Early life exposures and development of allergic disease in infants with familial risk: results from ongoing probiotic intervention trials

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Short title: Updated early allergy determinants

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ABSTRACT

Aim: We search revision of risk determinants of the ongoing allergy epidemic.

Methods: 433 children born to mothers with allergic disease or sensitization were selected from the three ongoing probiotic intervention trials for this case-control study. Children who developed atopic eczema or food allergy, had positive skin prick test results or had been prescribed inhaled corticosteroids by the age of two years were identified as cases (n=231) while children without allergic manifestations were the healthy controls (n=202). The data on early environmental exposures were collected from prospectively documented study records. The statistical analyses were adjusted for potential confounders.

Results: Determinants associated with the increased risk of atopic eczema were lower maternal prepregnancy BMI (aOR 0.15, 95% CI: 0.037 – 0.54) and maternal intrapartum antibiotic treatment (aOR 2.21, 95% CI 1.20 – 4.10), the latter also linked to obstructive respiratory symptoms (aOR 3.87, 95% CI 1.07 – 14.06). The risk of allergic sensitization was associated with lower maternal prepegnancy BMI (aOR 0.18, 95% CI 0.43 – 0.79) and intrapartum antibiotic treatment (aOR 2.13, 95% CI 1.07 – 4.22).

Conclusion: Based on our demonstrations, interventions such as personalized diets, can be optimized for specific subgroups and definite risk periods.

Keywords: Allergic sensitization; allergy; antibiotics; atopic eczema; body mass index

Key notes

Understanding the early determinants of allergic disease risk is instrumental in advising allergic families. Women in childbearing age should be counseled to aim for normal weight and if antibiotic treatment during childbirth or in early childhood is considered, should the potentially increased risk of allergies be taken into account.

INTRODUCTION

The allergy epidemic remains a serious health concern. The prevalence of allergic diseases has increased at least since the beginning of the 20th century but the reason remains largely obscure.[1,2,3] The most popular current explanation is the disappearing microbiota hypothesis,[4] extending the original hygiene hypothesis by Gerrard and colleagues and Strachan.[5,6]

We traditionally identify genetic factors, small family size, smoking, sex, breastfeeding duration and not living in a countryside as risk determinants favouring the emergence of the allergic phenotype.[3,5,6,7,8] Due to urbanization with improved or even exaggerated hygienic conditions, altered dietary intake and often more sedentary way of life our immediate environment and lifestyle has profoundly changed.[1,2,9] Today's mothers are more often obese, giving birth at an older age and more frequently by caesarean section; this worldwide trend seems to continue.[9,10,11] In modern medicine, antibiotics are used abundantly from early life without fully understanding the long-term effects.[12,13] Concurrently with urbanization, human contacts with animals have changed from contact with farm animals to pet ownership in city homes.[14,15] In view of the recent changes in our environmental exposures, our understanding of the causes of allergic diseases may need an update.

The present study aimed to improve our understanding of the ever-continuing allergy epidemic. In order to focus on environmental causes, we studied children at high genetic risk determined by existing maternal allergic disease or allergic sensitization. We hypothesized here that the altered environment and lifestyle carry previously unknown

and interrelated risk factors of atopic eczema, asthma and allergic sensitization in the modern society.

PATIENTS AND METHODS

This nested case – control study is based on three ongoing probiotic intervention trials aiming to reduce atopic disease risk [16,17,18] (ClinicalTrials.gov identifier NCT00167700). A total of 656 families participated in the original studies. To obtain uniform genetic risk determinants and target the study to the most vulnerable population in terms of genetic predisposition we included only the children born to mothers with atopic eczema, hay fever, asthma, food allergy or allergic sensitization. Twins, children with gestational age under 37 weeks or birthweight less than 2500 g, and children lost to follow-up before the age of 2 years, were excluded leaving 433 children in the analytic sample.

The primary clinical endpoints of the study consisted of allergic manifestations among the study population during the first two years of life. Atopic eczema (AE) was clinically diagnosed by the study physicians during scheduled control visits using prespecified criteria modified from those introduced by Hanifin including pruritus, typical morphology and distribution and chronic, relapsing course with the minimum of two eczema episodes with the duration of at least one month each or persisting chronic eczema without periods of remission.[19] Inhaled corticosteroid (ICS) use was applied as an objective representation of obstructive respiratory symptoms. The information of ICS purchases during the first two years of life was gathered from the nationwide Prescription Register maintained by The Social Insurance Institution of Finland. In Finland, ICSs are only available by prescription from a licensed physician. Allergic sensitization was assessed by skin prick testing (SPT) which were performed at the age of 6 months, 12 months and 24 months by trained study nurses. The SPT panels included cow's milk and egg white which are the most relevant food allergen sources for children under two years old. The information on doctor-diagnosed food allergies (FA) was collected from patient

records. In total, 231 children developed either AE, were prescribed ICSs, had positive SPT results during the first two years of life or had FA and thus, were identified as cases. 202 children with none of above allergic manifestations were identified as healthy controls.

The information of currently known risk factors for allergic diseases including child's sex, having older siblings, maternal smoking during pregnancy and breastfeeding duration and data on more novel exposures potentially affecting allergic disease risk including maternal prepregnancy body mass index (BMI), mode of delivery, maternal intrapartum antibiotic treatment, children's antibiotic treatment during the first 6 months of life, probiotic intervention during pregnancy and postnatally and having one or several furry pets during pregnancy or in early childhood were collected from the prospectively collected study records.

JMP Pro 16.0.0 was used for all statistical analyses. The continuous characteristics of the study population are presented as means with ranges and the categorical characteristics and the allergic manifestations as numbers and percentages. Differences between the groups of healthy children and children with specific allergic manifestations were assessed with Student's two-tailed t test for continuous variables and with Fisher's Exact Test for categorial variables.

Logistic regression was used to assess the association between exposures (maternal prepregnancy BMI, mode of delivery, maternal intrapartum antibiotic treatment, children's antibiotic treatment during the first 6 months of life, probiotic intervention and having a furry pet in a household) and the primary outcomes in a model adjusted for

child's sex, presence of older siblings, maternal smoking during pregnancy and breastfeeding duration. The results are expressed as odd's ratios (OR) with 95% confidence intervals (CI). P-values less than 0.05 were considered statistically significant.

RESULTS

During the first two years of life, altogether 166 (38%) children manifested AE, ICSs were prescribed to 23 (5%) children and 120 (28%) children exhibited atopic sensitization as assessed by positive SPT results. These manifestations exhibited marked overlap in the same individuals (Figure 1). Most commonly children were sensitized to egg white (104/120, 87%) followed by cow's milk (24/120, 20%), surpassing all other sensitizations. Altogether 65 (15%) children had been diagnosed FA. The most common food allergies were cow's milk allergy with 48 cases and allergy towards egg white with 25 cases.

The early circumstances and exposures from the immediate environment are presented in Table 1. In the entire study population, the mean maternal prepregnancy BMI was 23.3 kg/m^2. Altogether 63 (15%) mothers had received intrapartum antibiotic treatment and 64 (15%) children had been treated with antibiotics within the first 6 months of life. Probiotic intervention had been administered to 230 (53%) mother-child pairs. In all, 98 (23%) families reported owning a furry pet during pregnancy or during the first two years after birth.

Several early exposures were associated with the risk of atopic eczema during the first two years of life (Table 2). Lower prepregnancy BMI was associated with an increased risk of atopic eczema (P=0.0045). This association remained significant in a model with adjusting for original clinical study, child's sex, having older siblings, smoking during pregnancy and breastfeeding duration (adjusted OR 0.15, 95% CI 0.037 – 0.54, P=0.0038). Maternal intrapartum antibiotic treatment was also associated with an increased risk of atopic eczema (adjusted OR 2.21, 95% CI 1.20 – 4.10, adjusted

P=0.010), but an opposite association was seen if antibiotic treatment was given to children during the first 6 months of life (adjusted OR 0.50, 95% CI 0.26 – 0.95, P = 0.030). Probiotic intervention was associated with decreased risk of AE (adjusted OR 0.31, 95% CI 0.19 – 0.50, adjusted P<0.0001). In this study population, mode of delivery and having a furry pets showed no association with the risk of atopic eczema.

Maternal intrapartum antibiotic administration was associated with an increased risk of obstructive respiratory symptoms with the need for ICS medication (Table 3; adjusted OR 3.87, 95% CI 1.07 – 14.06, P = 0.047). Maternal BMI, mode of delivery, antibiotic treatment during early childhood, probiotic intervention and having pets in the family displayed no significant associations with obstructive respiratory symptoms.

Similar to the effects to the risk of atopic eczema, lower prepregnancy BMI and maternal intrapartum antibiotic administration were associated with an increased risk for developing allergic sensitizations in children (Table 4; adjusted OR 0.18, 95% CI 0.43 – 0.79, P = 0.018 and adjusted OR 2.13, 95% CI 1.07 – 4.22, P = 0.031, respectively). Antibiotic administration during early childhood was in turn associated with a decreased risk of allergic sensitization (adjusted OR 0.48, 95% CI 0.23 – 1.01, P=0.043). Exposure to pets during pregnancy or in the early childhood was associated with a decreased risk of atopic sensitization (adjusted OR 0.48, 95% CI 0.26 – 0.88, P=0.015).

DISCUSSION

Several early-life exposures were associated with allergic outcomes during the first 2 years of life in this study. Maternal intrapartum antibiotic administration was associated with an increased risk of childhood AE, obstructive respiratory symptoms and allergic sensitization. This finding highlights the potential impact of intrapartum antibiotic treatment on the risk of developing various atopic diseases later in life.

The result is consistent with previous studies concerning associations between antibiotic exposures during pregnancy, delivery and first days of life and allergic manifestations in children.[7,12,20,21,22]

In western countries, intrapartum antibiotics are used abundantly and nearly 40% of all neonates are exposed to intrapartum antibiotics, the purpose of which most often is to prevent the transmission of Group B *Streptococcus* from mother to the child or prevent maternal infections due to caesarean section.[13] Maternal antibiotic prophylaxis during vaginal and caesarean section delivery has been reported to cause critical changes in the infant's gut microbiota during the first weeks of life, particularly a temporary reduction in the abundance of the genus *Bifidobacterium*.[7,13,23] Not only has this dysbiosis been linked to allergic diseases, it has also been associated with both intestinal and systemic inflammation and dysregulation of the immune system.[7,24] As the infant's gut microbiota coevolves with the immune system, antibiotic exposure may perturb the compositional development of gut microbiota during critical period of maturation within which the microbiota and child immune phenotype are consolidated.

Antibiotic treatment in early childhood has been suggested to increase the risk of atopic manifestations, including the risk of asthma.[7,12,23,25] Broad-spectrum antibiotics, longer

duration of the exposure and multiple treatment periods seems to exaggerate the risk.[12,25] In our study, obstructive respiratory symptoms were more common in children exposed to antibiotics during the first six months of life, but the connection was not statistically significant, possible due to relatively small sample size. In contrast, antibiotic use during early infancy was associated with a lower risk of AE and allergic sensitization and this result unexpectedly differed from previously published studies.[7,12,23,25] The results may be explained by the fact that we focused on individuals at high hereditary risk and it is of note that these associations may not suggest a causal relationship or reliably separate cause from consequence. While some children manifested AE with allergic sensitization and some children only had allergic sensitization without clinical diseases, the risk factors for AE and allergic sensitization seem to be similar, whereas asthma appears to be more multifactorial.

Lower maternal prepregnancy BMI was associated with an increased risk of both atopic eczema and allergic sensitization and, in accordance with previous studies, pet ownership was linked to decreased risk of allergic sensitization.[23] Interestingly, both maternal weight status and having household pets have been linked with altered gut microbiota in infants.[23,26] According to current understanding, low-grade systemic inflammatory activation and proinflammatory changes in the maternal gut microbiota take place during healthy pregnancy, but maternal overweight and obesity cause excessive inflammatory responses that can be transmitted to the infant.[27] Therefore, our study invites the idea that our microbiome is the key regulator in the developmental trajectory to the child. This notion is corroborated by the finding that probiotic intervention showed a protective effect on atopic eczema. This result, published from the three original studies,[16,17,18] has since been repeated in several clinical probiotic trials.[28]

When assessing the allergy risk factors, not all hypotheses can be evaluated by intervention studies for ethical reasons, whereas case – control study is a safe method for epidemiological research. In this study, we used a nested case-control approach where we combined subjects from three different prospective original studies. All the studies were conducted in the same population and at the same research center with the same personnel and used a uniform follow-up protocol. The primary outcomes, that emerged during the follow-up, were identified with high reliability: AE was diagnosed by clinical history and examination performed by a licensed physician during a study visit. The purchases of ICSs, which in Finland are only available by prescription from a licensed physician, were obtained from a national register and the data are thus free of recall bias. Because asthma is difficult to diagnose at very young age, it is possible that ICSs may have been prescribed also to some infants who only manifested early wheezing without later developing actual asthma. SPTs were performed and interpreted by trained study personnel. The original study, from which the subjects were selected, was taken into account as a potential confounding factor in the statistical analyses. The rest of the confounding factors were chosen based on strong research evidence. The infants were followed up for 2 years, and it is possible that some children developed allergic manifestations later in life. However, in this study, the main goal was to assess the earliest risk factors for allergic diseases and a longer follow-up period would have increased the likelihood of confounding factors. Albeit the study population was relatively small, several statistically significant associations were found. The association between intrapartum antibiotic treatment and allergic outcomes cannot be explained by confounding by indication due to mostly prophylactic use of intrapartum antibiotics. In addition, the chronological order is evident. Antibiotic exposures within the first 6

months of life overlapped with the 2 years of follow-up period causing partial uncertainty in causality. AE is usually the first atopic manifestation and may appear before the age of 6 months, but in most children, symptoms emerge within the first year of life or later.[29] In this study, the indications for early childhood antibiotics or the types of antibiotics were not specified, but instead, we focused on the overall exposures. We find it unlikely that the observed association between furry pets and allergic sensitization might be explained by lower likelihood of pet ownership in allergic families given the fact that all included children had a family history of allergic disease.

This study reinforces the notion that pregnancy and early infancy represent a critical period of developmental plasticity when the scene is set for the consolidation of the child immunologic phenotype. Early exposures may facilitate adaption to the future environmental conditions but may concomitantly carry a heightened susceptibility to allergic disease, the earliest manifestation of non-communicable diseases. As the efficacy of preventive measures is dependent on the risk,[30] recognition of novel risk determinants with the information of existing allergic diseases in the family will help us to direct the counseling in the appropriate manner. We are only beginning to understand the potentially detrimental consequences of abundant antibiotic use and treacherously increasing maternal obesity to child's long-term health. Women in childbearing age should be counseled to aim for normal weight and if antibiotic treatment during childbirth or in early childhood is considered, should the potentially increased risk of allergies be taken into account.

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Conflict of interest

The authors have no conflict of interest.

Statement of ethics

Study was conducted ethically in accordance with the Declaration of Helsinki. The data were collected during clinical trials, which were approved by the Ethics Committee of the Hospital District of Southwest Finland. Oral and written informed consent was obtained from the caregivers.

Abbreviations

AE: atopic eczema

BMI: body mass index

CI: confidence interval

FA: food allergy

ICSs: inhaled corticosteroids

OR: odd's ratios

SPT: skin prick test

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TABLES

Table 1. The clinical characteristics of the study population.

	Healthy children n = 202	**Atopic eczema n = 166**	**P-value**	**ICS n = 23**	**P-value**	**Positive SPT n = 120**	**P-value**
Mother							
Mother's age in years, mean (range)	30.4 (18.8 – 43.5)	30.8 (18.8 – 42.7)	0.32	31.5 (25.9 – 38.7)	0.17	31.1 (18.8 – 43.9)	0.18
Mothers prepregnancy BMI [kg/m^2], mean (range)	23.8 (18.0 – 38.5)	22.7 (17.7 – 33.5)	**0.0031**	23.4 (19.0 – 30.8)	0.48	22.8 (18.2 – 35.3)	**0.012**
Maternal probiotic intervention, n (%)	125 (61.9)	67 (40.4)	**<0.0001**	13 (56.5)	0.66	63 (52.5)	0.10
Smoking during pregnancy, n (%)	10 (5.0)	9 (5.4)	0.84	0 (0.0)	0.60	3 (2.5)	0.38
Family							
Older siblings, n (%)	71 (35.1)	63 (38.0)	0.59	13 (56.5)	0.067	46 (38.3)	0.57
Pregnancy and childbirth							
Duration of pregnancy in weeks, mean (range)	40.2 (37.3 – 43)	40.0 (37.0 – 42.7)	0.090	40.0 (38.0 – 41.7)	0.26	40.2 (37.0 – 42.7)	0.88
Caesarean section, n (%)	25 (12.5)	23 (14.0)	0.76	2 (9.5)	0.70	14 (12.0)	0.89
Child's gender girl, n (%)	101 (50.0)	64 (38.6)	**0.035**	6 (26.1)	**0.046**	49 (40.8)	0.13
Birth weight in grams, mean (range)	3610.4 (2560 – 4860)	3657.3 (2580-4800)	0.29	3692.8 (2800 – 4800)	0.49	3631.4 (2800 – 4700)	0.67
Birth weight SD, mean (range)	0.0 (-2.2 – 2.1)	0.2 (-2.2 – 2.9)	0.10	0.1 (-2.1 – 2.9)	0.63	0.04 (-2.1 – 2.2)	0.77
Breastfeeding							

Breastfeeding duration in months, mean (range)	8.6 (0 – 27.0)	9.1 (0.5 – 24.0)	0.44	8.7 (0.3 – 18.0)	0.98	9.8 (0.5 – 36.0)	0.063
Furry pets							
Pets during pregnancy or in the first 24 months of life, n (%)	53 (27.6)	30 (18.5)	0.059	7 (30.4)	0.81	18 (15.7)	**0.018**
Antibiotic use							
During childbirth, n (%)	20 (10.3)	33 (20.6)	**0.010**	5 (25.0)	0.066	22 (20.0)	**0.026**
0-6 months of age, n (%)	35 (17.3)	17 (10.2)	0.070	8 (34.8)	0.053	11 (9.2)	**0.048**

Note: Differences between the groups of healthy children and children with specific allergic manifestations were assessed with Student's two-tailed t test for continuous variables and with Fisher's Exact Test for categorial variables. The bold values are statistically significant results ($p < 0.05$).

Abbreviations: ICS, inhaled corticosteroid; SPT, skin prick test; BMI, body mass index.

Table 2. Maternal, perinatal and early childhood's risk factors and association with the risk of atopic eczema during the first 24 months of life.

	Atopic eczema	No atopic eczema	Unadjusted p-value	Odds' ratio†	Lower confidence interval	Upper confidence interval	Adjusted p-value†
Mother							
Body Mass Index	22.7 (17.7 – 33.5)	23.8 (18.0 – 38.5)	**0.0045**	0.15	0.037	0.54	**0.0038**
Pregnancy and birth							
Caesarean section	23/164 (14.0)	25/200 (12.5)	0.76	1.11	0.60	2.06	0.74
Antibiotics during birth	33/160 (20.6)	20/194 (10.3)	**0.010**	2.21	1.20	4.10	**0.010**
Early childhood							
Antibiotics 0-6 months	17/166 (10.2)	37/202 (18.3)	0.070	0.50	0.26	0.95	**0.030**
Probiotics	67/166 (40.4)	125/202 (61.9)	**<0.0001**	0.31	0.19	0.50	**<0.0001**
Pets‡	30/162 (18.5)	53/192 (27.6)	0.059	0.60	0.35	1.01	0.051

Note: Body mass index has been presented as means and ranges and other early exposures as amounts and percentages. The associations between exposures and clinical outcomes were assessed with logistic regression. The results are expressed as odd's ratios with 95% confidence intervals. The bold values are statistically significant results ($p < 0.05$).

†Adjusted for original clinical study, child's sex, older siblings, smoking during pregnancy and breastfeeding duration.

‡Pets during pregnancy and the first 24 months of life.

Table 3. Maternal, perinatal and early childhood's risk factors and association with the usage of inhaled corticosteroids during the first 24 months of life.

	Inhaled corticosteroid	**No inhaled corticosteroid**	**Unadjusted p-value**	**Odds' ratio†**	**Lower confidence interval**	**Upper confidence interval**	**Adjusted p-value†**
Mother							
Body Mass Index	23.4 (19.0 – 30.8)	23.8 (18.0 – 38.5)	0.61	0.45	0.027	7.55	0.57
Pregnancy and birth							
Caesarean section	2/21 (9.5)	25/200 (12.5)	0.69	0.81	0.17	3.96	0.79
Antibiotics during birth	5/20 (25.0)	20/194 (10.3)	0.066	3.87	1.07	14.06	**0.047**
Early childhood							
Antibiotics 0-6 months	8/23 (34.8)	37/202 (18.3)	0.053	2.01	0.77	5.59	0.16
Probiotics	13/23 (56.5)	125/202 (61.9)	0.66	0.54	0.18	1.60	0.27
Pets‡	7/23 (30.4)	53/192 (27.6)	0.81	1.33	0.48	3.68	0.59

Note: Body mass index has been presented as means and ranges and other early exposures as amounts and percentages. The associations between exposures and clinical outcomes were assessed with logistic regression. The results are expressed as odd's ratios with 95% confidence intervals. The bold values are statistically significant results ($p < 0.05$).

†Adjusted for original clinical study, child's sex, older siblings, smoking during pregnancy and breastfeeding duration.

‡Pets during pregnancy and the first 24 months of life.

Table 4. Maternal, perinatal and early childhood's risk factors and association with the positive skin prick test by the age of 24 months.

	Positive skin prick test	Negative skin prick test	Unadjusted p-value	Odds' ratio†	Lower confidence interval	Upper confidence interval	Adjusted p-value†
Mother							
Body Mass Index	22.8 (18.2 – 35.3)	23.8 (18.0 – 38.5)	**0.017**	0.18	0.43	0.79	**0.018**
Pregnancy and birth							
Caesarean section	14/117 (12.0)	25/200 (12.5)	0.89	0.89	0.44	1.82	0.75
Antibiotics during birth	22/112 (19.6)	20/194 (10.3)	**0.026**	2.13	1.07	4.22	**0.031**
Early childhood							
Antibiotics 0-6 months	11/120 (9.2)	37/202 (18.3)	**0.048**	0.48	0.23	1.01	**0.043**
Probiotics	63/120 (52.5)	125/202 (61.9)	0.10	0.66	0.39	1.11	0.12
Pets‡	18/115 (15.7)	53/192 (27.6)	**0.018**	0.48	0.26	0.88	**0.015**

Note: Body mass index has been presented as means and ranges and other early exposures as amounts and percentages. The associations between exposures and clinical outcomes were assessed with logistic regression. The results are expressed as odd's ratios with 95% confidence intervals. The bold values are statistically significant results ($p < 0.05$).

†Adjusted for original clinical study, child's sex, older siblings, smoking during pregnancy and breastfeeding duration.

‡Pets during pregnancy and the first 24 months of life.

FIGURES

Figure 1. Allergic manifestations in children.