

Original article

Early childhood environment related to microbial exposure and the occurrence of atopic disease at school age

Background: There is a growing body of evidence that the early childhood environment with respect to day care attendance, older siblings, pet ownership, and early life airway infections may protect from developing atopic disease. Few studies have distinguished between atopic sensitization and symptoms, and none have evaluated independent contributions for all of these different environmental conditions.

Objective: Examine independent effects on atopic sensitization and symptoms of day care attendance, older siblings, pet ownership, and early infancy's airway disease.

Methods: A cross-sectional survey among 8–13-year-old school children with complete data for 1555 children.

Results: After adjustment for confounders, atopic sensitization occurred less frequently in children that had attended a day care centre (OR: 0.73, 95% CI: 0.55–0.98) or had a cat or dog before 2 years of age (OR: 0.78, 95% CI: 0.61–0.99). Having older siblings yielded a nonsignificant trend towards protection (OR: 0.88, 95% CI: 0.70–1.11). For symptoms, there was no relation with having older sibs, day care attendance and pet ownership, although there was a trend towards protection for the combination of atopy and symptoms. In contrast, children with doctors' treated airway disease before age 2, more frequently reported recent symptoms of wheeze, asthma, rhinitis, or dermatitis (all $P < 0.05$).

Conclusion: Early life environmental exposure to day care, or pets may protect against atopic sensitization. Protection against symptoms only occurred if atopic sensitization was present as well.

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The prevalence of childhood asthma has increased tremendously from the 1960s to the 1990s and nowadays is the most common chronic disease in children in Western Europe, Australia and New Zealand (1). In addition to this variation over time, recent studies have shown a geographical variation with higher prevalence rates in industrialized countries and an east–west gradient within Europe (2). These observations underpin the role of the environment in the causation of asthma. In particular, those factors that changed markedly in Western Europe and affluent English-speaking countries over the past 50 years.

Childhood asthma is considered an allergic disease, although specific stimuli differ between populations depending on its presence in the environment. In temperate humid climates, as in Western Europe, sensitization mostly occurs to house dust mite (HDM), pets

(cat or dog), and grass pollen (3, 4). Nowadays, the pathogenesis of allergic diseases is considered to be due to a persisting innate allergic type immune response (5). This suggests a key role for environmental conditions in the first years of life. Over the past 10–15 years much attention is being paid to the so-called 'hygiene hypothesis' in which a lack of microbial exposure in early life is presumed to prevent the maturation of the innate allergic immune response to a nonallergic one. Indirect evidence for this concept comes from observations of an inverse relationship between atopic disease and family size (6–9), or day care attendance (10–14), which both are considered to reflect microbial load (15–18). The reduced prevalence of atopic disease in farm children (19–26), and early life pet owners (27–31), may similarly be explained, considering animals as a source of microbial exposure as well (32, 33).

So far, most studies have focused on one factor of the early childhood environment and do not provide information about the relative contribution of each factor

separately. Furthermore, atopic disease has been defined inconsistently as atopic sensitization, asthma diagnosis or symptoms. Although asthma and atopy are strongly related, an environmental factor does not necessarily have the same effect on allergic sensitization or symptoms.

In this paper, we present the results of analyses on early childhood environment and atopic characteristics at age 8–13. Data were available for a number of early childhood environmental conditions such as pet ownership, day care attendance, older sibs, and a doctor's treated airway disease. We have evaluated separate and joint relationships to atopic sensitization, symptoms and an asthma diagnosis.

Methods

Study population and design

The population was a selection of school children that participated in cross-sectional studies on respiratory health effects of living close to a freeway or an international airport. The Medical Ethical Board of the University of Wageningen approved the study protocol and written informed consent was obtained for each child from a parent or legal locum.

Both studies were performed in 1997–98 by the same research team and used the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire to assess respiratory health. Children of 8 years and older ($n = 4111$) were invited to test atopic status. For the current analyses, we excluded 392 children that were born premature (< 37 weeks gestational age). In this initial population of 3719 subjects, atopic status was assessed in 2291 children. Another 102 children were excluded because of missing data for symptoms of wheeze ($n = 44$), asthma ($n = 25$), rhinitis ($n = 19$), and dermatitis ($n = 14$); 405 children were excluded because of missing data for day care attendance ($n = 12$), older sib(s) ($n = 71$), a cat or dog before age 2 ($n = 295$), and a doctor's treated airway disease before age 2 ($n = 27$). The final study population comprised 1784 children born at term, with complete data on atopic sensitization, symptoms, and indicators of early childhood environment.

Questionnaire

Respiratory health status was assessed using the ISAAC questionnaire. Current wheeze, rhinitis, and dermatitis were defined by a history of these symptoms in the past 12 months, all in the absence of a common cold. Current asthma was considered if a child had a diagnosis of asthma plus current wheeze or corticosteroid treatment. Symptoms were distinguished in atopic and nonatopic symptoms.

Data on early childhood environment comprised day care attendance before age 4, having older sibs, a cat or dog in the first 2 years of life, and a doctor's treated airway disease in the first 2 years of life. For day care attendance, information was collected for entry before and after age 2.

Additionally, detailed information was obtained for potential confounders such as parental characteristics (education, a history of asthma, Dutch descent), indoor environment (flooring, passive smoking, cooking fuel, moulds, moisture), and breast feeding. For the current analysis, a Dutch descent was considered if both parents and the child were born in the Netherlands.

Atopic status

Atopy was assessed by skin prick tests (SPT) and serum-specific immunoglobulin E (IgE). The SPTs were performed according to the ISAAC phase 2 standards to a panel of seven common allergens (ALK-Abello, Hørsholm, Denmark, the Netherlands). Serum-specific IgE was assessed by the CAP-assay (Pharmacia, Woerden, the Netherlands). The Phadiatop was used as a screening instrument for allergy to common inhalant allergens. Sera with a positive result were tested for specific IgE. Allergens tested in SPTs and/or IgE analysis comprised (i) mixed grass pollen (*Anthoxanthum odoratum*, *Avena eliator*, *Dactylis glomerata*, *Festuca pratensis*, *Holcus lanatus*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Secale cereale*), (ii) mixed tree pollen (*Alnus glutinosa*, *Betula verrucosa*, *Corylus avellana*, *Quercus alba*, *Salix caprea*), (iii) cat dander, (iv) dog dander, HDMs, (v) *Dermatophagoides farinae*, (vi) *D. pteronyssinus*, and (vii) moulds (*Alternaria tenuis*, *Cladosporium herbarum*, *Penicillium notatum*).

A positive SPT was defined as a mean wheal diameter ≥ 3 mm, and a positive test result on specific IgE as a titre of ≥ 0.35 kU/l. Children with either a positive result by SPT or by specific IgE were considered atopic. We distinguished indoor allergens as HDM, cat and dog dander, and outdoor allergens as moulds, grass and tree pollen.

Statistical analysis

Using the SAS 8.02 statistical software package, logistic regression was performed to test for the association between defined indicators of early childhood environment, atopic sensitization and symptoms. First, indicators were tested separately and subsequently independent effects were evaluated by including all indicators in one model. A basic set of confounders was defined as age, sex, parental history of asthma, mother's education, prolonged breast feeding (> 3 months), and passive smoking. Additionally, we tested if the results changed after adjustment for foreign descent, bedroom carpeting, bedroom sharing, gas cooking, house isolation, indoor moulds and moisture. Of these, evidence for confounding was found only for a child's descent and bedroom carpeting which changed the results slightly, and we therefore included these two variables in the multiple regression models.

Results

The initial population comprised 3719 children. Data on atopic sensitization, symptoms, and indicators of early childhood environment were obtained for 1784 children. The final study population comprised 1555 children with complete data for potential confounders as well. Table 1 describes characteristics of the final and initial study population. Children included in current analyses were more frequently of Dutch descent, had more frequently prolonged breast feeding, and were less frequently exposed to tobacco smoke in the home environment. In this study, atopic status was defined by results of either SPT's or serum-specific IgE, which yielded similar results for atopy (SPT 238/813, IgE 384/1326). In the final study population, children that had attended a day care centre, more frequently had a history of a doctor's treated airway disease (23 and 15%, $P < 0.05$), whereas day care attendance occurred less frequently in children

with older sibs compared to those without (44 and 58%, $P < 0.05$).

Table 2 shows the results of logistic regression analyses for each indicator of early childhood environment

Table 1. Characteristics of participants and nonparticipants ($n = 3719$)

	Nonparticipants		Participants	
	N/N _{total}	%	N/N _{total}	%
Atopy	234/736	32	471/1555	30
Current wheeze	342/2084	16	232/1555	15
Current asthma	111/2103	5	78/1555	5
Current rhinitis	515/2125	24	415/1555	27
Current dermatitis	333/2122	16	246/1555	16
Day care attendance	456/2132	21	330/1555	21
Older sibs	1080/2021	53	854/1555	55
Cat or dog <2 years	662/1592	42	628/1555	40
Airway disease <2 years	365/2092	17	263/1555	17
Girls	1131/2164	52	774/1555	50
Parental asthma	255/1799	14	188/1555	12
Dutch descent	1632/2145	76	1286/1555	83**
Education mother				
Low	954/2033	47	687/1555	44
Medium	555/2033	27	432/1555	28
High	524/2033	26	436/1555	28
Parental smoking	1435/2096	68	1009/1555	65*
Breast feeding >3 months	703/2127	33	631/1555	41**
Smooth floor child's bedroom	874/2106	42	673/1555	43

* $P < 0.05$, ** $P < 0.001$.

separately. Atopic sensitization occurred less frequently in children who had attended a day care facility, or had a cat or dog before 2 years of age. Neither of these was associated with symptoms. In contrast, children with a doctor's treated airway disease before 2 years of age more frequently reported current symptoms at age 8–13, whereas no association was found for atopic sensitization. Having an older sib was not associated with atopic sensitization or symptoms, and neither was the presence of at least two older sibs. Inclusion of all indicators of microbial exposure in the regression model resulted in a more pronounced protective relationship between day care attendance and current asthma (Table 3), although below the level of statistical significance ($P = 0.09$). Children that had attended a day care facility or had a pet in the first years of life were less likely to be sensitized at age 8–13. Results were similar for atopy defined by SPT's or IgE: for day care attendance respectively 0.72, 95% CI: 0.49–1.06 (SPT) and 0.74, 95% CI: 0.54–1.02 (IgE), and for having had a cat or dog respectively 0.70, 95% CI: 0.50–0.99 (SPT) and 0.79, 95% CI: 0.60–1.03 (IgE). Separate analyses for individual sensitizations showed protective odds ratios (OR) for all. Statistically significant associations were found for day care attendance and atopy to grass or dog, for older sibs and atopy to dog or moulds, and for early pet ownership and atopy to grass and tree pollen.

Table 2. Relationship between early childhood environmental characteristics (day care attendance, older sibs, furred pet, doctor's treated airway disease), and atopic sensitization and symptoms at age 8–13

	Day care		Older sibs		Cat or dog		Airway disease	
	No ($n = 1256$)	Yes ($n = 338$)	No ($n = 718$)	Yes ($n = 876$)	No ($n = 927$)	Yes ($n = 628$)	No ($n = 1324$)	Yes ($n = 270$)
Atopy								
%	31	28	31	29	33	27	29	34
OR _{crude} (95% CI)	0.86 (0.65–1.13)		0.89 (0.72–1.10)		0.76 (0.61–0.95)*		1.27 (0.96–1.68)	
OR _{adj} (95% CI)	0.74 (0.56–0.99)*		0.92 (0.73–1.15)		0.78 (0.61–0.99)*		1.13 (0.85–1.52)	
Current wheeze								
%	15	16	15	15	14	17	11	32
OR _{crude} (95% CI)	1.05 (0.75–1.48)		0.99 (0.75–1.31)		1.29 (0.98–1.71)		3.72 (2.73–5.07)***	
OR _{adj} (95% CI)	1.02 (0.72–1.46)		0.99 (0.74–1.32)		1.28 (0.95–1.73)		3.43 (2.49–4.72)***	
Current asthma								
%	5	4	5	5	5	5	3	14
OR _{crude} (95% CI)	0.80 (0.44–1.45)		1.19 (0.78–1.89)		1.15 (0.73–1.82)		5.00 (3.13–7.96)***	
OR _{adj} (95% CI)	0.68 (0.37–1.27)		1.24 (0.77–2.00)		1.11 (0.68–1.82)		4.09 (2.52–6.65)***	
Current rhinitis								
%	26	30	27	26	28	24	24	39
OR _{crude} (95% CI)	1.28 (0.98–1.67)		0.91 (0.73–1.14)		0.81 (0.64–1.02)		2.06 (1.56–2.72)***	
OR _{adj} (95% CI)	1.16 (0.86–1.53)		0.92 (0.73–1.17)		0.85 (0.66–1.09)		1.89 (1.42–2.52)***	
Current dermatitis								
%	16	15	15	16	16	15	14	24
OR _{crude} (95% CI)	0.99 (0.71–1.39)		1.04 (0.73–1.44)		0.95 (0.72–1.26)		1.96 (1.42–2.71)***	
OR _{adj} (95% CI)	0.93 (0.66–1.32)		1.03 (0.78–1.36)		1.06 (0.78–1.43)		2.04 (1.46–2.84)***	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Results of logistic regression analyses with separate models for each characteristic.

OR_{adj}: adjusted for age, sex, parental asthma, foreign descent, mother's education, breast feeding >3 months, passive smoking, smooth floor child's bedroom, cat or dog acquired after 2 years.

Table 3. Independent associations between early childhood environment, and atopic sensitization and symptoms at age 8–13

	Day care OR† (95% CI)	Older sibs OR‡ (95% CI)	Cat or dog OR§ (95% CI)
Atopy	0.73 (0.55–0.98)*	0.88 (0.70–1.11)	0.78 (0.61–0.99)*
Current wheeze	0.89 (0.62–1.29)	0.95 (0.71–1.28)	1.27 (0.93–1.73)
Current asthma	0.58 (0.30–1.10)	1.15 (0.71–1.88)	1.09 (0.66–1.81)
Current rhinitis	1.10 (0.82–1.46)	0.92 (0.72–1.16)	0.83 (0.65–1.07)
Current dermatitis	0.87 (0.61–1.24)	1.00 (0.75–1.32)	1.05 (0.78–1.42)

* $P < 0.05$.

Results of multiple logistic regression analyses with indicators included in one model.

†Adjusted for confounders as in Table 2, airway disease <2 years, older sibs, and cat/dog <2 years.

‡Adjusted for confounders as in Table 2, airway disease <2 years, day care, and cat/dog <2 years.

§Adjusted for confounders as in Table 2, airway disease <2 years, day care, and older sibs.

Table 4. Relationship between age of day care entry, and atopic sensitization and symptoms at age 8–13; reference no day care attendance

	0–2 years ($n = 113$) OR† (95% CI)	2–4 years ($n = 71$) OR† (95% CI)
Atopy	0.81 (0.51–1.28)	0.41 (0.21–0.81)*
Current wheeze	0.68 (0.36–1.30)	0.86 (0.41–1.83)
Current asthma	0.58 (0.19–1.77)	0.57 (0.13–2.53)
Current rhinitis	1.15 (0.72–1.81)	0.77 (0.42–1.41)
Current dermatitis	0.89 (0.51–1.55)	0.75 (0.57–1.52)

* $P < 0.05$.

†Adjusted for confounders as in Table 2, and airway disease <2 years, older sib(s), and cat/dog <2 years.

Information on age of entry to a day care facility was available for 184 children. As shown in Table 4, day care entry after 2 years of age yielded a stronger protection against atopic sensitization than day care entry before 2 years, although the difference was not statistically significant ($P = 0.10$). Adjustment for duration of day care attendance was not performed, since only five children quitted day care attendance. Protective ORs may be the result of selective avoidance of day care attendance because of a number of factors. If so, less protection would be expected after exclusion of children with such characteristics. In this population, we did not find evidence of a delayed day care entry because of a parental history of asthma, airway disease in early life, low-birth weight, or prolonged breast feeding (Table 5). For symptoms, the results did not change either.

Finally, Table 6 shows associations for a joint presence of atopy and symptoms, and for atopic sensitization only. Compared to nonatopic children without symptoms, there was a trend towards less atopic symptoms in children that had visited a day care centre and in those that had a cat or dog in early childhood. The pattern of associations differed for the two childhood environment characteristics. Symptoms of atopic dermatitis were

Table 5. Evaluation of selective avoidance for the relationship between age of day care entry, and atopic sensitization at age 8–13; reference no day care attendance

	n	0–2 years OR† (95% CI)	2–4 years OR† (95% CI)
All children	1400	0.81 (0.51–1.28)	0.41 (0.21–0.81)*
No parental asthma	1240	0.86 (0.52–1.40)	0.43 (0.21–0.86)*
No doctor's treated airway disease	1172	0.66 (0.38–1.15)	0.38 (0.18–0.80)*
Birth weight >2500 g	1290	0.68 (0.42–1.11)	0.37 (0.18–0.77)**
No breast feeding >3 months	826	0.53 (0.28–1.01)	0.41 (0.18–0.95)*

* $P < 0.05$; ** $P < 0.01$.

†Adjusted for confounders as in Table 2, airway disease <2 years, older sib(s), and cat/dog <2 years.

Table 6. Day care attendance or pet ownership in early childhood, and atopic symptoms or sensitization at age 8–13

	Day care OR† (95% CI)	Cat or dog OR‡ (95% CI)
Atopic wheeze	0.93 (0.56–1.53)	0.78 (0.50–1.20)
Atopic asthma	0.58 (0.27–1.24)	0.73 (0.39–1.36)
Atopic rhinitis	0.82 (0.55–1.22)	0.65 (0.46–0.92)*
Atopic dermatitis	0.58 (0.34–1.02)	0.78 (0.50–1.23)
Atopic symptoms	0.68 (0.47–0.98)*	0.74 (0.54–1.00)
Atopic sensitization only	0.73 (0.47–1.14)	0.77 (0.53–1.11)

* $P < 0.05$.

Results of multiple regression analyses, reference no atopic sensitization and no current symptoms or asthma.

†Adjusted for confounders as in Table 2, airway disease <2 years, older sibs, and cat/dog <2 years.

‡Adjusted for confounders as in Table 2, airway disease <2 years, older sibs, and day care.

negatively associated with day care attendance ($P < 0.10$), whereas atopic rhinitis occurred less frequently in children that had a cat or dog before age 2 ($P < 0.05$).

Atopic symptoms, defined by a joint occurrence of atopic sensitization and any symptom, occurred less frequently in children that visited a day care centre ($P < 0.05$) or had a cat or dog before age 2 ($P = 0.05$). In nonatopic children, there was a trend towards more wheeze if children had a cat or dog before 2 years of age (OR: 1.55, 95% CI: 0.97–2.47), while for day care attendance the relationship tended to be protective ($P > 0.10$). For other nonatopic symptoms, ORs were all close to 1.

Discussion

In this large population study, we observed less atopy if children had attended a day care centre or had a pet before age 2. Having an older sib was weakly associated with less atopy. There was no association with symptoms for any of these early childhood environmental characteristics. In contrast, symptoms were more often reported

for children that had a doctor's treated airway disease before age 2, whereas no relationship was found with atopic sensitization. This study adds to previous studies in its analysis of independent effects of a number of characteristics presumed to reflect microbial exposure in early life.

As in other studies, we observed less atopic sensitization in children that attended a day care centre (10–14), or had a pet in early childhood (27–31). Our results for a cat or dog before age 2 have previously been published (34), but were included to evaluate its relative contribution compared with other characteristics of the early childhood environment. The protective OR for day care attendance cannot be explained by selective avoidance of a day care centre or pets because of parental asthma, low-birth weight, or airway disease before age 2.

Children that attended a day care centre, more frequently reported a doctors' treated airway disease in early infancy. We did not obtain information on the nature of the airway disease, but assumed an infectious origin in most cases. However, our results for day care attendance and an airway disease in early life are conflicting. The latter proved a risk factor for symptoms at age 8–13, whereas no relationship was observed for day care attendance. This inconsistency may be due to misclassification if a substantial number of 'doctors' treated airway disease' actually should have been 'early onset wheeze'. Additionally, we cannot completely rule out bias if parents were more likely to report a doctors' treated airway disease before age 2 for symptomatic children.

Although pets are considered a source of microbial exposure, we did not observe a relationship between pet ownership and airway disease before age 2. This may be due to a difference in the nature of microbial exposure. Pet-related microbial exposure mainly constitutes of microbial cell wall components such as endotoxin, whereas whole viable microbes are a more common cause of acute respiratory infections. Taken together, our conflicting results of day care attendance and early life pet ownership yielding protective ORs, and early life airway disease being a risk factor may give further evidence to the hypothesis that mild upper airway infections confer protection against atopic disease, whereas more severe lower respiratory tract infections do not, or may even increase the risk (35, 36).

The protective effect of (older) sibs against childhood atopy as found in previous studies has been ascribed to infectious agents as well (8, 9). In this population, we did not find a relationship between older sibs and atopic sensitization or symptoms, except a reduced prevalence of grass pollen sensitization. Having older sibs was neither associated with an early childhood doctors' treated airway disease. The relation between sib size and infection rate is based upon the likelihood to encounter an infectious agent. This implies the involvement of other factors as well, like the time interval between sibs, number

of rooms, building characteristics, time and activities spent in house. Moreover, recent studies have suggested that the inverse relationship between family size and atopy may be explained by a change in the intrauterine environment by previous pregnancies (37, 38). Alternatively, atopic women might be less fertile as suggested by Sunyer et al. (39).

It is widely suggested that the time window of immune maturation towards a nonallergic immune response is restricted to the first 2 years of life. However, only few studies have actually evaluated age-specific relationships. In the former German Democratic Republic, Kramer et al. showed more atopic sensitization in children that entered a day care centre after age 1 (40). In our study population, the protection was independent of the age of day care entry. However, the Dutch and (former) GDR study populations presumably differ for a wide range of characteristics related to day care centres as well as atopic disease. Our study results may raise doubts about the time window of environmental susceptibility of the innate immune system, which is in line with a recent review on the protection of atopic disease by pet ownership (41). The intensity of day care attendance may play a role as well, which is mostly limited to 2–3 days a week in the Netherlands. However, no detailed data were obtained on day care intensity, number of children, etc. Atopic disease is a complex disorder in which multiple genes and environmental factors play a role. Presumably, the time window of susceptibility for gene–environment interaction may extend beyond the first years of life, depending on genotype, nature, dosage and timing of environmental factors.

In contrast to the protective associations with atopic sensitization, we did not find a consistent relationship with symptoms. Odds ratios for a joint presence of atopy and symptoms neither were consistently lower than those for atopic sensitization without symptoms. This suggests that a similar environmental factor may reveal opposite effects on the induction of atopic sensitization, or symptoms in previously sensitized subjects. Birth cohort studies may unravel the impact of environment throughout different periods of life on atopic sensitization and subsequent development of symptoms in different organs.

Summarized, atopic sensitization occurred less frequently in children that had attended a day care centre or had a pet before age 2, although no protection was observed against symptoms. Our results suggest that the time window of protective incitement may extend beyond 2 years of age. In contrast, children with a doctors' treated airway disease in early life more frequently had respiratory and dermal symptoms at school age whereas no relationship was observed with atopic sensitization.

We conclude that atopic sensitization occurs less frequently in children that attended a day care facility or had a pet in early infancy. The lack of a relationship with symptoms suggests a role for nonallergic pathways.

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