

Early life exposures and risk of atopy among Danish children

Simon F. Thomsen, M.D.,* Charlotte S. Ulrik, M.D., D.M.Sci.,# Celeste Porsbjerg, M.D.,* and Vibeke Backer, M.D., D.M.Sci.* (Denmark)

ABSTRACT

A large proportion of atopy develops in childhood and early life exposures are suspected to play a considerable role in the inception. The aim of this study was to examine the association between early life exposures and development of atopic disease in children. We performed a case-cohort study of a random population-based sample of children (n = 480) 7–17 years of age, living in urban Copenhagen, Denmark. Information on breast-feeding, supplementation, wheezy bronchitis, use of antibiotics, and parental smoking during pregnancy and in early life was obtained retrospectively by questionnaire. Skin test reactivity to 10 common aeroallergens was measured using standard techniques. Atopic disease was defined as a history of hay fever and/or asthma concomitantly with a positive skin-prick test. Logistic regression showed that parental atopy (odds ratio [OR] = 1.98; 95% confidence interval [CI], 1.12, 3.49; $p = 0.019$) and wheezy bronchitis before the age of 2 years (OR = 3.13; 95% CI, 1.63, 6.01; $p < 0.001$) were predictors of atopic disease, the latter especially when predisposition to atopy was present (OR = 8.63; 95% CI, 3.64, 20.44; $p < 0.001$). Duration of breast-feeding was longer in subjects with atopic heredity ($p = 0.017$), whereas smoking exposure during pregnancy ($p = 0.019$) and in the 1st year of life ($p = 0.018$) was less prevalent. Wheezy bronchitis was equally frequent among subjects with and without atopic predisposition ($p = 0.893$). Wheezy bronchitis before the age of 2 years seems to be independent of familial predisposition to atopic disease and significantly increases the likelihood for development of atopy in genetically susceptible individuals. Parental knowledge of atopic heredity significantly influences smoking and breast-feeding habits. (Allergy Asthma Proc 27:110–114, 2006)

A large proportion of atopic disease develops in childhood, and a genetic component is known to be essential.¹ In the face of this strong genetic liability, early life exposures are suspected to play a pivotal role in the initiation of atopy among genetically susceptible individuals.² However, the specific impact of early life exposures such as passive smoking, dietary factors, infections, and use of antibiotics are still contradictory.^{3–5} Although many studies tend to reproduce well-known harmful effects of traditional putative risk factors as the ones mentioned, still others document a protective effect, *i.e.*, an inverse relation between presumed adverse early life exposures and atopy that does not permit a straightforward interpretation. One example is preterm delivery, for which there has been established a beneficial effect.^{6,7} On the other hand, some surveys have found an increased risk of atopic disease among subjects who have been exposed to what previously have been thought of as protective environmental influences such as breast-feeding,⁸ and increased fetal growth.^{9,10} Alongside this ambiguity,

some confusion arises owing to contradictory results reported in studies using self-reported measures of allergic disease compared with studies that use objective measurements of atopic disease, such as skin test reactivity.¹¹

The aim of our study was to examine the association between early life exposures and development of atopic disease, in a random population sample of Danish children, 7–17 years of age. Additionally, we tested if there was a different distribution of early life exposures among subjects with familial predisposition to atopy compared with subjects without atopic heredity.

METHODS

Subjects

A sample of children and adolescents living in urban Copenhagen, Denmark, were examined in 2001. The ascertainment procedure has been reported in detail elsewhere.¹² In summary, all subjects were drawn at random from the civil registration list. The mean age was 12.1 years (age range, 7–17 years; SD = 2.9 years). A total of 480 subjects (33.3%) participated in the survey of whom 222 (46.3%) were boys. To assure the robustness of the sampled population, telephone interviews were conducted among 116 randomly selected subjects from the group of nonrespondents. The subjects, interviewed by telephone, did not differ significantly from the subjects originally included with re-

From the *Department of Internal Medicine, Bispebjerg Hospital, Denmark, and #Department of Respiratory Medicine, Hvidovre Hospital, Denmark

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Address correspondence and reprint requests to Simon Francis Thomsen, M.D., Department of Internal Medicine I, Bispebjerg Hospital, DK-2400 Copenhagen NV, Denmark

E-mail address: sft@city.dk

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Table 1 Factors Significantly Associated with Development of Atopic Disease, among 470 Danish Children, Who Were 7–17 Years of Age

Variable	OR	95% CI	p Value
Parental atopy	1.98	1.12, 3.49	0.019
Wheezy bronchitis <2 years of age	3.13	1.63, 6.01	<0.001

spect to sex, age, or prevalence of asthma, but there were significantly fewer children with symptoms of hay fever among the group of nonrespondents.¹²

Questionnaire

Information on possible risk factors was collected by means of a parental multi-item questionnaire. The questions were answered in the participating families' homes before testing, and in cases of doubt, consensus was reached with a physician on the day of testing. The exposures assessed included duration exclusively breast-fed, type and duration of supplementation with formula feeding, a diagnosis of wheezy bronchitis before 2 years of age, systemic antibiotics given before the age of 2 years, a history of maternal smoking during pregnancy, and exposure to domestic passive smoking in the 1st year of life.

Parental atopic disease was defined as symptoms of hay fever and/or asthma in at least one parent when exposed to allergens encountered in the standard testing panel described in the following sections. We used a method comparable with the one used in the European Community Respiratory Health Survey.¹³ Symptoms among participants were assessed with the same set of questions used to detect parental affectedness. These included the following: "Have you ever had hay fever?" "Have you ever had episodes of runny or stuffy nose or sneezing when not having a cold?" "Have you ever had asthma?" and "Have you ever had episodes of wheezing with shortness of breath?" This information was obtained on the day of examination by interview with a physician. Consensus was reached with the accompanying parent. Symptoms were defined as being present when a positive answer was provided to at least one of the two questions on hay fever and/or the two questions on asthma.

Skin-Prick Tests

Skin-prick tests (SPTs) were performed using standard dilutions of 10 common aeroallergens. The allergens used were birch, grass, mugwort, horse, dog, cat, house-dust mite (*Dermatophagoides pteronyssinus* and *D. farinae*), and two molds (*Alternaria iridis* and *Cladosporium herbarium*) with positive and negative references being histamine, 10 mg/mL in 50% glycerol and glycerol 50%, respectively. The concentrations of allergens were 10 Histamine Equivalent Prick (Soluprick SQ sys-

tem, ALK Abelló, Denmark). Reactions were read after 15 minutes. A positive test result was defined as a positive reaction to at least one of the allergens. The reaction to each of the allergens was regarded as positive if the mean wheal diameter $[(D1 + d2) \times \frac{1}{2}]$ was at least 3 mm.¹⁴ According to common protocol, the participants were told to stop using medications that contained antihistamine at least 3 days before skin testing.¹⁴

Statistical Analysis

Data were analyzed with the statistical package SPSS (SPSS, Inc., Chicago, IL). The associations between possible risk factors and the development of atopic disease were examined with logistic regression. Initially, we tested a full model, which included effects of all factors examined. This model was reduced by means of stepwise backward elimination of least-predictive variables. Subjects with a positive SPTs concomitantly with a history of hay fever and/or asthma were defined as having atopic disease and were used as response in the analysis.

To compare exposure status in subjects with and without familial predisposition to atopy, Fisher's exact test was used for binary variables. All risk factors measured on a continuous scale resembled a normal distribution when assessed by graphical evaluation and, hence, their means were compared by an independent sample *t*-test under the assumption of equal variances. A two-tailed value of $p < 0.05$ was considered to be statistically significant. The protocol was evaluated and approved by the local ethics committee and informed consent was obtained from all participating subjects and their parents.

RESULTS

Skin test reactivity was measured for 470 (98%) subjects of whom 91 (19%) had a positive test. A total of 110 (23%) and 56 (12%) individuals had symptoms of hay fever and asthma, respectively. A total of 63 (13%) subjects presented with symptoms of hay fever and/or asthma concomitantly with a positive SPT, *i.e.*, had atopic disease. Of those, 17 subjects had both asthma and hay fever; 44 subjects only had hay fever; and 2 subjects had asthma only.

The result of the logistic regression analysis is shown in Table 1. We found that parental atopy (odds ratio

Table 2 Distribution of Early Life Exposures among 470 Danish Children Who Were 7–17 Years of Age, According to Atopic Heredity

Variable	Predisposed Number (%)	Not Predisposed Number (%)	Mean Difference	95% CI*	p Value
Duration exclusively breast-fed [#]	4.16 (2.09)	3.62 (2.54)	−0.54	−0.99, −0.10	0.017
Maternal smoking during pregnancy	56 (29)	113 (40)		−0.48, 3.14	0.019
Number of cigarettes smoked*	9.31 (4.51)	10.64 (6.00)	1.33		0.150
Exposed to passive smoking <1 year of age	97 (51)	174 (62)		−0.55, 4.85	0.018
Number of cigarettes smoked§	13.66 (8.99)	15.81 (10.39)	2.15		0.118
Wheezy bronchitis <2 years of age	28 (15)	39 (14)			0.893
Antibiotics given <2 years of age	111 (58)	152 (54)			0.395

*Ninety-five percent confidence interval for the difference between means.

[#]Mean number of months (SD).

§Mean number of cigarettes smoked (SD).

Table 3 Impact of Wheezy Bronchitis on Risk of Atopic Disease in Danish Children Who Were 7–17 Years of Age, with and without Atopic Heredity

Wheezy Bronchitis <2 Years of Age Present (+)/ Absent (−)	Familial Predisposition Present (+)/ Absent (−)	Affected No.	Unaffected No.	OR	95% CI	p Value
(−)	(−)	22	219	1.00		
(+)	(−)	6	33	1.81	0.68, 4.79	0.248
(+)	(+)	13	15	8.63	3.64, 20.44	<0.001

[OR] = 1.98; 95% confidence interval [CI] [1.12, 3.49; $p = 0.019$) and wheezy bronchitis within the first 2 years of life (OR = 3.13; 95% CI, 1.63, 6.01; $p < 0.001$) were statistically significant determinants of atopic disease. We found no statistically significant associations between atopic disease and breast-feeding, supplementation with formula feeding, use of antibiotics before the age of 2 years, maternal smoking during pregnancy, and exposure to domestic passive smoking in the 1st year of life.

The distribution of early life exposures according to familial predisposition to atopy is shown in Table 2. The duration of exclusive breast-feeding was longer in subjects predisposed to atopic disease ($p = 0.017$), whereas maternal smoking during pregnancy ($p = 0.019$) and exposure to passive smoking during the 1st year of life ($p = 0.018$) was less prevalent among subjects with atopic heredity. A history of wheezy bron-

chitis before age two was equally frequent among subjects with and without atopic predisposition ($p = 0.893$).

To further explore our findings from the multivariate analysis, the effects of wheezy bronchitis were analyzed according to the presence or absence of familial predisposition to atopy. The result is shown in Table 3. We found that the risk of atopic disease among subjects with a history of wheezy bronchitis before the age of 2 years increased in a multiplicative fashion, *i.e.*, over eightfold when having a joined effect of exposure and familial predisposition, whereas the risk in subjects without atopic heredity was nonsignificantly increased.

DISCUSSION

This study showed a twofold increased risk of atopic disease among the offspring of parents with a history

of allergic disease. In addition, we found a strong positive association between atopic disease and wheezy bronchitis before the age of 2 years that seemed to be especially pronounced when familial predisposition to atopy was present. Finally, parental knowledge of atopic heredity predicts an increased duration of breast-feeding and a reduced magnitude of smoking during pregnancy and in early life of the child.

We found evidence of familial aggregation of atopic disease in this sample of children. It is well recognized that the liability to develop atopic disease is influenced by a major genetic component and that the principal mode of inheritance is multifactorial.¹ In line with this, several other studies examining this relationship find excess risk among first-degree relatives of atopic subjects.^{2,15}

Multivariate analysis showed a strong positive association between having had wheezy bronchitis in the first 2 years of life and risk of subsequent development of atopic disease. Wheezy bronchitis often is reported to be a risk factor for respiratory allergic disease,^{16–18} but whether this increased risk is caused by wheezy bronchitis being an independent environmental determinant or merely an expression of early disease is, however, still debatable.¹⁹ Martinez *et al.* argue that early wheezing when present in a transient form is rather benign and that the wheezing state is the result of infant airway anatomy. Still, when associated with parental atopy, exposure to household pets, and smoking these wheezing subjects are more likely to develop a more persistent form of wheezing accompanied by atopic sensitization.¹⁹ We speculate that if wheezy bronchitis indeed is an early disease manifestation, this condition would be present at a higher frequency in predisposed individuals compared with subjects with no familial history of atopic disease. We found an equal distribution of wheezy bronchitis in these two subgroups of children, indicating that it, in fact, could be an independent risk factor for atopic disease. In addition, when examining the risk of wheezy bronchitis differential on familial predisposition, we were able to show a multiplicative effect on the risk estimate among subjects with both determinants. This phenomenon is likely to have occurred because different subjects (different genotypes) are differentially sensitive to a given environment. Therefore, stratifying on predisposition is helpful when assessing exposures, *i.e.*, gives a clearer picture of the actual risk. However, some families might display aggregation of atopic disease because of genetic reasons that are different from other families, hence, the concept of complex inheritance. Consequently, our findings should not be interpreted as a direct interaction between a specific genetic marker and an exposure but rather a joined effect of overall risk genotype and the environment, or a coac-

tion. On the other hand, our finding is a crude measure of a genotype–environment interaction, and some evidence exists that a mediation effect of early respiratory tract infections with wheezing is biologically plausible.²⁰

This study has some limitations. First, the participation rate is fairly low. We attempted to correct for that by conducting telephone interviews with a random sample of nonrespondents to get a picture of whether the subjects studied were representative of the entire sample. We found a slightly lower prevalence of symptoms of hay fever among nonrespondents. For this reason, we can not entirely rule out the possibility of selection bias. Still, the present setting is built for detecting case–cohort relationships and thus might not be affected to a devastating degree by the nature of our ascertainment.

We examined several possible risk factors for the development of atopic disease as if we were using a prospective design when, in fact, the study is cross-sectional. The validity of the study is partly built on parental reporting of perinatal events, which have occurred several years before examination. One might suspect that parents who have children with atopic disease are more likely to report risk events to have taken place in early childhood trying to link these events to subsequent development of disease. This problem could have been avoided if the data had been collected prospectively. Whether the finding of a higher frequency of presumed disadvantageous early life events in affected individuals compared with unaffected is caused by true or false reporting or a combination of both remains ambiguous and must be considered when interpreting the results.

In addition, we used the parental phenotype as an imperfect indicator of inheritance and we have to recognize that this definition of atopic predisposition is a crude approximation of genetic risk. Because we did not have molecular genetic data or objective data on parental sensitization to aeroallergens, we chose to score parents as having atopic disease when experiencing wheezing and/or rhinitis on exposure to known stimuli encountered in the same testing panel as the one used to determine sensitization in the children. This procedure might have introduced a reporting bias, as in the case of parents with atopic children who are more likely to report their own symptoms, thus inflating the magnitude of familial effects.

Knowing that risk genotype might promote certain behaviors, we examined the distribution of risk factors in predisposed individuals to see if it differed from that in the rest of the population. This would give a feeling of whether parents (mothers) who have a risk genotype adopt healthy lifestyles to try to prevent the phenotype from being expressed. We found that parental smoking and breast-feeding habits are indeed influenced by

knowledge of risk status. If these findings were not the cause of reporting bias it would explain why we did not pick up an elevated risk of exposure to passive smoking in fetal life and in the 1st year of life. In fact, we found a decreased but statistically insignificant risk in subjects exposed to passive smoking, presumably because of this skewed distribution of risk factors in predisposed subjects compared with those without that determinant.

We chose an outcome measure, which is based on clinical symptoms of allergic airway disease concomitantly with an objective verification of atopic sensitization. This leaves subjects with only clinical symptoms and individuals with only positive SPTs unaccounted for. The reason for collapsing these two entities into one phenotype is in our view providing the most solid measure of atopic disease but, on the other hand, it is unarguably leaving information behind. In line with this, some previous reports document a different risk profile for these two outcomes being unable to determine exposures associated with positive SPT as the sole diagnostic criterion.¹¹ In the face of these results, it is evident that only using self-reported symptoms enables one to study larger sample sizes but at the expense of missing information on sensitization and physician-based interviews.⁶ On the contrary, studies using objective tests, *i.e.*, a more reliable diagnosis, often end up being insufficiently powered to detect effects of subtle variation in environmental influences. Following this, this survey is a good example of why we need larger studies with refined methodology to make solid inferences about environmentally ubiquitous risk factors.¹³

We conclude that genetic variation is the major driving force behind the development of atopic disease in childhood. Wheezy bronchitis before the age of 2 years seems to be independent of familial predisposition to atopic disease and significantly increases the likelihood for development of atopy in genetically susceptible individuals. Parental knowledge of atopic heredity significantly influences smoking and breast-feeding habits. However, this change in lifestyle does not seem sufficient to prevent transmission of disease.

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