MS STEFANIE METZLER (Orcid ID : 0000-0003-0720-7365)

DR AMANDINE DIVARET-CHAUVEAU (Orcid ID : 0000-0002-2492-9864)

Article type : Original

Association between antibiotic treatment during pregnancy & infancy and the development of allergic diseases

Stefanie Metzler[1,3], Remo Frei PhD[3,4], Elisabeth Schmaußer-Hechfellner BSc[6], Erika von Mutius, MD[6,7,8], Juha Pekkanen PhD[9,10], Anne M. Karvonen[9], Pirkka V. Kirjavainen[9], Jean-Charles Dalphin PhD[11], Amandine Divaret-Chauveau MD[12,13,14], Josef Riedler MD[15], Roger Lauener MD[3,5], Caroline Roduit MD, PhD[2,3,5]; on behalf of the PASTURE/EFRAIM study group*

Affiliations:

1 University of Zurich, Zurich, Switzerland

2 University Children's Hospital Zurich, Switzerland

3 Christine Kühne-Center for Allergy Research and Education, Davos, Switzerland

4 Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland

5 Children's Hospital St Gallen, St Gallen, Switzerland

6 Helmholtz Zentrum München - German Research Center for Environmental Health, Institute for Asthma and Allergy Prevention, Neuherberg, Germany

7 Dr von Hauner Children's Hospital, Ludwig Maximilian University, Munich, Germany

8 CPC-M, Member of the German Center for Lung Research

9 Environment Health Unit, National Institute for Health and Welfare, Kuopio, Finland

10 Department of Public Health, University of Helsinki, Helsinki, Finland

11 University of Besançon, Department of Respiratory Disease, UMR/CNRS 6249 Chrono-environment, University Hospital, Besançon, France

12 Pediatric Allergy Department, University Hospital of Nancy, Nancy, France

13 EA3450 Développement Adaptation et Handicap (DevAH), University of Lorraine, Nancy, France

14 UMR 6249 Chrono-environment, CNRS and University of Franche-Comté, Besançon, France

15 Children's Hospital Schwarzach, Kardinal Schwarzenbergplatz 1, Schwarzach, Austria, Teaching Hospital of Paracelsus Medical Private University Salzburg

*The PASTURE study group:

The members of the PASTURE study group are (in alphabetical order by study center):

Hyvärinen A, Remes S., Roponen M. (Finland); Chauveau A., Dalphin ML., Kaulek V. (France); Ege M., Genuneit J., Illi S., Kabesch M., Schaub B., Pfefferle P. (Germany); Doekes G. (The Netherlands).

Running title: Pre-/postnatal antibiotics and allergies

Corresponding author:

Dr Caroline Roduit, MD

Kinderspital Zurich

Steinwiesstrasse 75, 8032 Zurich, Switzerland

email: Caroline.Roduit@kispi.uzh.ch

Tel.: +41 (0)44-266-3119

Fax: +41 (0)44-266-7984

Material in the electronic repository:

- Table E1. Association between prenatal exposure to antibiotics and sensitization at the age of 4 years
- Table E2. Association between prenatal exposure to antibiotics and sensitization at the age of 1 and 6 years
- Table E3. Association between exposure to antibiotics in the first year of life and atopic dermatitis up to 4 years in relation to indication

Conflicts of Interest:

The authors declare that they have no conflicts of interest.

Funding:

This work was supported by European Union research grants PASTURE/EFRAIM (QRLT4-CT 2001-00250, KBBE-2-2-06) and the Kühne-Foundation.

Author Contributions:

RF, ESH, EVM, AK, JP, JCD, JR, RL and CR designed the study and were responsible for the acquisition of data. SM, RL and CR designed this subproject. SM, CR were involved in the analysis and interpretation of data. SM drafted the article. All authors revised the manuscript critically for important intellectual content, approved of the final version of the manuscript.

Abstract

Background: Allergies are a serious public health issue and prevalences are rising worldwide. The role of antibiotics in the development of allergies has repeatedly been discussed, as results remain inconsistent.

The aim of this study was to investigate the association between pre- & postnatal antibiotic exposure and subsequent development of allergies (atopic dermatitis, food allergy, asthma, atopic sensitization and allergic rhinitis).

Methods: A total of 1080 children who participated in a European birth cohort study (PASTURE) were included in this analysis. Data on antibiotic exposure during pregnancy and/or first year of life and allergic diseases were collected by questionnaires from pregnancy up to 6 years of age and analysed by performing logistic regressions. To take into account reverse causation, we included models, where children with diagnosis or symptoms of the respective disease in the first year of life were excluded.

Results: Antibiotic exposure in utero was significantly and positively associated with atopic dermatitis and food allergy. The strongest effect was on diseases with onset within the first year of life (for atopic dermatitis: aOR 1.66, 95% CI 1.11–2.48; and for food allergy: aOR 3.01, 95% CI 1.22-

7.47). Antibiotics in the first year of life were positively associated with atopic dermatitis up to 4 years (aOR 2.73, 95% CI 1.66-4.49), and also suggest a dose-response relationship. A tendency was observed with asthma between 3 & 6 years (aOR 1.65, 95% CI 0.95-2.86).

Conclusions: Our findings show positive associations between exposure to antibiotics and allergies, mainly with atopic dermatitis and food allergy within the first year of life, after prenatal exposure and, atopic dermatitis and asthma after postnatal exposure to antibiotics in children born in rural settings.

Key words

allergy; antibiotics; children; atopic dermatitis; food allergy; asthma

Abbreviations used

aOR: Adjusted odds ratio

CI: Confidence interval

ISCED: International Standard Classification of Education

OR: Odds ratio

PASTURE: Protection against Allergy-Study in Rural Environments

SCORAD: Scoring atopic dermatitis

Introduction

A rise in the prevalence of allergic diseases has been observed worldwide over the last decades. Primary prevention remains a challenge, as the reasons for development of allergies are still incompletely understood.[1]

A trend for an increased antibiotic use paralleling the epidemics of asthma has been observed.[2] Therefore, a potential role of antibiotics in the development of allergic diseases has been discussed.

Three biological mechanisms were suggested that support the link between antibiotics and allergy. Firstly, antibiotics change the course of infection, which may cause the immune system to shift towards an allergic pathway.[2] Secondly, experimental studies showed that certain types of antibiotics suppress mediators of the Th1 response, which might lead to a deviation towards the Th2 response and therefore promote the development of allergy.[3] Lastly, animal studies have shown that perinatal exposure to vancomycin impacts the gut microbiome, which subsequently increases the susceptibility to asthma.[4]

However, in spite of numerous studies on this subject, the results remain inconsistent.

It has been discussed that such analysis is very susceptible to confounding, as well as reverse causation.[2,5,6]

Data from the PASTURE birth cohort, prospectively collected, allows us to study the relationship between prenatal and postnatal exposures and the development of allergic diseases.[7] Here, the objective was to study the relationship between antibiotic use during pregnancy or the first year of life and the development of allergic diseases.

Methods

Study design and population

The PASTURE study is a prospective birth cohort including children living in rural areas of five European countries (Austria, Finland, France, Germany and Switzerland). The design of this cohort has been described in detail elsewhere.[7] Overall, 1133 children were included in this study. Questionnaires were based on previous studies.[8-10] They were administered in interviews or self-administered to the mothers. Children with data available on antibiotic exposure in utero (n=1080) or in the first year of life (n=1019) were included in these analyses.

Definitions

Data on antibiotic exposure during pregnancy ("ever use of antibiotics") was obtained from questionnaires which were administered in the third trimester of pregnancy and when the child was 2 months old. Information on antibiotic exposure during the first year of life including information on indication (bronchitis/pneumonia vs. other) was obtained from the 2-month ("ever use of antibiotics") and 12-month ("use of antibiotics since last visit") questionnaires. Data on allergic symptoms and doctor-diagnosis of allergic diseases was obtained from questionnaires at the age of 12, 18 and 24 months and then yearly up to 6 years of age.

Atopic dermatitis was defined as reported doctor-diagnosis up to 6 years of age and/or diagnosis made during the medical examination at 1 year of age (all these children had a positive SCORAD score (>0)). We also used the point prevalence of atopic dermatitis, defined as a doctor-diagnosis at 12, 18 and 24 months and then yearly up to 6 years.

Food allergy was defined as doctor-diagnosis up to 6 years of age, with point prevalences reported in the questionnaires at 12, 18 and 24 months and then yearly up to 6 years, except at 4 years.

Asthma was defined as doctor-diagnosis or at least 2 doctor-diagnosed episodes of obstructive bronchitis in the last 12 months in the year 4, 5 or 6 questionnaires, independently of diagnosis reported in the first 3 years.

Allergic rhinitis was defined as doctor-diagnosis or presence of symptoms (itchy, runny or blocked nose, without a cold, and associated with red itchy eyes) between year 5 and 6, reported in the 6-year questionnaire.

Atopic sensitization at the age of 1, 4.5 and 6 years was defined as positive specific IgE antibodies (cut-off 0.35 kU/L and 0.7 kU/L) against seasonal allergens (alder, birch, hazel, grass, rye, mugwort, Alternaria), perennial allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, horse, dog) and food allergens (hen's egg, cow's milk, peanut, hazelnut, carrot, plantain, wheat).

Data on potential confounders was obtained from the questionnaires in the third trimester of pregnancy and when the child was 2 and 12 months old. Parental history of allergies was defined as self-reported asthma, allergic rhinitis and/or atopic dermatitis. According to the UNESCO International Standard Classification of Education (ISCED) 2011, we defined a low education level as ISCED levels 0 & 1, a mid level as ISCED level 2 and a high level of education as ISCED levels 3-8.

Presence of infections was recorded in weekly diaries, in particular occurrence of rhinitis (common cold or runny nose), fever (at least 38.5°C), otitis, pneumonia, cough or wheeze for at least 2 of the last 7 days, in a given week between week 8 and 53 of life.[11]

Statistical Analysis

Data analysis was conducted by using IBM SPSS Statistics Version 23 (IBM Corp., Armonk, NY, USA). Differences in patient characteristics between exposed and unexposed were analysed by using Pearson's chi-square test and expressed as p-values. Logistic regressions were used to investigate the association between exposure to antibiotics during pregnancy & infancy and the development of allergic diseases and atopy. From these analyses, odds ratios (OR) with 95% confidence intervals (CI)

were reported. Two models were used to evaluate the association between antibiotic exposure and allergy. Model 1 shows the crude results and model 2 was additionally adjusted for potential confounders. To consider reverse causation with postnatal exposure, we used a third model: From model 2, we additionally excluded children with the respective allergic disease in the first year of life with the exception of asthma where we excluded all cases with wheeze in the first year of life.

For this analysis the statistical significance level was defined by a p-value < 0.05.

Ethical approval

The research protocol was approved by the local research ethics committees of each study centre. Moreover, written informed consent was obtained from the parents.

Results

Patient characteristics

288 (26.7%) of the overall 1080 children, who were included in the analysis for prenatal antibiotics, were exposed in utero (Table I). For postnatal exposure, we included 1019 children of whom 419 children (41.1%) received antibiotics in the first year of life. 136 of these 419 children were also exposed in utero.

The percentage of smoking mothers was almost twice as high in the group who received antibiotics during pregnancy compared to mothers who were not exposed. Furthermore, a significant relationship between maternal education and antibiotic exposure was observed, with women in the higher educated group being exposed more frequently whereas women with a mid level of education received fewer antibiotics. Children who received antibiotics in the first year of life were less likely to be born by caesarean section, more likely to have siblings and tended to be breastfed during a shorter

period of time compared to the unexposed group. No major difference regarding antibiotic use was observed between farmers and non-farmers.

Children exposed to prenatal antibiotics had a significantly higher cumulative prevalence of atopic dermatitis (39.9% vs. 29.8%) and food allergy (13.2% vs. 7.7%) in the first 6 years of life compared to non-exposed. For antibiotic intake in the first year of life, a significantly higher prevalence of atopic dermatitis up to 6 years of life (44.6% vs. 23.9%) was observed.

Prenatal antibiotics and allergic diseases & atopy

Associations between prenatal antibiotic exposure and doctor-diagnosis of atopic dermatitis & food allergy according to time of onset are presented in Table II. The analysis showed that children who were exposed to antibiotics in utero were significantly more likely to develop atopic dermatitis in the first year of life. A positive trend between exposure and atopic dermatitis was found for the later point prevalences up to 5 years. This analysis was further stratified for parental atopy, and no significant difference between the two groups could be seen *(p-value for the interaction term = 0.67).*

The association reached a positive statistical significance for diagnosis of food allergy in the first year and between year 3 and 5.

We categorized the children into four subgroups in order to study the two diseases separately: children who were diagnosed with both food allergy and atopic dermatitis up to 5 years of age, children only suffering from atopic dermatitis, children only having food allergy and children without either allergic disease as reference. The risk of developing both was significantly higher after maternal intake of antibiotics during pregnancy, with a 2.6-fold increase (Table III). We also found a positive trend for the development of only atopic dermatitis. However, no association was seen with only food allergy (small number of cases in this subgroup).

A significant increased risk of becoming sensitized to any allergen at the age of 4 years, especially to seasonal and food allergens, was observed after prenatal exposure (see Supplementary Table E1), with a remaining positive tendency when using a higher cut-off (0.7kU/L). No significant associations were found for sensitization at the age of 1 and 6 years (Supplementary Table E2).

No association was found between exposure to antibiotics in utero and the prevalence of asthma (aOR 1.19, 95% CI 0.69-2.05) between year 3 & 6 or current allergic rhinitis (aOR 1.18, 95% CI 0.64-2.19) at 6 years of age.

Antibiotics in the first year of life and allergic diseases & atopy

Antibiotics in the first year of life increased the risk of atopic dermatitis for the different time points up to the age of 4 years (Table IV). After exclusion of children with atopic dermatitis in the first year of life, we still observed a significant increased risk. Stratification for wheeze in the first year of life showed similar results between the two groups (*p-value for the interaction term = 0.82*). Additionally, adjusting for infections (cough, wheeze, rhinitis, otitis, fever, pneumonia) in the first year of life had no major impact on this association (data not shown). When we looked separately at the type of indication (bronchitis/pneumonia vs. other indication) no major difference between the two groups was detected (see Supplementary Table E3). A strong dose-response relationship was observed for the number of antibiotic courses (prescribed for pneumonia/bronchitis) on the cumulative prevalence of atopic dermatitis up to 4 years (Figure I).

A borderline significant association was found for children with antibiotic intake in the first year of life and asthma between the age of 3 & 6 years (Table IV). This risk was higher for antibiotics prescribed for bronchitis or pneumonia compared to other indications and also for an increasing number of antibiotic courses (p-value for trend = 0.028). The association weakened after additionally adjusting for infections (aOR 1.41, 95% CI 0.74-2.68).

With regards to antibiotic intake in the first year of life, we did not find a significantly increased risk for food allergy (aOR 1.33, 95% CI 0.76-2.33) up to 6 years, allergic rhinitis (aOR 0.58, 95% CI 0.31-1.10) or sensitization (aOR 1.04, 95% CI 0.73-1.47) at the age of 6.

In table V we analysed four antibiotics' exposure categories: children exposed both in utero and in the first year, only exposed in utero or only in the first year and never exposed as reference. The highest risk of atopic dermatitis up to 6 years or asthma between 3 & 6 years was seen with a cumulative exposure to antibiotics in utero as well as in the first year of life.

Discussion

Our results show that exposure to prenatal antibiotics is positively associated with atopic dermatitis and food allergy in childhood with the strongest effect on disease developing in the first year of life. No associations were found between prenatal antibiotic exposure and asthma or allergic rhinitis.

A strong dose-response relationship with the number of antibiotic courses in the first year of life was observed on atopic dermatitis up to 4 years. Children who were exposed to three or more courses during the first year of life have been associated with a 7-fold increased risk to develop atopic dermatitis. A tendency of a positive association between antibiotic intake in the first year of life and asthma between 3 & 6 years was observed, but only with antibiotics prescribed for bronchitis or pneumonia. No significant associations were found between antibiotic exposure in the first year of life and food allergy, sensitization and allergic rhinitis.

Similar to our results, several studies found significant positive associations between atopic dermatitis and antibiotic exposure in utero [12,13] or in the first year of life[14-16]. In contrast, the study by Bisgaard et al.[17], where in contrast to our study only children of mothers with history of asthma were included,

did not see an increased risk for atopic dermatitis after maternal use of antibiotics in pregnancy. Dom et al. [13], who defined atopic dermatitis as parent-reported symptoms, did not find a relationship with antibiotics in the first year of life.

Most studies [5,12,14,16,18-21], which have been carried out on antibiotic use during pregnancy & infancy, found an increased risk for asthma whereas our results only provide evidence for a weak positive association after antibiotic use in the first year of life but not with prenatal exposure. Discrepancies in these findings might be explained by different outcome definitions. We used asthma diagnosis between 3 & 6 years whereas other studies on prenatal antibiotics frequently used asthma diagnosis starting from birth. Moreover, most significant positive results for postnatal antibiotics tended to come from retrospective or database studies, which are susceptible to recall biases and confounding by indication, what we observed in our results.[5]

To our knowledge only one study has so far analysed the association between prenatal antibiotics and food allergy.[22] In contrast to our results, they did not find a relationship between maternal antibiotics and food allergy, which might be explained as they only included a small number of children who were exposed and developed food allergy.

It is well known that atopic dermatitis and food allergy are closely related.[23,24] Here, we showed that prenatal exposure to antibiotics is strongly associated with an increased risk of developing both, atopic dermatitis and food allergy.

Major strengths of our study are the large study population and prospective design, which allow studying the timing of exposures with occurrence before the development of allergic diseases and taking into account major confounding factors.

One limitation of this study is that the study population was selected from rural areas of Europe, so the results might not be representative for urban areas as well as non-European countries. Another limitation is the small case numbers for some of the models (especially for food allergy). Even though

reverse causation is unlikely in the analysis of prenatal antibiotics, it is a major concern when looking at postnatal exposure to antibiotics. We carefully tried to take this into account by using a third model, where we excluded children who already had been diagnosed or had symptoms of the respective disease in the first year of life. Even though it helps to reduce reverse causation, this effect can still not completely be excluded. We observed a strong positive association between exposure to postnatal antibiotics for other indications than bronchitis/pneumonia and atopic dermatitis, which further provided evidence that not the whole effect can be due to reverse causation. The results found for asthma may be explained by confounding by indication as the only real association was found for respiratory tract infections.

Apart from allergic rhinitis, we only included doctor-diagnosed cases in our study. This might result in an underestimation of the prevalence of the respective allergic disease (with the exception of food allergy) but reduces detection bias. Moreover, this misclassification of the outcome is most likely independent of the exposure (non-differential misclassification) and might lead to an underestimation of the OR. Regarding food allergy, the prevalence might be overestimated, as an oral food challenge should be carried out to make an appropriate diagnosis. A dilution effect would be induced by an overestimation of the prevalence and would shift the OR towards 1 (no effect). Furthermore, we do not expect misclassification of the exposures here.

Maternal infections during pregnancy like chorioamnionitis have been associated with an increased risk of childhood asthma.[12,25,26] It was suggested that the association between prenatal exposure to antibiotics and asthma is caused by other mechanisms like a heritable predisposition to infections rather than representing a causal link.[20] Our results support this statement as adjusting for infections in the first year of life did weaken the association between postnatal exposure and asthma. However, the positive association between antibiotic exposure in the first year of life and atopic dermatitis remained significant independent of infections in the first year.

The mechanism underlying the suggested increased risk for allergies due to antibiotic exposures remains unclear. One potential mechanism might be the influence of antibiotic treatment on microbiota, as several studies have found an association between gut microbiota composition and allergic disease.[27,28,29] Furthermore, Yassour et al.[30] found that antibiotic treatment in early life reduces microbial diversity at 3 years.

In summary, our results show positive associations between exposure to antibiotics and allergic diseases, mainly with atopic dermatitis and food allergy in the first year of life after prenatal exposure, and atopic dermatitis and asthma after postnatal exposure to antibiotics in children born in rural settings. Keeping this and the increasing microbial resistance in mind, appropriate prescription of antibiotics is all the more important.

Acknowledgements:

We thank all the fieldworkers and other PASTURE/EFRAIM team members.

1. Pawankar R, Canonica GW, Holgate ST, Lockey RF, Blaiss MS. WAO White Book on Allergy 2013 Update, Executive Summary. In. Milwaukee, Wisconsin: World Allergy Organization; 2013.
2. Dharmage SC, Lodge CJ, Lowe AJ, Allen KJ. Antibiotics and risk of asthma: a debate that is set to continue. *Clin Exp Allergy.* 2015;45(1):6-8.
3. Kuo CH, Kuo HF, Huang CH, Yang SN, Lee MS, Hung CH. Early life exposure to antibiotics and the risk of childhood allergic diseases: an update from the perspective of the hygiene hypothesis. *J Microbiol Immunol Infect.* 2013;46(5):320-329.
4. Russell SL, Gold MJ, Willing BP, Thorson L, McNagny KM, Finlay BB. Perinatal antibiotic treatment affects murine microbiota, immune responses and allergic asthma. *Gut Microbes.* 2013;4(2):158-164.
5. Murk W, Risnes KR, Bracken MB. Prenatal or early-life exposure to antibiotics and risk of childhood asthma: a systematic review. *Pediatrics.* 2011;127(6):1125-1138.

6. Kummeling I, Thijs C. Reverse causation and confounding-by-indication: do they or do they not explain the association between childhood antibiotic treatment and subsequent development of respiratory illness? *Clin Exp Allergy.* 2008;38(8):1249-1251.

7. von Mutius E, Schmid S, Group PS. The PASTURE project: EU support for the improvement of knowledge about risk factors and preventive factors for atopy in Europe. *Allergy.* 2006;61(4):407-413.

8. Alfvén T, Braun-Fahrländer C, Brunekreef B, et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle--the PARSIFAL study. *Allergy.* 2006;61(4):414-421.

9. Riedler J, Braun-Fahrländer C, Eder W, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet.* 2001;358(9288):1129-1133.

10. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J.* 1995;8(3):483-491.

11. Loss G, Depner M, Ulfman LH, et al. Consumption of unprocessed cow's milk protects infants from common respiratory infections. *J Allergy Clin Immunol.* 2015;135(1):56-62.

12. McKeever TM, Lewis SA, Smith C, Hubbard R. The importance of prenatal exposures on the development of allergic disease: a birth cohort study using the West Midlands General Practice Database. *Am J Respir Crit Care Med.* 2002;166(6):827-832.

13. Dom S, Droste JH, Sariachvili MA, et al. Pre- and post-natal exposure to antibiotics and the development of eczema, recurrent wheezing and atopic sensitization in children up to the age of 4 years. *Clin Exp Allergy.* 2010;40(9):1378-1387.

14. Mitre E, Susi A, Kropp LE, Schwartz DJ, Gorman GH, Nylund CM. Association Between Use of Acid-Suppressive Medications and Antibiotics During Infancy and Allergic Diseases in Early Childhood. *JAMA Pediatr.* 2018:e180315.

15. Tsakok T, McKeever TM, Yeo L, Flohr C. Does early life exposure to antibiotics increase the risk of eczema? A systematic review. *Br J Dermatol.* 2013;169(5):983-991.

16. McKeever TM, Lewis SA, Smith C, et al. Early exposure to infections and antibiotics and the incidence of allergic disease: a birth cohort study with the West Midlands General Practice Research Database. *J Allergy Clin Immunol.* 2002;109(1):43-50.

17. Bisgaard H, Halkjaer LB, Hinge R, et al. Risk analysis of early childhood eczema. *J Allergy Clin Immunol.* 2009;123(6):1355-1360.e1355.

18. Örtqvist AK, Lundholm C, Kieler H, et al. Antibiotics in fetal and early life and subsequent childhood asthma: nationwide population based study with sibling analysis. *BMJ.* 2014;349:g6979.

19. Marra F, Lynd L, Coombes M, et al. Does antibiotic exposure during infancy lead to development of asthma?: a systematic review and metaanalysis. *Chest.* 2006;129(3):610-618.

20. Stokholm J, Sevelsted A, Bønnelykke K, Bisgaard H. Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. *Lancet Respir Med.* 2014;2(8):631-637.

21. Zhao D, Su H, Cheng J, et al. Prenatal antibiotic use and risk of childhood wheeze/asthma: A meta-analysis. *Pediatr Allergy Immunol.* 2015;26(8):756-764.

22. Eggesbø M, Botten G, Stigum H, Nafstad P, Magnus P. Is delivery by cesarean section a risk factor for food allergy? *J Allergy Clin Immunol.* 2003;112(2):420-426.

23. Roduit C, Frei R, Depner M, et al. Phenotypes of Atopic Dermatitis Depending on the Timing of Onset and Progression in Childhood. *JAMA Pediatr.* 2017;171(7):655-662.

24. Eigenmann PA, Sicherer SH, Borkowski TA, Cohen BA, Sampson HA. Prevalence of IgE-mediated food allergy among children with atopic dermatitis. *Pediatrics.* 1998;101(3):E8.

25. Xu B, Pekkanen J, Järvelin MR, Olsen P, Hartikainen AL. Maternal infections in pregnancy and the development of asthma among offspring. *Int J Epidemiol.* 1999;28(4):723-727.

26. Kumar R, Yu Y, Story RE, et al. Prematurity, chorioamnionitis, and the development of recurrent wheezing: a prospective birth cohort study. *J Allergy Clin Immunol.* 2008;121(4):878-884.e876.

27. Penders J, Stobberingh EE, van den Brandt PA, Thijs C. The role of the intestinal microbiota in the development of atopic disorders. *Allergy.* 2007;62(11):1223-1236.

28. Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol.* 2012;129(2):434-440, 440.e431-432.

29. Bisgaard H, Li N, Bonnelykke K, et al. Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *J Allergy Clin Immunol.* 2011;128(3):646-652.e641-645.

30. Yassour M, Vatanen T, Siljander H, et al. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. *Sci Transl Med.* 2016;8(343):343ra381.

Tables

Table I. Patient characteristics and prevalence of atopy and allergic diseases in relation to antibiotic exposure in utero or first year of life

	Overall study population		Antibiotics during pregnancy					Antibiotics in 1st year of life				
			No (n=792)		Yes (n=288)			No (n=600)		Yes (n=419)		
	N	%	N	%	N	%	p-value*	N	%	N	%	p-value*
Farmer							0.949					0.879
Yes	530	46.8	373	47.1	135	46.9		285	47.5	197	47.0	
No	603	53.2	419	52.9	153	53.1		315	52.5	222	53.0	
Sex							0.411					0.036
Female	530	48.6	389	49.1	130	46.3		310	51.7	188	45.0	
Male	560	51.4	403	50.9	151	53.7		290	48.3	230	55.0	
Parental history of atopic disease							0.074					0.735
Yes	595	54.1	405	51.7	165	57.9		327	54.7	223	53.6	
No	504	45.9	378	48.3	120	42.1		271	54.3	193	46.4	
Siblings							0.280					0.006
0	413	36.5	298	37.6	94	32.6		239	39.8	128	30.5	
1-2	604	53.3	411	51.9	163	56.6		300	50.0	245	58.5	
3-4	98	8.6	72	9.1	24	8.3		48	8.0	42	10.0	
5+	18	1.6	11	1.4	7	2.4		13	2.2	4	1.0	
Maternal education							0.022					<0.001
Low	202	17.8	142	17.9	47	16.3		101	16.8	69	16.5	
Mid	487	43.0	357	45.1	108	37.5		289	48.2	152	36.3	
High	444	39.2	293	37.0	133	46.2		210	35.0	198	47.3	
Caesarean section							0.422					0.024
Yes	192	17.7	136	17.3	54	19.4		119	20.0	60	14.5	
No	890	82.3	651	82.7	224	80.6		477	80.0	355	85.5	
Pets (cats & dogs) in the 1st year of life							0.983					0.149
Yes	598	57.2	436	57.1	152	57.1		356	59.3	229	54.8	
No	447	42.8	328	42.9	114	42.9		244	40.7	189	45.2	
Pets (cats & dogs) during pregnancy							0.579					0.311
Yes	663	58.6	462	58.3	162	56.4		355	59.2	234	56.0	
No	469	41.4	330	41.7	125	43.6		245	40.8	184	44.0	
Breastfeeding							0.493					<0.001
Never	100	9.6	71	9.3	29	10.9		42	7.0	57	13.6	
>0 - 2 months	170	16.3	122	16.0	48	18.0		76	12.7	87	20.8	
3 - 6 months	289	27.7	202	26.5	78	29.3		158	26.4	122	29.2	
7 - 9 months	221	21.2	169	22.1	51	19.2		139	23.2	76	18.2	
10+ months	264	25.3	199	26.1	60	22.6		184	30.7	76	18.2	
Maternal smoking during pregnancy							<0.001					0.167
Yes	158	13.9	89	11.2	61	21.2		71	11.8	62	14.8	
No	975	86.1	703	88.8	227	78.8		529	88.2	357	85.2	
Centre							<0.001					<0.001
Austria	220	19.4	174	22.0	40	13.9		122	20.3	76	18.1	
Switzerland	242	21.4	189	23.9	43	14.9		173	28.8	46	11.0	
France	203	17.9	123	15.5	71	24.7		45	7.5	130	31.0	
Germany	254	22.4	198	25.0	47	16.3		172	28.7	53	12.6	
Finland	214	18.9	108	13.6	87	30.2		88	14.7	114	27.2	
Prevalence allergic diseases												
Atopic dermatitis up to 6 years							0.005					<0.001
Yes	288	32.7	192	29.8	89	39.9		121	23.9	154	44.6	

No	593	67.3	452	70.2	134	60.1		385	76.1	191	55.4	
Food allergy up to 6 years							0.016					0.081
Yes	80	9.2	49	7.7	29	13.2		38	7.5	37	11.0	
No	788	90.8	585	92.3	191	86.8		468	92.5	299	89.0	
Asthma between 3 & 6 years							0.417					0.106
Yes	79	8.9	55	8.5	23	10.3		38	7.4	35	10.6	
No	804	91.1	591	91.5	200	89.7		477	92.6	296	89.4	
Any sensitization at 1 year (cut-off: 0.35 kU/L)							0.589					0.199
Yes	264	28.1	187	27.7	73	29.6		137	26.3	115	30.2	
No	675	71.9	487	72.3	174	70.4		384	73.7	266	69.8	
Any sensitization at 6 years (cut-off: 0.35 kU/L)							0.724					0.777
Yes	401	53.8	283	53.5	111	55.0		221	53.5	167	54.6	
No	344	46.2	246	46.5	91	45.0		192	46.5	139	45.4	
Allergic rhinitis at 6 years							0.392					0.453
Yes	60	6.4	40	5.9	18	7.5		38	7.1	21	5.8	
No	873	93.6	637	94.1	223	92.5		497	92.9	339	94.2	

*Based on the chi-square test

Table II. Association between prenatal exposure to antibiotics and doctor-diagnosis of atopic dermatitis & food allergy at different time points up to 6 years of age

	Atopic Dermatitis					Food Allergy				
	Model 1: crude			Model 2: adjusted[†]		Model 1: crude			Model 2: adjusted[†]	
	n/N	OR	95% CI	aOR	95% CI	n/N	OR	95% CI	aOR	95% CI
Diagnosis in the first year of life	148					24				
Prenatal exposure to antibiotics: yes	52/260	1.72	1.19-2.50	1.66	1.11-2.48	13/265	3.53	1.56-7.98	3.01	1.22-7.47
Prenatal exposure to antibiotics: no, reference	96/757	1.00		1.00		11/764	1.00		1.00	
Diagnosis between 1&1.5 years	79					32				
Prenatal exposure to antibiotics: yes	24/243	1.35	0.82-2.23	1.26	0.73-2.18	10/246	1.38	0.64-2.95	na[§]	na[§]
Prenatal exposure to antibiotics: no, reference	55/732	1.00		1.00		22/738	1.00		1.00	
Diagnosis between 1.5&2 years	74					24				
Prenatal exposure to antibiotics: yes	23/250	1.37	0.82-2.29	1.26	0.73-2.19	9/252	1.80	0.78-4.17	2.19	0.89-5.40
Prenatal exposure to antibiotics: no, reference	51/740	1.00		1.00		15/744	1.00		1.00	
Diagnosis between 2&3years	71					21				
Prenatal exposure to antibiotics: yes	27/248	1.87	1.13-3.09	1.54	0.90-2.63	9/248	2.22	0.93-5.34	2.28	0.89-5.83
Prenatal exposure to antibiotics: no, reference	44/718	1.00		1.00		12/720	1.00		1.00	
Diagnosis between 3&4[‡] years	61					15				
Prenatal exposure to antibiotics: yes	21/248	1.59	0.92-2.76	1.43	0.80-2.56	8/244	3.39	1.21-9.44	3.45	1.13-10.49
Prenatal exposure to antibiotics: no, reference	40/729	1.00		1.00		7/706	1.00		1.00	
Diagnosis between 4&5 years	45									
Prenatal exposure to antibiotics: yes	17/245	1.81	0.97-3.36	1.42	0.74-2.75					
Prenatal exposure to antibiotics: no, reference	28/706	1.00		1.00						
Diagnosis between 5&6 years	19					8				
Prenatal exposure to antibiotics: yes	4/213	0.76	0.25-2.32	0.64	0.20-2.03	3/194	1.84	0.44-7.76	1.53	0.33-7.20
Prenatal exposure to antibiotics: no, reference	15/611	1.00		1.00		5/590	1.00		1.00	
Diagnosis up to 6 years	281					78				
Prenatal exposure to antibiotics: yes	89/223	1.56	1.14-2.15	1.19	0.69-2.05	29/220	1.81	1.11-2.95	1.80	1.05-3.07
Prenatal exposure to antibiotics: no, reference	192/644	1.00		1.00		49/634	1.00		1.00	

[†] adjusted for farmer, centre, parental atopic status, gender, smoking during pregnancy, number of siblings, pets (dogs & cats) during pregnancy, caesarean section, maternal education
[‡] diagnosis between 3&5 years for food allergy
[§] zero count for one of the centres

Table III. Association between prenatal exposure to antibiotics and doctor-diagnosis of atopic dermatitis and/or food allergy up to the age of 5 years

	Sensitization at 4 years (Cut-off: 0.7kU/L)			Prenatal antibiotics (Cases=213)				
	Any allergen	Food allergens	Inhalant allergens	Model 1: crude			Model 2: adjusted[†]	
	%	%	%	n/N	OR	95% CI	aOR	95% CI
Atopic dermatitis and food allergy up to 5 years	58.8	41.2	47.1	18/40	2.90	1.51-5.56	2.63	1.29-5.39
Only atopic dermatitis up to 5 years	43.1	20.8	34.0	57/187	1.55	1.08-2.24	1.41	0.95-2.09
Only food allergy up to 5 years	56.3	37.5	31.3	5/22	1.04	0.38-2.88	1.05	0.36-3.08
No atopic dermatitis & no food allergy up to 5 years, reference	45.1	27.5	33.0	133/604	1.00		1.00	

[†]adjusted for farmer, centre, parental atopic status, gender, smoking during pregnancy, number of siblings, pets (dogs & cats) during pregnancy, caesarean section, maternal education

Table IV. Association between exposure to antibiotics in the first year of life and doctor-diagnosis of atopic dermatitis at different time points & asthma.

	Model 1: crude			Model 2: adjusted†		Model 3: adjusted† and excluded‡	
	n/N	OR	95% CI	aOR	95% CI	aOR	95% CI
Atopic dermatitis between 1&1.5 years	76						
Exposure to antibiotics (1st year): yes	41/384	1.82	1.13-2.91	1.66	0.96-2.88	2.71	1.14-6.48
Exposure to antibiotics (1st year): no, reference	35/567	1.00		1.00		1.00	
Atopic dermatitis between 1.5&2 years	70						
Exposure to antibiotics (1st year): yes	39/394	1.91	1.17-3.13	1.99	1.14-3.50	2.30	1.06-5.00
Exposure to antibiotics (1st year): no, reference	31/571	1.00		1.00		1.00	
Atopic dermatitis between 2&3years	66						
Exposure to antibiotics (1st year): yes	39/386	2.19	1.32-3.65	1.94	1.07-3.50	2.58	1.13-5.91
Exposure to antibiotics (1st year): no, reference	27/554	1.00		1.00		1.00	
Atopic dermatitis between 3&4 years	57						
Exposure to antibiotics (1st year): yes	39/387	3.40	1.91-6.04	2.68	1.40-5.12	4.11	1.69-10.03
Exposure to antibiotics (1st year): no, reference	18/564	1.00		1.00		1.00	
Atopic dermatitis between 4&5 years	40						
Exposure to antibiotics (1st year): yes	24/373	2.31	1.21-4.42	1.61	0.77-3.33	1.85	0.71-4.82
Exposure to antibiotics (1st year): no, reference	16/554	1.00		1.00		1.00	
Atopic dermatitis between 5&6 years	17						
Exposure to antibiotics (1st year): yes	8/309	1.44	0.55-3.77	0.95	0.31-2.88	1.28	0.40-4.03
Exposure to antibiotics (1st year): no, reference	9/496	1.00		1.00		1.00	
Atopic dermatitis up to 6 years	275						
Exposure to antibiotics (1st year): yes	154/345	2.57	1.91-3.44	1.91	1.36-2.67	2.65	1.69-4.16
Exposure to antibiotics (1st year): no, reference	121/506	1.00		1.00		1.00	
Asthma between 3&6 years of age	73						
Exposure to antibiotics (1st year): yes	35/331	1.48	0.92-2.40	1.65	0.95-2.86	1.33	0.63-2.83
Antibiotics because of other indication than bronchitis/pneumonia	18/220	*0.96*	*0.54-1.69*	*1.03*	*0.55-1.91*	*0.96*	*0.43-2.13*
Antibiotics because of bronchitis/pneumonia	12/89	*1.68*	*0.85-3.30*	*1.85*	*0.86-3.99*	*1.32*	*0.34-5.15*
Exposure to antibiotics (1st year): no, reference	38/515	1.00		1.00		1.00	

†adjusted for farmer, centre, parental atopic status, gender, smoking during pregnancy, number of siblings, pets (dogs & cats) during pregnancy or first year of life, months of breastfeeding, caesarean section

‡ Exclusion of children with atopic dermatitis in the 1st year of life for atopic dermatitis and exclusion of children with wheeze in the 1st year of life for asthma

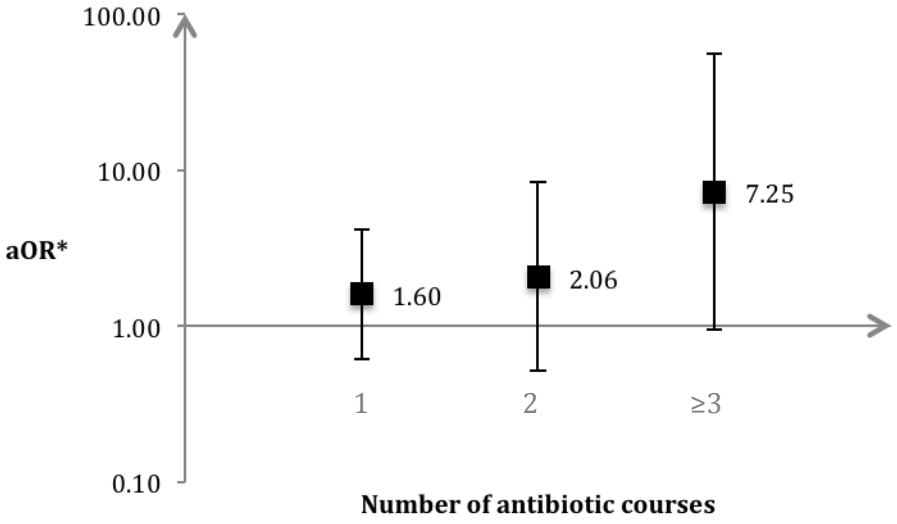
Table V. Association between exposure to antibiotics during pregnancy & first year of life and allergic diseases & atopy

	Atopic dermatitis up to 6 years			**Asthma between 3&6 years**			**Food allergy up to 6 years**			**Allergic rhinitis at 6 years**			**Any sensitization at 6 years (cut-off: 0.35 kU/L)**		
		Model 2: adjusted[†]			Model 2: adjusted[†]			Model 2: adjusted[†]			Model 2: adjusted[†]			Model 2: adjusted[†]	
Antibiotic exposure	n/N	aOR	95% CI	n/N	aOR	95% CI	n/N	aOR	95% CI	n/N	aOR	95% CI	n/N	aOR	95% CI
Yes															
Pregnancy & 1st year	53/111	2.49	1.44-4.30	12/109	2.32	0.96-5.60	14/110	1.42	0.59-3.37	6/121	0.47	0.16-1.40	62/105	1.01	0.58-1.77
Only pregnancy	29/102	1.23	0.73-2.09	9/102	1.36	0.59-3.11	13/101	2.39	1.08-5.26	11/108	1.55	0.69-3.46	45/88	0.97	0.58-1.61
Only 1st year	97/228	1.92	1.29-2.86	23/217	1.68	0.87-3.21	22/220	1.47	0.73-2.98	14/233	0.70	0.33-1.51	102/195	1.06	0.70-1.60
No (reference)	90/398	1.00		28/407	1.00		25/399	1.00		26/421	1.00		173/320	1.00	

[†]adjusted for farmer, centre, parental atopic status, gender, smoking during pregnancy, number of siblings, pets (dogs & cats) during pregnancy, caesarean section, maternal education

Figure legend

Figure I. Association between atopic dermatitis up to 4 years and number of antibiotic courses (prescribed for pneumonia/bronchitis) in the first year of life



* adjusted for farmer, centre, parental atopic status, gender, smoking during pregnancy, number of siblings, pets (dogs & cats) during pregnancy or first year of life, months of breastfeeding, caesarean section after exclusion of children with atopic dermatitis in the first year of life