Maternal antibiotic use during pregnancy and asthma in children: population-based cohort study and sibling design

Natalie C. Momen and Xiaoqin Liu

Affiliation: National Centre for Register-based Research, Aarhus University, Aarhus, Denmark.

Correspondence: Xiaoqin Liu, National Centre for Register-based Research, Aarhus University, Fuglesangs Allé 26, 8210 Aarhus V, Denmark. E-mail: lxq@econ.au.dk

@ERSpublications
Prenatal exposure to antibiotics is not associated with asthma in children. The associations observed by previous studies are likely to be explained by confounding factors shared within families.
https://bit.ly/3gbJbOs

Cite this article as: Momen NC, Liu X. Maternal antibiotic use during pregnancy and asthma in children: population-based cohort study and sibling design. *Eur Respir J* 2021; 57: 2000937 [https://doi.org/10.1183/13993003.00937-2020].

ABSTRACT Antibiotic use during pregnancy may affect asthma risk in offspring. However, epidemiological studies yielded conflicting findings, with an observed association possibly confounded by shared familial factors. We sought to assess the association between maternal antibiotic use during pregnancy and childhood asthma in the offspring, by accounting for time-stable familial factors.

We conducted a population-based cohort study and sibling study using data from Danish nationwide registers, which comprised 407 804 liveborn singletons from 2005 to 2011. Antibiotic use during pregnancy was defined as at least one antibiotic prescription filled by the mother from 1 month prior to pregnancy up until delivery, identified in the National Prescription Registry. First-time asthma in the offspring was determined by hospital treatment or asthma medication treatment after age 5 years. We estimated hazard ratios (HRs) of asthma using Cox regression in the population-based cohort and stratified Cox regression in the sibling cohort.

Approximately 36.5% of pregnant women redeemed antibiotic prescriptions. Antibiotic use during pregnancy was associated with childhood asthma in cohort analyses (HR 1.21, 95% CI 1.18–1.24), but not in sibling analyses (HR 0.96, 95% CI 0.90–1.03). In the population-based analyses, higher risks of asthma were seen with longer duration of maternal antibiotic use, a higher number of prescriptions and prescriptions of multiple types of antibiotics. All these associations disappeared in the sibling analyses.

The associations observed by previous studies for prenatal exposure to antibiotics and offspring asthma risk are likely to be due to confounding factors shared within families.

This article has supplementary material available from erj.ersjournals.com

Received: 31 March 2020 | Accepted after revision: 24 July 2020

Introduction

Antibiotics are commonly prescribed to pregnant women, with ∼40% redeeming at least one antibiotic prescription during pregnancy [1, 2]. Some antibiotics can reach the fetal circulation by transferring across the placenta [3]; additionally, prenatal exposure to antibiotics may impact the child's intestinal microbiome [4], altering the balance of beneficial and pathogenic bacteria, and thus increase the child's risk of developing asthma.

Several epidemiological studies have reported an increased asthma risk among children exposed to antibiotics prenatally [2, 5–7]. However, the role of confounding, for example by the mother's general propensity for infections and shared familial factors, has been postulated [2, 8, 9]. Örtqvist *et al.* [10] found an increased risk of asthma among children exposed prenatally to antibiotics using a cohort design, but when sibling analyses were employed, this increase was not seen. As the sibling design controls for potential confounding due to genetic predisposition and environmental factors, these findings suggest that genetic and/or environmental factors could account for the associations previously observed for prenatal antibiotics and offspring asthma in cohort studies. However, while this study [10] highlights issues with confounding, it included asthma diagnosed before 5 years, as others have done [5, 9]. Asthma diagnosis is often unspecific and challenging in young children. Consequently, these studies reflected the association of *in utero* antibiotic exposure with wheezing, rather than asthma.

Our primary objective was to assess the association between maternal antibiotic use during pregnancy and asthma in children aged ⩾5 years, by accounting for time-stable familial factors using two designs: 1) population-based cohort and 2) sibling design. We hypothesised that if the associations observed in the population-based cohort design were due to shared familial factors, the associations would attenuate or disappear in the sibling analysis. Otherwise, similar associations would be observed in the two analyses.

Secondary objectives were to examine the association by the timing of antibiotic exposure, antibiotic type, number and duration, and infection type. The evidence available to date on the time-specific effect of antibiotic exposure is conflicting, with an elevated risk reported in the second or third trimester only [7, 11, 12], or the first trimester only [13], as well as no trimester-specific effect [2, 9]. While the type of antibiotic could affect the association between prenatal antibiotic exposure and asthma, results so far have been conflicting, with most studies underpowered for this analysis [2, 5, 6]. Furthermore, women could take multiple antibiotics or antibiotics for different periods of time, which has not been accounted for in previous studies [5, 9]. To our knowledge, no research paper to date on the association of prenatal antibiotic exposure with childhood asthma has included all these aspects in one study, which are important to examine. While we expected that there would be a positive association between duration of maternal antibiotic use and offspring asthma, it is challenging to hypothesise about the effects of the other aspects. For the type of antibiotic and infection, there is a lack of evidence to support the hypotheses. Finally, for the trimester of exposure, the role of the placenta and vulnerability of the fetus changes throughout gestation [14].

Methods

Study population

We conducted a population-based cohort study. We identified 428 781 liveborn singletons born between 2005 and 2011 from the Danish Medical Birth Register [15] (identification ended in 2011 so that children could reach 5 years of age before the end of follow-up). We excluded 8196 children with missing or unfeasible gestational age (*i.e.* gestational age <154 days or >315 days), as it would not be possible to ascertain the gestational period (the exposure window) accurately. We excluded 7131 children who emigrated and 1571 children who died before their 5th birthday, in order to ensure we were able to retrieve information on asthma diagnosis after age 5 years. Furthermore, we excluded 4079 children who could not be linked to their biological fathers.

In comparison to studies in unrelated individuals, sibling analysis can be a powerful tool to account for unmeasured within-family genetic, social and lifestyle confounders [16]. Out of 407 804 singletons, 202 812 singletons born by 97 703 mothers with at least one sibling born during 1993–2007 were identified through the Danish Medical Birth Register, by identification of the mother. Both full and half siblings on the mother's side were included. We included all the siblings from a family that had discordant exposure statuses to provide a more accurate estimate within families. Siblings discordant on exposure and where at least one of them developed asthma during the study period contributed to the analysis. Therefore, 119 746 singletons with no discordant sibling to be paired to were excluded. After exclusion, 83 066 singletons born by 39 327 mothers remained for the sibling analyses. Overall, 89.3% of the discordant pairs included two siblings, 10.2% included three siblings and 0.5% included four or five siblings.

Use of antibiotics

Antibiotic use during pregnancy was defined as at least one antibiotic prescription (Anatomical Therapeutic Chemical (ATC) code J01) filled by the mother from 1 month prior to conception up until delivery. Information on antibiotic use during pregnancy was identified in the Danish National Prescription Registry [17], which holds information on ATC codes and dispensation dates for all prescriptions dispensed in all Danish pharmacies since 1995, with treatment indication from April 1, 2004 onwards. The prescriber, *e.g.* a general practitioner or a hospital physician, may select an indication code from a drop-down menu containing a list of indications, or they can include the indication as free text, in which case no code is recorded [12]. ~50.0% of antibiotic prescriptions were redeemed with indications as missing (12.6%), unspecified (10.1%) or unspecified infection or inflammation (29.1%). We further determined treatment indications according to the ATC codes modified from Almqvist *et al.* [18]: urinary tract infections (pivmecillinam (J01CA08), short-acting sulfonamides (J01EB) and nitrofuran derivatives (J01XE)), respiratory tract infections (penicillins with extended-spectrum (J01CA), β-lactamase sensitivity penicillins (J01CE) and macrolides (J01FA), excluding pivmecillinam (J01CA08)) and skin or soft-tissue infections (clindamycin (J01FF01) and flucloxacillin (J01CF05)).

To consider whether there was a trimester-specific effect, we further categorised exposure to antibiotics into 1st trimester only (1 month before pregnancy to 90 days after the last menstrual period (LMP)), 2nd trimester only (91–180 days after the LMP), 3rd trimester only (181 days after the LMP to childbirth) and more than one trimester. In addition, we considered the type of antibiotic: β-lactam penicillins (J01C), cephalosporins (J01D), sulfonamides (J01E), macrolides (J01F) and other classes (J01 excluding J01C, J01D, J01E and J01F) to examine the association between subtypes of antibiotics and asthma.

Childhood asthma

Accurate diagnosis of asthma in children aged <5 years is challenging. Therefore, asthma was defined as meeting at least one of two criteria after age 5 years [19]: 1) at least one hospital contact for asthma (10th revision of the International Classification of Diseases (ICD-10) codes J45 and J46) or 2) at least two prescriptions for asthma medication within a 1-year period. Information on hospital contacts was retrieved from the Danish National Patient Register [20] and information on asthma medication from the Danish National Prescription Registry. The ATC classification codes for asthma medications were inhaled β_2-agonists (R03AC02, R03AC03, R03AC04, R03AC12 and R03AC13), inhaled glucocorticoids (R03BA01, R03BA02 and R03BA05), fixed-dose combination of inhaled β_2-agonists and glucocorticoids (R03AK06 and R03AK07), and leukotriene receptor antagonists (R03DC03). The date of asthma onset was defined as the date of the first prescription for anti-asthma medication or first hospital contact for asthma, whichever came first.

Statistical analysis

The children were followed from 5 years of age until the first onset of asthma, emigration, death or December 31, 2016 (last day of data availability), whichever came first. Cox proportional hazard regression models were used for the population-based cohort to estimate the hazard ratio (HR) of receiving an asthma diagnosis in children with a sandwich estimator of standard errors to account for the dependence within siblings. The proportional hazards assumption was tested using log-log plots. For these sibling analyses, stratified Cox models were used. Children born by the same mother constituted a separate stratum, and each stratum had its baseline hazard function. Analyses were performed to consider the timing and duration of antibiotic exposure, the number of prescriptions, combination treatment, type of antibiotics prescribed and indications for antibiotic treatment. Types of antibiotics or indications for antibiotic treatment were mutually adjusted for in the models.

Both crude and adjusted results are reported. The following covariates were selected *a priori*: maternal age at delivery (<25, 25–34 or ⩾35 years), parity (1st, 2nd or greater), smoking during pregnancy (yes, no), cohabitation status (married/cohabiting, single, divorced or widowed), highest education attained at delivery (elementary school or above elementary school), diabetes during the index pregnancy (yes, no), maternal atopic disposition (yes, no), paternal atopic disposition (yes, no) and calendar year of birth (2005–2006, 2007–2008 or 2009–2011). Mothers were considered to have diabetes during pregnancy if they received a diagnosis of pre-gestational diabetes (ICD-8 codes 249–250; ICD-10 codes E10–E14, O24, H36.0 excluding O24.4) or gestational diabetes during the index pregnancy (ICD-10 code O24.4) registered in the Danish National Patient Register. We defined parental atopic disposition if any of the following disorders were recorded in the National Patient Register: allergic rhinitis (507 in ICD-8 and J30.1, J30.2, J30.3 or J30.4 in ICD-10), asthma (493 in ICD-8 and J45–J46 in ICD-10), or atopic dermatitis (691.0 in ICD-8 and L20 in ICD-10) [21]. Data on covariates were extracted from the registers mentioned earlier, as well as from Statistics Denmark's registers on education [22]. Data were missing in 5.6% of the subjects for one or more potential confounders, and we applied 20 imputations using the Markov chain

Monte Carlo technique for imputing missing values [23]. Data processing was conducted in Stata (version 15.0; StataCorp, College Station, TX, USA).

Sensitivity analysis

To test the robustness of our results, we performed four sensitivity analyses. First, to account for the antibiotics received during inpatient treatment, we excluded children born to mothers who received diagnoses for certain infections (appendicitis K35; sepsis A40–A41; meningitis A87, G00–G03; pneumonia J18; skin infection L08; cystitis N30; and pyelonephritis N10) during the index pregnancy. This was done to test whether associations changed when we remove children whose mothers had been exposed to antibiotics as an inpatient, but may have been misclassified as unexposed, as the prescription register does not include medications received in hospitals. Second, as antibiotic prophylaxis is often administered to women with premature rupture of membranes or those undergoing caesarean section [24, 25], we further adjusted for premature rupture of membranes (yes, no) and caesarean section (yes, no) in the models. Information on the premature rupture of membranes (ICD-10 code O42) and caesarean section (ICD-10 code O82) was obtained from the Danish National Patient Register. Third, to address shared environmental factors within the family, we evaluated paternal antibiotic exposure as a negative control. The linkage of the children to their fathers employed the Danish Civil Registration System [26], which contains information on the identity of the parents. Fourth, we applied a design considering older/younger siblings to account for an age-specific family environment and birth order. More specifically, we employed sibling design in two ways. Analysis 1 consisted of 11 997 sibling pairs, where the first-born child was considered "exposed" (mother filled a prescription for antibiotics during pregnancy), and the second-born child was considered "unexposed" (mother did not fill a prescription for antibiotics). Analysis 2 consisted of 17 239 sibling pairs where the first-born child was "unexposed" and the second-born child "exposed".

TABLE 1 Demographic characteristics of the study population

	Antibiotic-exposed group	Unexposed group
Subjects	148 782	259 022
Maternal age at delivery years		
<25	27 156 (10.5)	21 545 (14.5)
25–34	181 006 (69.9)	99 489 (66.9)
⩾35	50 860 (19.6)	27 748 (18.6)
Parity		
1	62 026 (41.7)	121 091 (46.7)
⩾2	86 756 (58.3)	137 931 (53.3)
Maternal smoking during pregnancy		
Yes	24 948 (16.8)	31 941 (12.3)
No	120 709 (81.1)	221 957 (85.7)
Unknown	3125 (2.1)	5124 (2.0)
Maternal cohabiting status		
Married or cohabiting	127 215 (85.5)	227 519 (87.8)
Single, divorced or widowed	20 824 (14.0)	28 114 (10.9)
Unknown	743 (0.5)	3389 (1.3)
Maternal highest education attained at delivery		
Elementary school	31 122 (20.9)	37 120 (14.3)
Above elementary school	113 206 (76.1)	211 299 (81.6)
Unknown	4454 (3.0)	10 603 (4.1)
Maternal diabetes during pregnancy	4187 (2.8)	5794 (2.2)
Premature rupture of membranes	9109 (6.1)	16 185 (6.3)
Caesarean section	25 955 (17.4)	42 992 (16.6)
Maternal atopic disposition	9427 (6.3)	11 699 (4.5)
Paternal atopic disposition	6910 (4.6)	11 138 (4.3)
Paternal antibiotic use during the index pregnancy	33 028 (22.2)	42 692 (16.5)
Preterm birth	7591 (5.1)	12 523 (4.8)
Calendar year of birth		
2005–2006	41 626 (28.0)	77 655 (30.0)
2007–2008	43 546 (29.3)	75 546 (29.2)
2009–2011	63 610 (42.8)	105 821 (40.9)

Data are presented as n or n (%).

Ethics

The study was approved by the Danish Data Protection Agency (J.nr. 2013-41-2569). No informed consent is required for purely register-based studies on the basis of encrypted data in accordance with the legislation in Denmark.

Results

Our study population for the population-based analysis consisted of 407 804 mother–child dyads with a median follow-up time of 3.3 years (interquartile range 1.5–5.1 years). Overall, 36.5% of children were exposed to antibiotics *in utero*. The characteristics of children with and without antibiotic exposure during pregnancy are presented in table 1. Children whose mothers took antibiotics during pregnancy were more likely to have older mothers who were multiparous, smoked, single, with short education, and atopic disposition. There were no differences in antibiotic use during pregnancy by premature rupture and preterm delivery.

The characteristics of those included in the sibling analysis, compared to those in the population-based cohort, are described in supplementary table S1. While the two groups are not dissimilar for most characteristics, a higher proportion of children in the population-based cohort were born to mothers in the oldest age group (⩾35 years at delivery).

Primary outcome

By the end of follow-up for the population-based analysis, asthma had been diagnosed for 7.3% (n=10 847) of children born to mothers with antibiotic use during pregnancy, compared to 6.0% (n=15 490) children born to mothers with no antibiotic use. Asthma was 21% (95% CI 18–24%) higher among exposed children compared to unexposed children.

In order to disentangle the effect of antibiotic treatment from the effect of an underlying maternal disorder and shared familial factors, we performed a sibling analysis among 39 327 discordant sibling pairs. The mean±SD age was 8.5±2.0 years for the unexposed sibling and 8.1±2.0 years for the exposed sibling. The mean age difference among siblings was 0.4±3.0 years. We found that the risk of asthma in children exposed to antibiotics was similar to their unexposed siblings (HR 0.96, 95% CI 0.90–1.03). As can be seen in figures 1 and 2, the risk did not differ with trimester of exposure, the number of antibiotics prescribed, duration of treatment in the sibling analyses, generic names of antibiotics or indications for antibiotic treatment.

Secondary outcomes

Out of all children, 91 054 (22.3%) were born to mothers who redeemed one prescription, 34 220 (8.4%) two prescriptions and 23 508 (5.8%) three or more prescriptions. Exposure to only one type of antibiotic was seen for 108 921 children (26.7%), 30 734 (7.5%) were exposed to two types of antibiotics and 9126 (2.2%) were exposed to three or more types of antibiotics. Overall, 31 089 (7.6%) of children were born to mothers with antibiotic use in the first trimester only, 32 992 (8.1%) in the second trimester only, 46 684 (11.5%) in the third trimester only and 38 022 (9.3%) in two or more trimesters. The crude HRs of childhood asthma by the number of prescriptions, timing, duration, combination treatment and type of antibiotic exposure are presented in supplementary tables S2 and S3. After adjustment for covariates, we did not find any difference in asthma risk with maternal antibiotic prescription redemption by trimester, but the association was stronger for children whose mothers had redeemed more than one antibiotic prescription or multiple types of antibiotics (figure 1).

Penicillins, cephalosporins, sulfonamides, macrolides and other antibiotics were all non-differentially associated with a higher risk of childhood asthma in the offspring. The HRs for childhood asthma were increased among children whose mothers used antibiotics to treat urinary tract infections or airway infections, whereas this elevated association was not seen among children whose mothers used antibiotics for skin or soft-tissue infections (figure 2).

Sensitivity analysis

Overall, 3888 children were born to mothers who had hospital contact for infectious diseases during the index pregnancy. The magnitude of risk remained almost identical after excluding these children (HR 1.20, 95% CI 1.17–1.23 in the population-based cohort and HR 0.96, 95% CI 0.90–1.02 in the sibling analysis). The results remained similar after further adjustment for premature membrane rupture and caesarean section (supplementary tables S4 and S5). Altogether, 75 720 (18.6%) of children were born to fathers who used antibiotics during the index pregnancy. Paternal antibiotic use was also associated with increased risk in childhood asthma in the population-based analysis (HR 1.10, 95% CI 1.06–1.13), but not sibling analysis (HR 0.99, 95% CI 0.92–1.07). In the sibling analysis comparing a first "exposed" child with a

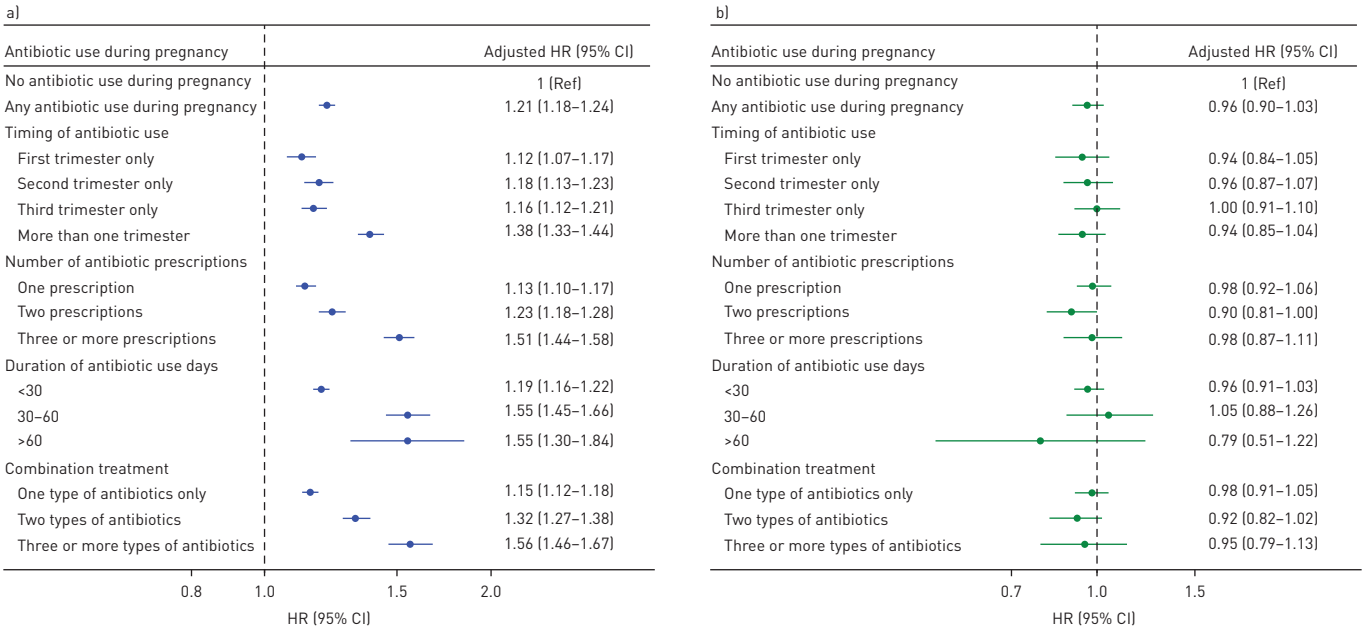

FIGURE 1 Association between prenatal antibiotics and childhood asthma in the a) population-based analysis and b) sibling design. Models for adjusted hazard ratios (HRs) include maternal age at delivery, parity, maternal smoking during pregnancy, maternal cohabiting status, maternal education status at delivery, maternal diabetes during pregnancy, maternal atopic disposition, paternal atopic disposition, paternal antibiotic use during the index pregnancy and calendar year of birth.

second "unexposed" child, the HR was 1.01 (95% CI 0.78–1.31). When a second "exposed" child was compared to a first "unexposed" child, the HR was 0.67 (95% CI 0.53–0.83).

Discussion

In this study, we found that over one-third of women received at least one antibiotic prescription during pregnancy. Exposure to antibiotics during pregnancy was associated with a small increased risk of asthma

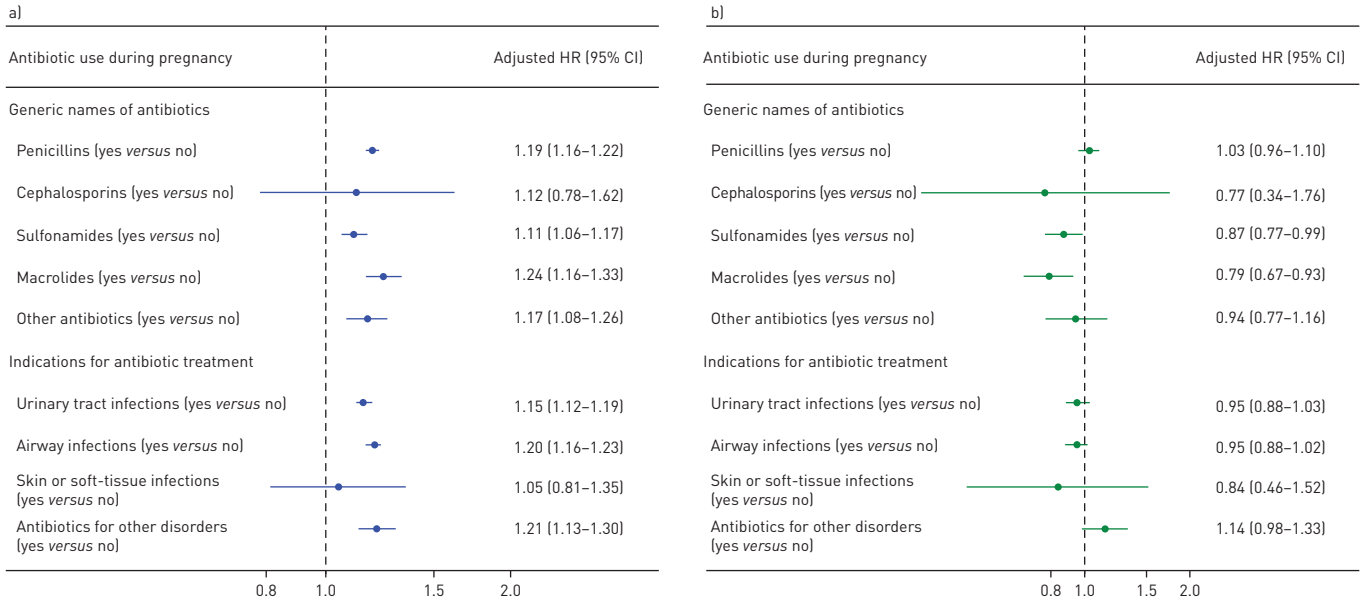

FIGURE 2 Association between prenatal antibiotics and childhood asthma in the a) population-based analysis and b) sibling design by types of antibiotics. Models for adjusted hazard ratios (HRs) include maternal age at delivery, parity, maternal smoking during pregnancy, maternal cohabiting status, maternal education status at delivery, maternal diabetes during pregnancy, maternal atopic disposition, paternal atopic disposition, paternal antibiotic use during the index pregnancy and calendar year of birth. The types of antibiotics in terms of generic names were mutually adjusted for in the models. The indications for antibiotic treatment were mutually adjusted for in the models.

in the population-based cohort. In the sibling analysis, the association was attenuated, to the level of no increased risk among those exposed, suggesting that the increased asthma risk observed in the population based-cohort can be attributed to shared familial factors.

Our finding of a 21% increased risk of asthma among children exposed prenatally to antibiotics in the population-based analysis was consistent with those from previous epidemiological studies, which reported increased risks of between 18% and 37% when a mother had taken an antibiotic at any point during her pregnancy [2, 5–7]. Similar to some of the previous studies, we did not observe a trimester-specific effect [2, 9]. Offspring asthma risk was increased for all types of antibiotics, with the exception of cephalosporins, and for all antibiotic treatment of all indications, apart from skin and soft-tissue infections.

All associations became statistically insignificant when the sibling design was employed. In this sibling study, siblings with discordant exposure status were included. This gives a reference group that will be as similar as possible with regards to environmental and genetic factors to the children exposed to antibiotics *in utero*, minimising confounding from these sources. While lack of statistical significance may in part be due to less precise confidence intervals corresponding to the reduction in power, all estimates were attenuated, moving towards the null. This lends support to suggestions in previous studies that shared familial factors, the influence of which would have been minimised in the sibling analyses, play a large role in the association between prenatal antibiotic exposure and asthma [2, 8, 9].

To examine whether the associations between maternal antibiotic use and childhood asthma were confounded by shared environmental or genetic variables, paternal antibiotic use was also considered, as a negative control. For the population-based cohort design, while the association of childhood asthma with paternal antibiotic use was attenuated compared with that of maternal antibiotic use, a positive association was observed, supporting the idea that the estimates for this design are subject to confounding.

Strengths and limitations

Our study has several methodological strengths, including the use of the population-based registers of Denmark. These allow for the inclusion of the entire population and almost complete follow-up, and contain prospectively collected data on exposure and outcome. The use of two designs (population-based analysis and sibling comparison) and paternal antibiotic use as a negative control improve causal inference. Implementation of the sibling comparison highlights the caution that should be used when interpreting the results of other studies, as our findings from this design suggest that confounding could be responsible for positive associations seen in population-based analyses. The inclusion of a variety of designs and comparison aids the assessment of our findings [27, 28].

Our study has some limitations. First, although using the Danish registers minimises selection bias by providing data on the entire population, we excluded 4.8% of children (those with missing/unfeasible gestational age, who died or emigrated before their 5th birthday or who could not be linked to their biological father). This may have introduced some selection bias, but any impact is likely to be small. It should be noted that as paternity is recorded as reported to the Civil Registration System, and is not confirmed by genetic testing, a small proportion of incorrect links can be expected, and the findings on the association between paternal antibiotic use and childhood asthma would have been diluted. Nonetheless, the association still exists, regardless of the misclassification of biological fathers and thus exposure status in fathers, which further confirms our conclusion that shared family factors confound the associations between maternal antibiotic use and childhood asthma. Second, misclassification of exposure status cannot be ruled out. Redeemed prescriptions for antibiotics may not reflect actual use. A study of Swedish women found low agreement (20.5%) between filled prescriptions for antibiotics during pregnancy recorded in the registers and reported use [29], with prescriptions for antibiotics dispensed for more women than reported using them. Therefore, women either under-reported taking antibiotics or chose not to take them after redeeming a prescription. Thus, we may misclassify women with no antibiotic use as exposed and make these two groups more similar, meaning estimates would become more conservative. Furthermore, prescription medications received in hospitals are not included in the prescription register, which may add to misclassification. We attempted to account for this through our sensitivity analyses, but, again, this may contribute to more conservative estimates. We determined the treatment indications both based on an indication variable and the ATC codes. However, ∼50% of antibiotic prescriptions were redeemed with indications as missing/unspecified. The majority of the missing/unspecified data were between 2004 and 2005 when the register started collecting information on the indication variable. Therefore, these values are most likely to be missing at random. However, caution is warranted in interpreting the results. Third, we determined asthma cases using the Danish National Prescription Register and the Danish National Patient Register. A validation study reported sensitivity of 83.3% and specificity of 66.0% for defining asthma using these two registers [30]. This indicates that some

children with mild symptoms receiving no treatment were consequently categorised as not having asthma. Conversely, not all children who received treatment had asthma. This misclassification is expected to be non-differential and would have biased the findings toward the null [31]. We would expect the HR to be >1.22 observed in the population-based cohort and <0.96 in the sibling cohort, were subjects correctly classified as asthma cases *versus* non-cases, which would not change our conclusion. Fourth, while we attempted to account for confounding by indication, it is not well recorded in the registers, so we may not have dealt with this fully. Finally, although we adjusted for several covariates in our models, we cannot completely rule out residual confounding, even for the sibling analysis. While the sibling design controls for familial factors, the associations could be confounded by non-shared factors [32]. It should be noted that due to the size of the population in the study and its power, confidence intervals tend to be small. As always, they should be interpreted with caution, along with the magnitude of the estimates.

Conclusions

While the findings from the population-based analysis indicated that maternal antibiotic use during pregnancy increased risk of asthma in the offspring, the association disappeared when a sibling-based design was applied. This suggests that previous studies that have concluded an increased risk could be confounded by genetic and/or familial environmental confounders.

Conflict of interest: N.C. Momen has nothing to disclose. X. Liu has nothing to disclose.

Support statement: X. Liu is supported by the Danish Council for Independent Research (DFF-5053-00156B) and the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement number 891079. N.C. Momen is supported by Niels Bohr Professorship Grant from the Danish National Research Foundation and the Stanley Medical Research Institute. The funders had no role in the study design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. Funding information for this article has been deposited with the Crossref Funder Registry.

References

1 Ingstrup KG, Liu X, Gasse C, *et al.* Prescription drug use in pregnancy and variations according to prior psychiatric history. *Pharmacoepidemiol Drug Saf* 2018; 27: 105–113.
2 Loewen K, Monchka B, Mahmud SM, *et al.* Prenatal antibiotic exposure and childhood asthma: a population-based study. *Eur Respir J* 2018; 52: 1702070.
3 Nahum GG, Uhl K, Kennedy DL. Antibiotic use in pregnancy and lactation: what is and is not known about teratogenic and toxic risks. *Obstet Gynecol* 2006; 107: 1120–1138.
4 Tanaka S, Kobayashi T, Songjinda P, *et al.* Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. *FEMS Immunol Med Microbiol* 2009; 56: 80–87.
5 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.
6 Metsälä J, Lundqvist A, Virta LJ, *et al.* Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood. *Clin Exp Allergy* 2015; 45: 137–145.
7 Mulder B, Pouwels KB, Schuiling-Veninga CC, *et al.* Antibiotic use during pregnancy and asthma in preschool children: the influence of confounding. *Clin Exp Allergy* 2016; 46: 1214–1226.
8 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.
9 Bai L, Zhao D, Cheng Q, *et al.* Trimester-specific association between antibiotics exposure during pregnancy and childhood asthma or wheeze: the role of confounding. *Ann Epidemiol* 2019; 30: 1–8.
10 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.
11 Popovic M, Rusconi F, Zugna D, *et al.* Prenatal exposure to antibiotics and wheezing in infancy: a birth cohort study. *Eur Respir J* 2016; 47: 810–817.
12 Lapin B, Piorkowski J, Ownby D, *et al.* Relationship between prenatal antibiotic use and asthma in at-risk children. *Ann Allergy Asthma Immunol* 2015; 114: 203–207.
13 Hughes CH, Jones RC, Wright DE, *et al.* A retrospective study of the relationship between childhood asthma and respiratory infection during gestation. *Clin Exp Allergy* 1999; 29: 1378–1381.
14 Koren G, Ornoy A. The role of the placenta in drug transport and fetal drug exposure. *Expert Rev Clin Pharmacol* 2018; 11: 373–385.
15 Bliddal M, Broe A, Pottegård A, *et al.* The Danish Medical Birth Register. *Eur J Epidemiol* 2018; 33: 27–36.
16 Susser E, Eide MG, Begg M. Invited commentary: the use of sibship studies to detect familial confounding. *Am J Epidemiol* 2010; 172: 537–539.
17 Kildemoes HW, Sørensen HT, Hallas J. The Danish National Prescription Registry. *Scand J Public Health* 2011; 39: Suppl. 7, 38–41.
18 Almqvist C, Wettermark B, Hedlin G, *et al.* Antibiotics and asthma medication in a large register-based cohort study – confounding, cause and effect. *Clin Exp Allergy* 2012; 42: 104–111.
19 Liu X, Andersen SL, Olsen J, *et al.* Maternal hypothyroidism in the perinatal period and childhood asthma in the offspring. *Allergy* 2018; 73: 932–939.
20 Lynge E, Sandegaard JL, Rebolj M. The Danish National Patient Register. *Scand J Public Health* 2011; 39: Suppl. 7, 30–33.

21 Liu X, Agerbo E, Li J, *et al.* Maternal pregestational or gestational diabetes and childhood wheezing: a population-based cohort study. *Allergy* 2018; 73: 2247–2250.
22 Petersson F, Baadsgaard M, Thygesen LC. Danish registers on personal labour market affiliation. *Scand J Public Health* 2011; 39: Suppl. 7, 95–98.
23 Royston P, White IR. Multiple imputation by chained equations (MICE): implementation in Stata. *J Stat Softw* 2011; 45: 1–20.
24 Passos F, Cardoso K, Coelho AM, *et al.* Antibiotic prophylaxis in premature rupture of membranes at term: a randomized controlled trial. *Obstet Gynecol* 2012; 120: 1045–1051.
25 Tita AT, Szychowski JM, Boggess K, *et al.* Adjunctive azithromycin prophylaxis for cesarean delivery. *N Engl J Med* 2016; 375: 1231–1241.
26 Schmidt M, Pedersen L, Sørensen HT. The Danish Civil Registration System as a tool in epidemiology. *Eur J Epidemiol* 2014; 29: 541–549.
27 Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. *Int J Epidemiol* 2016; 45: 1866–1886.
28 Cohen JM, Wood ME, Hernández-Díaz S, *et al.* Paternal antidepressant use as a negative control for maternal use: assessing familial confounding on gestational length and anxiety traits in offspring. *Int J Epidemiol* 2019; 48: 1665–1672.
29 Stephansson O, Granath F, Svensson T, *et al.* Drug use during pregnancy in Sweden – assessed by the Prescribed Drug Register and the Medical Birth Register. *Clin Epidemiol* 2011; 3: 43–50.
30 Stensballe LG, Klansø L, Jensen A, *et al.* The validity of register data to identify children with atopic dermatitis, asthma or allergic rhinoconjunctivitis. *Pediatr Allergy Immunol* 2017; 28: 535–542.
31 Rothman KJ, Greenland S, Lash TL. Validity in epidemiologic studies. *In*: Rothman KJ, Greenland S, Lash TL, eds. Modern Epidemiology. Philadelphia, Lippincott Williams & Wilkins, 2008; pp. 162–185.
32 Frisell T, Öberg S, Kuja-Halkola R, *et al.* Sibling comparison designs: bias from non-shared confounders and measurement error. *Epidemiology* 2012; 23: 713–720.