD (if relevant). In case the child was enrolled in a day-care institution the child was censored from the date of enrolment in day-care. The risk estimates for the effect of maternal employment in a child-care institution was adjusted for all variables listed in Table 1. Both adjusted and unadjusted analyses were performed. The estimates for maternal employment in child-care institutions were essentially the same in adjusted and unadjusted analyses. For some variables (urbanization and season) the level was allowed to depend on age, while for the rest of the variables the level was constant during the

15 months. Separate analyses were performed for first-born infants and infants with older siblings. An interaction term between maternal employment in child-care and sibling status (first-born or not) was tested in the model including all children. The model was checked with use of Schoenfeld residuals and this model checks did not indicate any contradictions between the observations and the model assumptions. All data management and statistical analysis were performed in SAS v 8.02 (SAS Inc, Cary, NC, USA). For the Cox regression SAS procedure phreg was used.

Results

Table 1 shows the characteristics of mothers employed in child-care institutions and mothers not employed in a child-care institution. Mothers employed in child-care institutions had lower household income and higher prevalence of asthma and AD compared with mothers not employed in child-care institutions. Furthermore, their children were enrolled in a day care at an older age than other infants. There was no excess fever episodes among mothers employed in child-care institutions. Urinary tract infection during pregnancy was significantly more commonly reported by mothers not employed in child-care institutions compared with mothers employed in child-care institutions.

Table 2 shows the adjusted estimates for the relative risk of wheeze and AD in first-born infants in relation to maternal employment in child-care institution. The unadjusted estimates were essentially similar to the adjusted estimates (unadjusted estimates not shown).

Compared with first-born infants of mothers not employed in a child-care institution, first-born infants of mothers employed in child-care institutions had a slightly, but significantly, higher risk of recurrent wheeze. The adjusted relative risk of wheeze, recurrent wheeze, and AD in first-born infants of mothers employed in child-care institutions was 1.14 (95% CI: 0.96–1.37), 1.37 (95% CI: 1.05–1.77), and 1.03 (95% CI: 0.81–1.31), respectively (Table 2). In contrast to first-born infants, there was no effect of maternal employment in child-care institutions on the risk of infant wheeze among infants with older siblings (Table 3) and p-values for interaction between maternal employment in child-care institution and sibling status (first-born or not) were 0.11, 0.12, and 0.52 for wheeze, recurrent wheeze, and AD, respectively. Thus, this difference between first-borns and infants with older siblings in the effect of maternal

	Infants of mothers employed in child-care institutions	Infants of mothers not employed in child-care institutions	
Risk factors	n = 2215, n (%)	n = 29256, n (%)	p-value (chi-square test)
Infant parameters			
Gender			
Boy	1139 (51.4)	14853 (50.8)	0.553
Girl	1076 (48.6)	14403 (49.2)	
Birth weight			
<2500 g	52 (2.3)	675 (2.3)	0.234
2500–2999 g	186 (8.4)	2407 (8.2)	
3000–3499 g	591 (26.7)	8487 (29.0)	
3500–3999 g	829 (37.4)	10648 (36.4)	
≥4000 g	557 (25.1)	7039 (24.1)	
Head circumference			
<34 cm	315 (14.2)	4008 (13.7)	0.769
34–37 cm	1387 (62.6)	18369 (62.8)	
≥ 37 cm	513 (23.2)	6879 (23.5)	
Exclusive breastfeeding			
≥2 months	361 (16.3)	4835 (16.5)	0.772
3–4 months	832 (37.6)	11153 (38.1)	
>4 months	1022 (46.1)	3268 (45.4)	
Number of older siblings			
0	1000 (45.1)	12893 (44.1)	0.615
1	825 (37.2)	11272 (38.5)	
2	324 (14.6)	4171 (14.3)	
≥3	66 (3.0)	920 (3.1)	
Premature <37 wk			
No	2124 (95.9)	28147 (96.2)	0.452
Yes	91 (4.1)	1109 (3.8)	
Age at first day care outside home			
0–3 months	3 (0.1)	27 (0.1)	<0.001
3–9 months	728 (32.9)	12199 (41.7)	
9–15 months	977 (44.1)	11009 (37.6)	
>15 months	507 (22.9)	6021 (19.1)	
Season of birth			
Autumn	428 (19.3)	6127 (20.9)	0.053
Winter	559 (25.2)	6870 (23.5)	
Spring	690 (31.2)	9502 (32.5)	
Summer	538 (24.3)	6757 (23.1)	
Maternal parameters			
Maternal age when giving birth			
<25 yr	180 (8.1)	2342 (8.0)	0.037
25–29 yr	909 (41.0)	11206 (38.3)	
30–34 yr	814 (36.7)	11096 (37.9)	
≥ 35 yr	312 (14.1)	4612 (15.8)	
Maternal asthma			
No	1989 (89.8)	26738 (91.4)	0.010
Yes	226 (10.2)	2518 (8.6)	
Maternal atopic dermatitis			
No	1931 (87.2)	25926 (88.6)	0.040
Yes	284 (12.8)	3330 (11.4)	
Maternal hay fever			
No	1630 (73.6)	21666 (74.1)	0.629
Yes	585 (26.4)	7590 (25.9)	
Maternal fever during pregnancy			
No	1615 (72.95)	21593 (73.3)	0.356
Yes	600 (27.1)	7663 (26.2)	
Maternal urinary tract infection during pregnancy			
No	2144 (96.8)	27972 (95.6)	0.008
Yes	71 (3.2)	1284 (4.4)	
Maternal education above primary school			
Missing data	0 (0.0)	1 (0.0)	0.874
No	742 (33.5)	9752 (33.3)	
Yes	1473 (66.5)	19503 (66.7)	

Table 1. Characteristics of infants in relation to maternal employment in child-care institutions. Danish National Birth Cohort (n = 31471)

Maternal employment in child-care institutions and risk of infant wheeze and AD

Table 1. Continued

Risk factors	Infants of mothers employed in child-care institutions	Infants of mothers not employed in child-care institutions	p-value (chi-square test)
	n = 2215, n (%)	n = 29256, n (%)	
Smoking during pregnancy			
No	1817 (82.0)	24414 (83.4)	0.084
Yes	398 (18.0)	4842 (16.6)	
Paternal parameters			
Paternal asthma			
No	2036 (91.9)	26923 (92.0)	0.858
Yes	179 (8.1)	2333 (8.0)	
Paternal atopic dermatitis			
No	2112 (95.3)	27810 (95.1)	0.540
Yes	103 (4.7)	1446 (4.9)	
Paternal hay fever			
No	1674 (75.6)	21588 (73.8)	0.065
Yes	541 (24.4)	7668 (26.2)	
Residential parameters			
Household income			
Low	1060 (47.9)	10837 (37.0)	<.001
High	803 (36.3)	14784 (50.5)	
Unknown	352 (15.9)	3635 (12.4)	
Farm residence			
No	2055 (92.8)	27036 (92.4)	0.531
Yes	160 (7.2)	2220 (7.6)	
Pets in the home			
No	1271 (57.4)	16816 (57.5)	0.929
Yes	944 (42.6)	12440 (42.5)	
Citizens per km ² at birth time			
Missing data	2 (0.1)	4 (0.0)	0.162
≥4000	340 (15.3)	4588 (15.7)	
2000–3999	446 (20.1)	5317 (18.2)	
500–1999	825 (37.2)	10931 (37.4)	
50–499	363 (16.4)	5163 (17.6)	
0–49	239 (10.8)	3253 (11.1)	

Table 2. Cumulated incidence and relative risk of wheeze, recurrent wheeze, and atopic dermatitis in relation to maternal employment in child-care institutions. First-born infants from Danish National Birth Cohort (n = 13883)

	Wheeze		Recurrent wheeze		Atopic dermatitis	
	Incidence‡	Risk†	Incidence‡	Risk†	Incidence‡	Risk†
Mother employed in child-care institution	26.1% (261/1000)	1.14 (0.96–1.37)	14.0% (140/993)*	1.37 (1.05–1.77)	9.7% (97/1000)	1.03 (0.81–1.31)
Mother not employed in child-care institution	24.4% (3150/12893)	1.00 (Reference)	11.0% (1420/12844)*	1.00 (Reference)	9.9% (1281/12893)	1.00 (Reference)

*Missing data about recurrent wheeze in 49 infants.

†Adjusted hazard ratio with 95% confidence interval.

‡Cumulated incidence proportion (Number of cases/number of participants).

employment in child-care institution on the risk of wheeze and AD was not statistically significant.

Furthermore, we investigated whether the effect of maternal employment on the risk of wheeze, recurrent wheeze and AD was the same in boys and girls (Table 4). The relative risk of wheeze and recurrent wheeze was statistically significant for sons, 1.28 (1.02–1.59) and 1.53

(1.13–2.07), respectively, but not for daughters, although none of these gender differences were statistically significant (p-values for test for interaction are shown in Table 4). There was no sex-difference for AD.

Additionally, we adjusted our results for maternal fever and urinary tract infection during pregnancy. This additional adjustment had no major effects on the risk estimates for maternal

Table 3. Adjusted risk (*) of wheeze, recurrent wheeze, and atopic dermatitis among infants of mothers employed in child-care institutions compared to infants of mothers otherwise employed depending on whether the infant was first-born or not

	First-born infants, n = 13883	Infants with older siblings, n = 17262
Wheeze ever	1.15 (0.96–1.37)	0.94 (0.82–1.09)
Recurrent wheeze	1.37 (1.05–1.77)	0.95 (0.78–1.16)
Atopic dermatitis	1.03 (0.81–1.31)	0.95 (0.77–1.19)

*Adjusted hazard ratio with 95% confidence interval. Hazard ratios have been obtained in a proportional hazard model with 'wheeze ever', 'recurrent wheeze', or 'atopic dermatitis' as the dependent variables.

Table 4. Adjusted risk of wheeze, recurrent wheeze, and atopic dermatitis in first-born infants of mothers employed in child-care institutions compared to first-born infants of mothers otherwise employed stratified by sex

n = 13883	Overall relative risk*	p-value (no sex difference)†
Wheeze ever		
Boy	1.28 (1.02, 1.59)	0.11
Girl	0.95 (0.71, 1.28)	
Recurrent wheeze		
Boy	1.53 (1.13, 2.08)	0.20
Girl	1.07 (0.67, 1.70)	
Atopic dermatitis		
Boy	1.09 (0.81, 1.46)	0.52
Girl	0.93 (0.63, 1.37)	

*Relative risk (95% confidence intervals) obtained in a proportional hazard model with 'wheeze ever', 'recurrent wheeze', and 'atopic dermatitis' as the dependent variables.

†The p-value represents the test for whether the effect of maternal employment can be assumed to differ between boys and girls (test for interaction effect).

employment in child-care institution. The independent effect of maternal fever during pregnancy on wheeze, recurrent wheeze, and AD was 1.17 (1.00–1.38), 1.38 (1.12–1.72), and 1.05 (0.80–1.38), respectively. The effect of urinary tract infection was 1.23 (1.14–1.32), 1.27 (1.14–1.42), and 1.07 (0.95–1.21), respectively.

Discussion

The proposed hypothesis that maternal exposure to common infections, as reflected by maternal employment in child-care institutions before and during pregnancy provides protection against infant wheeze and AD in the offspring was not supported by our data. In contrast, having a mother employed in a child-care institution seemed to increase the risk of recurrent wheeze in first-born infants.

We assumed the effect of maternal employment in child-care institutions would be more evident among first-born infants, whose mothers

were not exposed to infections transmitted from older siblings. This assumption appeared to be correct (Table 3) albeit in the opposite direction than expected, as we observed that maternal employment in child-care institutions increased the risk of recurrent wheeze in first-born infants. However, it should be recognized that infant wheezing may not be part of the atopic phenotype. Thus, previously published data from the same birth cohort showed that most risk factors had differential effects on infant wheeze and AD indicating a different aetiology (23). Thus, the most important atopic outcome in the present study was probably AD.

We examined the offspring of mothers employed in child-care institutions on the assumption that because of their constant exposure to infections their off-spring would have a lower incidence of atopic diseases. However, we had not taken into consideration that the mother may also be important as a transmitter of infections resulting in increased risk of virus-induced infant wheezing. Several previous studies have suggested that mothers may be an important source of infectious agents for their own infants (27, 28). It may be that mothers with employment in child-care institutions have more contact with children in general and therefore are more likely to transmit infections to their off-spring. From this perspective, it was interesting that we found the male/female morbidity ratios for wheeze and recurrent wheeze to be higher among children of mothers employed in child-care institutions. This finding is consistent with our previous studies showing that mothers might be more likely to transmit infections to sons than daughters (28, 29). Nevertheless, it should be recognized that this gender difference was not significant from a statistical point of view, and may be a by chance finding.

The effects of maternal microbial exposure could be of short duration (AD and wheeze) and of longer duration (asthma and rhinitis). In the present study, the infants had been followed from birth to 18 months of age. Thus, we cannot exclude that there could be long-term effects of maternal employment in child-care institutions on the incidence of asthma or rhinitis later in life.

The observation that maternal fever and urinary tract infection during pregnancy increased the risk of recurrent wheeze in their off-spring lends some support to the hypothesis that the intra-uterine environment plays a role in the development of infant wheeze.

Several potential limitations of the present study should be considered. First, mothers employed in child-care institutions may differ

from other mothers with regard to factors that may be related to the risk of atopic diseases. Clearly, a few such differences were seen, e.g., affluence as reflected by household income and history of maternal atopic disease (Table 1). Although, we did attempt to take into account such differences by multivariable analyses, it cannot be excluded that the slightly higher risk of recurrent wheeze among children of mothers employed in child-care institutions could be because of residual confounding by these factors. Secondly, maternal employment in child-care institutions might not be a good marker of exposure to infections or may not reflect exposure to the specific infections relevant to any possible protective effect against atopic diseases. Of note is, that self-reported urinary tract infection was slightly but significantly more frequent among mothers not employed in child-care institutions compared with mothers employed in child-care institutions. Thirdly, no objective diagnosis of AD and wheeze was available, although the diagnosis of AD was developed based on a specialist evaluation of a sub-sample of children. However, objective assessment of disease is rarely achievable in a large-scale epidemiological setting.

Most studies have found that affluence is associated with higher risk of atopy. Maternal employment in child-care institutions was clearly associated with lower household income, but despite this a higher incidence of recurrent wheeze. However, it should be considered that wheezing in infancy is probably not an atopic phenotype (23). It should also be noted that within some countries, the relationship between affluence and atopy may have changed over time. Thus, in another Scandinavian country (Sweden), it was found that low socio-economic class was associated with lower prevalence of asthma with allergic rhinitis during 1952–1961 and a higher prevalence during 1972–1977 (30). Hence, the association with affluence may be changing in some countries and the socio-economic gradient may not anymore be that strong. Of course, the observed slightly higher frequency of maternal atopic disease among mothers employed in child-care institutions could also be an explanation for the slightly higher incidence of recurrent wheeze in their off-spring.

In conclusion our hypothesis that maternal exposure to infections before or during pregnancy provides protection for their infants against wheeze and AD was not supported by the present study. On the contrary, maternal employment in child-care institution seems to

slightly increase the risk of recurrent wheeze among first-born infants.

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