

MS SIGNE TIMM (Orcid ID : 0000-0002-5862-7784)

Received Date : 11-Aug-2016

Revised Date : 10-Feb-2017

Accepted Date : 01-Mar-2017

Article type : Original Article-Epidemiology of Allergic Disease

Title: Prenatal antibiotics and atopic dermatitis among 18-month old children in the Danish National Birth Cohort

Short title: Prenatal antibiotics and atopic dermatitis in the Danish National Birth Cohort

Authors: Signe Timm¹, Vivi Schlünssen^{1,2}, Jørn Olsen³, Cecilia Høst Ramlau-Hansen¹

Affiliations:

1: Department of Public Health, Aarhus University, Denmark

2: National Research Center for Working Environment, Copenhagen, Denmark □

3: Department of Clinical Epidemiology, Aarhus University, Denmark

Corresponding author:

Cecilia Høst Ramlau-Hansen

Department of Public Health, Aarhus University

Bartholins Allé 2, Building 1260

8000 Aarhus C

Denmark

Telephone: +45 87168036 / +45 26295715

E-mail: chrh@ph.au.dk

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cea.12916

This article is protected by copyright. All rights reserved.

Abstract

Background: Despite extensive research, the aetiology of atopic dermatitis remains largely unknown, but reduced intestinal microbiota diversity in neonates has been linked to subsequent atopic dermatitis. Consequently, postnatal antibiotics have been proposed as a risk factor, but a potential association between prenatal antibiotics and atopic dermatitis is not well studied. Overall, the current evidence suggests a positive association between exposure to prenatal antibiotics and atopic dermatitis.

Objective: To investigate the association between prenatal antibiotics and atopic dermatitis among 18-month old children.

Methods: This study conducted within the Danish National Birth Cohort included 62,560 mother-child pairs. Data on maternal prenatal antibiotics were collected in the 30th gestation week and 6 months post partum, and offspring atopic dermatitis 18 months post partum through telephone interviews. Antibiotic use was categorised by the timing of exposure as 1st-2nd trimester (gestation week 0-29), 3rd trimester (gestation week 30-birth), all three trimesters or none. Data were analysed by logistic regression analyses adjusting for potential confounders.

Results: Exposure to antibiotics prenatally was associated with increased odds of atopic dermatitis among children born by atopic mothers but only when used in both 1st-2nd and 3rd trimester (OR_{adj} 1.45, 95 % CI 1.19-1.76). The findings were consistent using different definitions of atopic dermatitis.

Conclusions and Clinical Relevance: Prenatal exposure to antibiotics throughout pregnancy was associated with an increased risk of atopic dermatitis but only within the first 18 months of life among children born by atopic mothers. The clinical usefulness of this finding must rest on corroboration in independent data sources.'

Introduction

In 1989, Strachan et al. observed that the prevalence of atopic dermatitis was negatively associated with the number of older siblings in the family [1]. This inspired to the "Hygiene Hypothesis" which proposes impaired immunological competence following low microbial stimulation in early childhood. The human microbiome is established in utero and during the first 2-3 years of life and several environmental factors including conditions during the prenatal period, mode of delivery, antibiotic use, breastfeeding and household environment play a key role in this process [2, 3]. The development of a healthy and diverse microbiome is crucial for immune maturation, and reduced intestinal microbiota diversity during the first months of life has been linked to subsequent allergic diseases including atopic dermatitis [2, 4, 5].

The prevalence of atopic dermatitis has increased rapidly during the last 30-40 years and now affects up to 15-20 % of children in affluent countries [6, 7]. Atopic dermatitis is often referred to as the first manifestation of the "atopic march" followed by food allergy, asthma and allergic rhinitis occurring throughout childhood and adolescence [8]. Despite an extensive amount of research, the aetiology of atopic dermatitis still remains largely unsolved and the opportunities for primary prevention are therefore limited. One genetic mutation, filaggrin mutation, has been identified as a strong risk factor for atopic dermatitis, as this mutation is strongly related to loss of skin barrier function [9, 10]. Filaggrin mutation is generally present among approximately 50 % of patients with atopic dermatitis compared to 10 % in the general population, and cases with filaggrin mutations tend to have an earlier onset [9].

In parallel with the increase in atopic dermatitis, the use of antibiotics has increased markedly and is now at the highest level since the Statens Serum Institut in Denmark started to monitor the use in 1995 [11]. Maternal use of antibiotics in pregnancy influences the maternal

microbiome and hence the very early colonization of the child's own microbiome during pregnancy, birth and breast-feeding in early life [2]. Perinatal and postnatal exposure to antibiotics has almost consistently been associated with an increased risk of atopic dermatitis in both human and murine models [12–17]. However, evidence for a role of prenatal antibiotic exposure is scarce, inconsistent and does not take timing of exposure into account [10, 18–21]. Four studies found an increased risk of atopic dermatitis among children exposed to prenatal antibiotics, and one study found no association [18]. Furthermore, one study found an indication of an additive effect from prenatal antibiotic exposure and caesarean delivery on atopic dermatitis at 1 year of age [22].

The aim of this study was therefore to investigate the association between prenatal antibiotic exposure and atopic dermatitis among children at 18 months, taking the timing of exposure in pregnancy, mode of delivery and maternal atopy into account.

Materials and methods

Study population: The Danish National Birth Cohort

The Danish National Birth Cohort (DNBC) is a population-based cohort study aiming to investigate pre- and postnatal origins of a various number of health outcomes appearing during pregnancy, childhood and adulthood [23]. The source population for DNBC was all Danish-speaking women with a clinically confirmed pregnancy, who lived in Denmark and intended to carry their pregnancy to term. Recruitment took place in 1996-2002 and involved the whole country. All women received an invitation to participate from their general practitioners, as their general practitioner would usually be the women's first contact with the antenatal care system in Denmark. However, a large portion of general practitioners did not participate in the recruitment, and hence around 50 % of the eligible women were never invited to the study [23, 24]. The number of participants among the invited women remains

unknown, as general practitioners did not report their participation rate, however, it has been estimated that a maximum of 60 % of the invited women accepted the invitation, corresponding to approximately 30 % of the eligible population. The total number of women included in the DNBC was 101,042.

All mothers gave their written consent before they were included in the cohort, and children participated on their mother's consent.

Variables and data measurement

Data was obtained from four assisted telephone interviews with the mother and from the Danish Birth Registry. The telephone interviews took place twice during pregnancy (Int-1 around 12th and Int-2 around 30th gestation week) and twice after birth (Int-3 when the child was 6 months and Int-4 when the child was 18 months old). Data sources and measurements for all covariates are shown in Table 1.

Information on prenatal antibiotic exposure was derived from the second and third interview, where mothers reported their past antibiotic use in gestation weeks 0-29 (defined as 1st-2nd trimester) and gestation week 30 to birth (defined as 3rd trimester), respectively. Exposure categories were defined as taking antibiotics in 1st-2nd trimester, in 3rd trimester, in both periods (1st-2nd + 3rd trimester) or in none of the periods. Outcome was defined as an affirmative answer to either an itchy rash defined by the mother as atopic dermatitis and/or ever atopic dermatitis at the fourth interview when the child was 18 months. An affirmative answer to one of the two was sufficient to make the child categorised as having atopic dermatitis. Furthermore, the mothers reported if a doctor diagnosed the presence of atopic dermatitis. Maternal atopy was defined as an affirmative answer to either asthma or allergy (including hay fever, food allergy and eczema) or both. Mothers reported current education and work situation on behalf of themselves and the child's father.

Statistical methods

Data was analysed in logistic regression models and presented as odds ratios (OR) with corresponding 95 % confidence intervals (95 % CI). The analyses were performed as complete case analyses. As mothers were allowed to participate with more than one pregnancy, robust standard errors (SE) were estimated as SE taking clusters into account. To ensure the validity of the statistical model, the assumptions were tested. Since maternal atopy may be considered as a potential effect measure modifier, all analyses were stratified by maternal atopic status. Analyses were adjusted for prenatal smoking, household socioeconomic status and older siblings. Covariates were selected on the basis of a directed acyclic graph (DAG) depicting the potential causal network of associations within this particular research field based on current evidence and assumptions (supplementary material, Figure S1). Inclusion of prenatal smoking and socioeconomic status in the DAG were based on published evidence, and older siblings were based partly on evidence (the association with outcome) and partly on assumptions (the association with exposure) [1, 25–28]. The reference was set at no maternal smoking during pregnancy, no older siblings and highest household socioeconomic status (higher grade professionals). Additional analyses included test for interaction using Wald tests and stratification by mode of delivery. Sensitivity analyses were performed on (maternal-reported) doctor-diagnosed atopic dermatitis and using the The International Study of Asthma and Allergies in Childhood (ISSAC) definition of atopic dermatitis. The latter defines atopic dermatitis as having an itchy rash affecting one or more of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes [7]. Furthermore, sensitivity analyses were performed on different classes of antibiotics (penicillins, macrolides, antibiotics against urine tract infections and other types of antibiotics including tetracyclines, beta-laktam

antibiotics, quinolones) and when excluding mothers receiving antibiotics that potentially could be related to their own atopic disease.

Statistics were calculated using STATA 13 (STATA Corp, College Station, Texas, USA).

Results

Basic characteristics of the study population (N = 62,560) are shown in Table 2 and in Figure

1. The study population included 10,253 children with atopic dermatitis corresponding to a prevalence of 16 % at 18 months. Mothers exposed and not exposed to prenatal antibiotics were comparable with regard to age at baseline, household socioeconomic status, number of older siblings and mode of delivery. Prenatal smoking and maternal atopy was more prevalent among the exposed, especially for the group exposed to antibiotics in both 1st-2nd trimester and 3rd trimester (Table 2).

As seen in Table 3, antibiotics in both the 1st-2nd and 3rd trimester showed higher odds of offspring atopic dermatitis among atopic mothers (OR_{adj} 1.45, 95 % CI 1.19-1.76), but not among non-atopic mothers (OR_{adj} 1.01, 95 % CI 0.83-1.22). Prenatal antibiotics in the 1st-2nd or 3rd trimester alone was not associated with higher odds of offspring atopic dermatitis before 18 months of age, Table 3. . Mode of delivery tended to modify the association for atopic mothers exposed to antibiotics in both the 1st-2nd and 3rd trimester, as the observed association was stronger for caesarean born children (OR_{adj} 2.13, 95 % 1.34-3.38) compared to vaginally born children (OR_{adj} 1.34, 95 % 1.08-1.66), Table 4. Any use of prenatal antibiotics, independent of timing, showed increased odds of atopic dermatitis only among children born by atopic mothers (OR_{adj} 1.09, 95 % CI 1.00-1.18) (data not shown). Sensitivity analyses on doctor-diagnosed atopic dermatitis and atopic dermatitis using the ISAAC definition revealed similar results (data not shown). Sensitivity analyses excluding mothers

receiving antibiotics potentially related to their atopic disease (6,815 of 12,688 exposed mothers) essentially did not change the results except for attenuating the estimate for mothers exposed the whole pregnancy (OR_{adj} 1.10, 95 % CI 0.74-1.65). Sensitivity analyses on classes of antibiotics (penicillins, macrolides, antibiotics against urine tract infections and other types of antibiotics including tetracyclines, beta-laktam antibiotics, quinolones) did not suggest that the potential effect between prenatal antibiotics and atopic dermatitis are different between different classes of antibiotics (data not shown).

Discussion

Main findings

Among children born by atopic mothers, use of prenatal antibiotics was associated with increased odds of atopic dermatitis, if antibiotics were used in all three trimesters. The association was not present among children born by mothers with no atopy, or if antibiotics had been used in only 1st-2nd or 3rd trimester. These findings were consistent across different definitions of atopic dermatitis. The association tended to be stronger among children born by caesarean section by atopic mothers exposed to antibiotics in both the 1st-2nd and 3rd trimester compared to those born by a vaginal birth.

Strengths and limitations

The follow-up design is the most important strength of this study. To our knowledge, this is also the largest study conducted to date. Furthermore, this is the first study to take into account timing of exposure in pregnancy and modification by maternal atopy.

Almost all variables are reported by the mother and the study is therefore limited by potential risk of recall bias. With regards to outcome, 81 % of the cases had eczema in one of the locations typical for atopic dermatitis using the definition from the ISAAC study (the folds of

the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes), which indicates that the mothers reports are valid [7]. However, the mothers also reported the locations of the lesions. The prevalence of atopic dermatitis in our study is comparable or slightly higher than other studies among 1-2 year old children in Northern and Central Europe [8, 29, 30]. This slightly higher prevalence may be due to a true higher prevalence in our study population, or due to over-reporting or earlier onset of atopic dermatitis. We believe that the mother's reports reflect their actual antibiotic use, which may not be completely overlapping with the prescribed antibiotics as recorded in The Danish National Prescription Registry, where non-compliance poses an uncertainty [31]. Usually, the recorded timing of exposure may be subject to recall bias, but since the mothers were asked twice to recall earlier antibiotic use (once during pregnancy and once 6 months post partum), we believe that they were able to report the timing quite accurately. Unfortunately, we were not able to take filaggrin mutations among the children into account. Filaggrin is strongly related to the skin barrier function and a strong risk factor for atopic dermatitis as well as asthma and allergies [9, 10]. Filaggrin mutation is generally present among approximately 50 % of cases with atopic dermatitis compared to 10 % in the general population, and cases with filaggrin mutations tend to have an earlier onset [27]. As maternal atopy is not equally distributed across exposure groups, there is a potential risk that the prevalence of filaggrin mutations is not equally distributed either, and hence may bias our results. Stratifying the analyses by maternal atopy may, however, to some extent separate out the children predisposed for filaggrin mutations and minimize the potential bias. The sensitivity analyses excluding mothers receiving antibiotics potentially related to their own atopy attenuated the result for mothers exposed the whole pregnancy. This could support that the mentioned bias is present or may merely be a result of random error caused by exclusion of half of the exposed mothers. The sensitivity analyses on classes of antibiotics did, however, not show

any evidence that the association between prenatal antibiotics and atopic dermatitis are different between different classes of antibiotics.

Only 58 % of the mothers in the original cohort had complete data and were included in this study. As antibiotic use is more prevalent among lower socioeconomic groups, the drop-out may be related to exposure [27]. Participant drop-out occurring before birth may be most likely not related to atopic dermatitis in the child, but we cannot rule out the occurrence of participant drop-out following birth being related to the outcome. However, atopic dermatitis is not a severe disease and we believe that bias from this scenario is small.

Multiple testing produces “significant p-values” which should be interpreted with caution. In our main analysis (Table 3), we tested hypotheses stated in the protocol, and we tested the double number when stratifying on type of birth (Table 4). However, since we followed an a priori planned analytic strategy and found results that are rather consistent in both crude, adjusted and stratified analyses, we assess the results to be robust and not due to multiple testing errors. The traditional Bonferroni adjustment will produce severe bias towards the null hypothesis. Still, p-values in a study like this provide no proof of causal associations and repetition in an independent data source should be done. Change in use of antibiotics is not at present justified based on observational findings of this type.

Comparison with other studies

The findings from this study are overall in line with the current evidence. McKeever et al. conducted a cross-sectional study of 24,690 children in the United Kingdom and found that prenatal antibiotics were associated with atopic dermatitis in a dose-response related manner [32]. In line with our findings, the study showed the effect from prenatal antibiotics to be strongest after exposure to >2 prescriptions [32]. Sariachvili et al. investigated 1,128 1-year-

old infants in a prospective birth cohort in Belgium and found a significant increased risk of atopic dermatitis after prenatal exposure to antibiotics [33]. Dom et al. studied the same cohort at 4 years of age and confirmed the results. The association persisted after adjustment for postnatal antibiotics [19]. In a pilot study in Krakow, Poland, Jedrychowski et al. studied 102 1-year old infants and found an increased risk of atopic dermatitis after prenatal antibiotic exposure, however, this was not statistically significant [21]. Bisgaard et al. investigated antibiotics in the 3rd trimester only, and found no association with atopic dermatitis at 3 years of age among 411 children in the prospective clinical study Copenhagen Study on Asthma in Childhood [10]. However, none of the studies mentioned above investigated the effect of prenatal antibiotics and offspring atopic dermatitis in atopic and non-atopic mothers separately. The higher prevalence of filaggrin mutations among atopic mothers may contribute to explain the modification of the estimates [10]. In line with our findings, Thorsteinsdottir et al. found a protective effect from domestic dog exposure around birth on the incidence of atopic dermatitis only among children born by atopic mothers [34]. It has been suggested, that maternal allergy may amplify the effects of prenatal environmental exposures via epigenetic modulations which may affect foetal gene expression and immune function [35].

This study repeated the tendency of an additive effect from prenatal antibiotics and caesarean section observed by Lee et al. in a Korean prospective birth cohort of 412 1-year old infants, although we only observed this among atopic mothers [22]. Wegienka et al. found a protective association from vaginal birth compared to caesarean section was attenuated if the mother used systemic antibiotics or vaginal antifungal medication during pregnancy [36]. In our study, the prevalence of children born by caesarean section was similar across exposure groups in this study, and caesarean section in itself is not found to be associated with atopic dermatitis in offspring in a recent systematic review [37]. We therefore do not believe that the

Accepted Article

results are due to differentiated bias. A possible explanation for the findings might be, that maternal atopic disease is a strong risk factor for offspring atopic dermatitis and asthmatic mothers are more likely to give birth by caesarean section [38, 39].

It remains uncertain whether these findings reflect a causal relationship, and the results should be interpreted with caution. Stensballe et al. found similar results for prenatal antibiotics and asthma in two population-based cohorts of Danish children, but Stockholm et al. challenged the results in a registry-based study investigating maternal antibiotic use in 80 weeks before and after pregnancy among 910,301 mother-child pairs [40, 41]. The study showed a similar association with maternal antibiotics in the 80 weeks before and after pregnancy independent of antibiotics in pregnancy, and the authors concluded that maternal propensity for infections due to insufficient immune function is may be the underlying link between prenatal antibiotics and offspring asthma [41]. These findings argue against the hypothesis that the association between prenatal antibiotics and atopic dermatitis acts through the microbiome of the mother during pregnancy, birth and breast-feeding. However, in favour of the hypothesis, Dom et al. found that the association between prenatal antibiotics and atopic dermatitis persisted even after adjustment for postnatal antibiotics [19]. Our study did not take postnatal antibiotics and indirect antibiotic exposure through breast-feeding into account. This may have led to bias of our results, if the findings from Stockholm et al. apply [41].

The external validity and generalizability is perhaps influenced by the underrepresentation of mothers with low socioeconomic status. However, the internal validity of this study does not seem to be limited by these selection patterns [24].

Conclusions

In conclusion, use of prenatal antibiotics throughout pregnancy was associated with an increased risk of atopic dermatitis, but only among children born by atopic mothers. This finding is in support of the hypothesis that reduced intestinal microbiota diversity during the first months of life may be linked to subsequent allergic diseases, including atopic dermatitis.

However, since the indication for prescription of antibiotics is infection and other potentially serious conditions, this study will often not justify that use antibiotics is abandoned. Use of antibiotics in pregnancy should take all important consequences into consideration as the microbiome theory should be discussed as one of these conditions.

Acknowledgement

The Danish National Research Foundation has established the Danish Epidemiology Science Centre that initiated and created the Danish National Birth Cohort. The cohort is furthermore a result of a major grant from this Foundation. Additional support for the Danish National Birth Cohort is obtained from the Pharmacy Foundation, the Egmont Foundation, the March of Dimes Birth Defects Foundation, the Augustinus Foundation, and the Health Foundation.

Conflict of interest

The authors have no conflicts of interest to declare.

References

- [1] Strachan DP. Hay fever, hygiene and household size. *BMJ* 1989; 299: 1259–1260.
- [2] Munyaka PM, Khafipour E, Ghia J-E. External influence of early childhood establishment of gut microbiota and subsequent health implications. *Front Pediatr* 2014; 2: 109.
- [3] Arrieta M-C, Stiemsma LT, Amenyogbe N, et al. The Intestinal Microbiome in Early Life: Health and Disease. *Front Immunol* 2014; 5: 1–18.
- [4] Abrahamsson TR, Jakobsson HE, Andersson AF, et al. Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol* 2012; 129: 434–40.
- [5] Ismail IH, Oppedisano F, Joseph SJ, et al. Reduced gut microbial diversity in early life is associated with later development of eczema but not atopy in high-risk infants. *Pediatr Allergy Immunol* 2012; 23: 674–681.
- [6] Larsen A, Schlünssen V, Christensen B, et al. Exposure to Psychosocial job strain during pregnancy and odds of asthma and atopic dermatitis among 7-year old children – a prospective cohort study. *Scand J Work Environ Heal* 2014; 40: 639–648.
- [7] Asher MI, Montefort S, Björkstén B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 2006; 368: 733–43.
- [8] Wahn U, Sampson HA. *Allergy, immunity and tolerance in early childhood - the first steps of the atopic march*. Elsevier Inc., 2016.
- [9] Sandilands A, Sutherland C, Irvine AD, et al. Filaggrin in the frontline: role in skin barrier function and disease. *J Cell Sci* 2009; 122: 1285–94.
- [10] Bisgaard H, Halkjær LB, Hinge R, et al. Risk analysis of early childhood eczema. *J Allergy Clin Immunol* 2009; 123: 1355–1360.e5.

- [11] Statens Serum Institut, National Veterinary Institute, Technical University of Denmark, et al. *DANMAP 2013 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark*. 2013.
- [12] Atanabe JW, Ujiwara RF, Asajima NS, et al. Administration of Antibiotics during Infancy Promoted the Development of Atopic Dermatitis-Like Skin Lesions in NC / Nga Mice. *Biosci Biotechnol Biochem* 2010; 74: 358–363.
- [13] Kusel M, Klerk N De, Holt PG, et al. Antibiotic use in the first year of life and risk of atopic disease in early childhood. *Clin Exp Allergy* 2008; 1921–1928.
- [14] Wang J, Liu L, Chen C, et al. Acetaminophen and / or antibiotic use in early life and the development of childhood allergic diseases. *Int J Epidemiol* 2013; 42: 1087–1099.
- [15] Wohl D, Curry W, Mauger D, et al. Intrapartum Antibiotics and Childhood Atopic Dermatitis. *J Am Board Fam Med* 2015; 28: 82–89.
- [16] Schmitt J, Nm S, Kirch W, et al. Early exposure to antibiotics and infections and the incidence of atopic eczema : A population-based cohort study. *Pediatr Allergy Immunol* 2010; 292–300.
- [17] Madhok V, Futamura M, Thomas KS, et al. What ' s new in atopic eczema ? An analysis of systematic reviews published in 2012 and 2013 . Part 1 . Epidemiology , mechanisms of disease and methodological issues. *Clin Exp Dermatol* 2015; 238–242.
- [18] Tsakok T, McKeever TM, Yeo L, et al. Does early life exposure to antibiotics increase the risk of eczema? A systematic review. *Br J Dermatol* 2013; 169: 983–991.
- [19] Dom S, Droste JHJ, Sariachvili M a, 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: 1378–87.
- [20] McKeever TM, Lewis S a., 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: 43–50.

- [21] Jedrychowski W, Gałaś A, Whyatt R, et al. The prenatal use of antibiotics and the development of allergic disease in one year old infants. A preliminary study. *Int J Occup Med Environ Health* 2006; 19: 70–76.
- [22] Lee S-Y, Yu J, Ahn K-M, et al. Additive effect between IL-13 polymorphism and cesarean section delivery/prenatal antibiotics use on atopic dermatitis: a birth cohort study (COCOA). *PLoS One* 2014; 9: e96603.
- [23] Olsen J, Melbye M, Olsen SF, et al. The Danish National Birth Cohort - its background, structure and aim. *Scand J Public Health* 2001; 300–307.
- [24] Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? *Epidemiology* 2006; 17: 413–8.
- [25] Wang IJ, Chen SL, Lu TP, et al. Prenatal smoke exposure, DNA methylation, and childhood atopic dermatitis. *Clin Exp Allergy* 2013; 43: 535–543.
- [26] Steinberg MB, Akincigil A, Kim EJ, et al. Tobacco Smoking as a Risk Factor for Increased Antibiotic Prescription. *Am J Prev Med* 2015; 50: 692–698.
- [27] Thrane N, Olesen C, Schønheyder HC, et al. Socioeconomic factors and prescription of antibiotics in 0- to 2-year-old Danish children. *J Antimicrob Chemother* 2003; 51: 683–689.
- [28] Schmitz R, Atzpodien K, Schlaud M. Prevalence and risk factors of atopic diseases in German children and adolescents. *Pediatr Allergy Immunol* 2012; 23: 716–723.
- [29] Deckers IAG, McLean S, Linssen S, et al. Investigating international time trends in the incidence and prevalence of atopic eczema 1990-2010: A systematic review of epidemiological studies. *PLoS One*; 7. Epub ahead of print 2012. DOI: 10.1371/journal.pone.0039803.

- Accepted Article
- [30] Nissen SP, Kjaer HF, Høst A, et al. The natural course of sensitization and allergic diseases from childhood to adulthood. *Pediatr Allergy Immunol* 2013; 24: 549–55.
 - [31] Kildemoes HW, Sørensen HT, Hallas J. The Danish National Prescription Registry. *Scand J Public Health* 2011; 39: 38–41.
 - [32] McKeever TM, Lewis S a, Smith C, et al. 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: 827–32.
 - [33] Sariachvili M, Droste J, Dom S. Is breast feeding a risk factor for eczema during the first year of life? *Pediatr Allergy Immunol* 2007; 18: 410–17.
 - [34] Thorsteinsdottir S, Thyssen JP, Stokholm J, et al. Domestic Dog Exposure at birth reduces the Incidence of Atopic Dermatitis. *Allergy*. Epub ahead of print 2016. DOI: 10.1111/all.12980.
 - [35] Prescott S, Saffery R. The role of epigenetic dysregulation in the epidemic of allergic disease. *Clin Epigenetics* 2011; 2: 223–232.
 - [36] Wegienka G, Havstad S, Zoratti EM, et al. Combined effects of prenatal medication use and delivery type are associated with eczema at age 2 years. *Clin Exp Allergy* 2015; 45: 660–668.
 - [37] Panduru M, Panduru M N, Ion DA. Caesarian Section delivery and atopic dermatitis - meta-analysis of observational studies. *Gineco Ro* 2012; 8: 196–198.
 - [38] Demissie K, Breckenridge MB, Rhoads GG. Infant and maternal outcomes in the pregnancies of asthmatic women. *Am J Respir Crit Care Med* 1998; 158: 1091–1095.
 - [39] McKeever TM, Lewis SA, Smith C, et al. Siblings, multiple births, and the incidence of allergic disease: a birth cohort study using the West Midlands general practice research database. *Thorax* 2001; 56: 758–62.
 - [40] Stensballe LG, Simonsen J, Jensen SM, et al. Use of antibiotics during pregnancy

increases the risk of asthma in early childhood. *J Pediatr* 2013; 162: 832–838.e3.

- [41] Stokholm J, Sevelsted A, Bønnelykke K, et al. Maternal propensity for infections and risk of childhood asthma: A registry-based cohort study. *Lancet Respir Med* 2014; 2: 631–637.

Tables

Table 1: Data sources and measurements. Data from the four telephone interviews (Int1, Int-2, Int-3, Int-4) are reported by the mother.

Data source	Covariate	Measurement
Int-1: 12th gestation week	Maternal prenatal smoking	<i>Have you smoked during your pregnancy (please also think about the very early stage of the pregnancy)?</i>
	Maternal atopy	<i>Have you ever had asthma? Have you ever had allergy (including hay fever, food allergy or eczema)?</i>
	Household socioeconomic status	<i>What is your/your partners current education? What is your/your partners current work situation? Covariate constructed on maternal and paternal status</i>
	Older siblings	<i>How many children is in your household?</i>
Int-2: 30th gestation week	Maternal prenatal smoking	<i>Have you smoked since the last interview?</i>
	Maternal prenatal antibiotics	<i>Have you (while pregnant) taken any medicine against infections i.e. antibiotics?</i>
Int-3: child 6 months old	Maternal prenatal antibiotics	<i>Did you take any medicine against infections during the last part of your pregnancy i.e. antibiotics?</i>
Int-4: child 18 months old	Offspring atopic dermatitis	<i>Has your child had an itchy rash on his/her skin? If yes, was it childhood eczema? AND/OR</i>
		<i>Has your child ever had childhood eczema? Has a doctor told you, that your child has eczema?</i>
Danish Birth Registry	Mode of delivery	Recorded by midwife, doctor or nurse at the hospital

Table 2: Characteristics of the study population

	No antibiotics	Antibiotics 1st-2nd trimester	Antibiotics 3rd trimester	Antibiotics 1st-2nd+3rd trimester	All
Subjects, N	49,872	9,136	2,161	1,391	62,560
Children with atopic dermatitis					
Self-reported, N (%)	8,070 (16 %)	1,571 (17 %)	346 (16 %)	266 (19 %)	10,253 (16 %)
Doctor-diagnosed, N (%)	6,856 (14 %)	1,348 (15 %)	305 (14 %)	224 (16 %)	8,733 (14 %)
Maternal age at baseline, mean $\pm$ SD	29.93 $\pm$ 4.23	30.04 $\pm$ 4.28	29.92 $\pm$ 4.34	29.91 $\pm$ 4.18	29.95 $\pm$ 4.24
Maternal prenatal smoking, N (%)	12,309 (25 %)	2,534 (28 %)	574 (27 %)	400 (29 %)	15,817 (25 %)
Maternal atopy *, N (%)	16,080 (32 %)	3,272 (36 %)	756 (35 %)	557 (40 %)	20,665 (33 %)
Household highest socioeconomic status					
Higher grade professionals	11,754 (24 %)	2,197 (24 %)	534 (25 %)	305 (22 %)	14,790 (24 %)
Lower grade professionals	15,839 (32 %)	2,934 (32 %)	701 (33 %)	444 (32 %)	19,918 (32 %)
Skilled work	14,207 (28 %)	2,507 (28 %)	571 (26 %)	395 (28 %)	17,680 (28 %)
Unskilled work	6,594 (13 %)	1,214 (13 %)	295 (14 %)	206 (15 %)	8,309 (13 %)
Studying	1,071 (2 %)	213 (2 %)	41 (2 %)	33 (2 %)	1,358 (2 %)
Outside the workforce	295 (1 %)	52 (1 %)	14 (1 %)	6 (1 %)	367 (1 %)
Number of older siblings, mean $\pm$ SD	0.74 $\pm$ 0.82	0.80 $\pm$ 0.81	0.78 $\pm$ 0.82	0.80 $\pm$ 0.84	0.75 $\pm$ 0.82
Mode of delivery					
Vaginal birth	42,493 (85 %)	7,710 (84 %)	1,828 (85 %)	1,169 (84 %)	53,200 (85 %)
Caesarean section	7,379 (15 %)	1,426 (16 %)	333 (15 %)	222 (16 %)	9,360 (15 %)

* Maternal self-reported asthma, hay fever, food allergy or eczema

Table 3: Odds ratios (OR) for maternal-reported atopic dermatitis according to prenatal antibiotic exposure for prenatal smoking, socioeconomic status and older siblings stratified by maternal atopy

	No maternal atopy (N=41,895)		Maternal atopy ^ (N=20,665)	
	Crude	Adjusted	Crude	Adjusted
Antibiotic exposure				
No antibiotics	1	1	1	1
Antibiotics 1st-2nd trimester *	1.05	1.06 (0.98-1.14)	1.07	1.07 (0.98-1.18)
Antibiotics 3rd trimester "	1.01	1.01 (0.87-1.17)	0.93	0.94 (0.78-1.13)
Antibiotics both 1st-2nd + 3rd trimester	1.00	1.01 (0.83-1.22)	1.42	1.45 (1.19-1.76)

* 1st-2nd trimester was defined gestation week 0-29, " 3rd trimester was defined gestation week 30-birth

^ Maternal atopy = Maternal self-reported asthma, hay fever, food allergy or eczema

Table 4: Odds ratios (OR) for maternal-reported atopic dermatitis according to prenatal antibiotic exposure adjusted for prenatal smoking, socioeconomic status and older siblings stratified by maternal atopy and mode of delivery

	Vaginal birth (N=53,200)		Caesarean section (N=9,360)	
	No maternal atopy	Maternal atopy ^	No maternal atopy	Maternal atopy ^
Antibiotic exposure				
No antibiotics	1	1	1	1
Antibiotics 1st-2nd trimester *	1.06 (0.97-1.15)	1.07 (0.97-1.19)	1.03 (0.84-1.25)	1.07 (0.85-1.35)
Antibiotics 3rd trimester "	1.05 (0.89-1.24)	0.95 (0.78-1.16)	0.79 (0.53-1.18)	0.87 (0.52-1.45)
Antibiotics both 1st-2nd + 3rd trimester	1.03 (0.84-1.28)	1.34 (1.08-1.66)	0.87 (0.52-1.43)	2.13 (1.34-3.38)

* 1st-2nd trimester was defined gestation week 0-29, " 3rd trimester was defined gestation week 30-birth

^ Maternal atopy = Maternal self-reported asthma, hay fever, food allergy or eczema

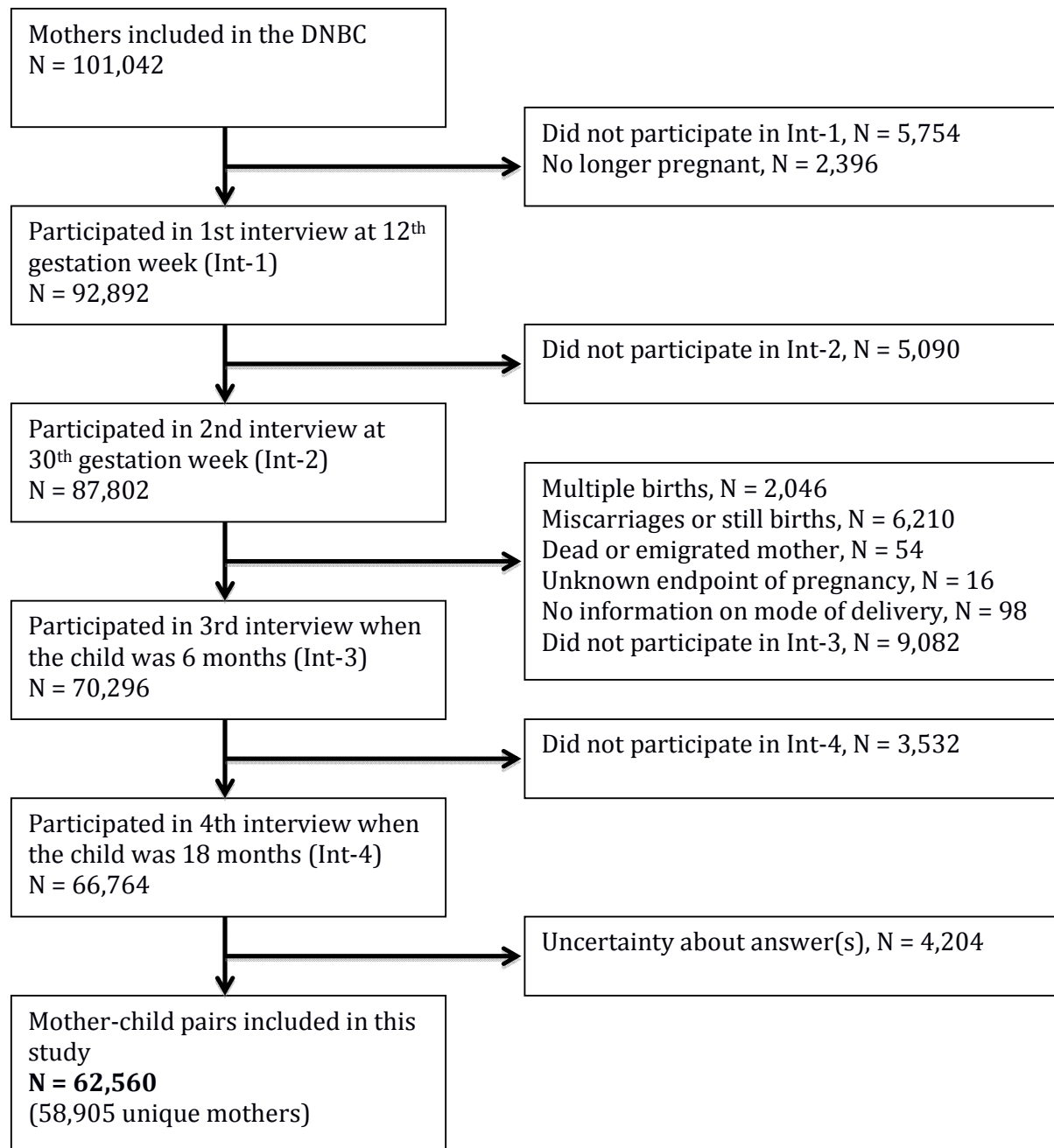


Figure 1: Flow chart of the study population - The Danish National Birth Cohort. Each woman was allowed to participate with more than one child in the recruitment period 1996-2002.