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Early-life antibiotic use and risk of asthma and eczema: results of a discordant twin study

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Abstract

Rationale

Early-life antibiotic use has been associated with development of atopic diseases, but the aetiology remains unclear. To elucidate aetiology, we used a discordant twin design to control for genetic and environmental confounding.

Methods

We conducted a retrospective cohort study in twins (3-10 years) from the Netherlands Twin Register (NTR, n=34,352) and a replication study at age 9 in the Childhood and Adolescent Twin Study in Sweden (CATSS, n=7,906). Antibiotic use was recorded at 0-2 years. Doctor diagnosed asthma and eczema were reported by parents when children were 3-12 years in both cohorts. Individuals were included in unmatched analyses and in co-twin control analyses with disease discordant twin pairs.

Results

Early-life antibiotic use was associated with increased risk of asthma (NTR OR 1.34 95%CI 1.28-1.41; CATSS 1.45 95%CI 1.34-1.56) and eczema (NTR OR 1.08 95%CI 1.03-1.13; CATSS 1.07 95%CI 1.01-1.14) in unmatched analyses. Co-twin analyses in mono- and dizygotic twin pairs showed similar results for asthma (NTR 1.54 95%CI 1.20-1.98 and CATSS 2.00 95%CI 1.28-3.13), but opposing results for eczema in NTR (0.99 95%CI 0.80-1.25) and CATSS (1.67 95%CI 1.12-2.49). The risk of asthma increased for antibiotics prescribed for respiratory infections (CATSS 1.45 95%CI 1.34-1.56), but not for antibiotics commonly used for urinary tract/skin infections (CATSS 1.02 95%CI 0.88-1.17).

Conclusion

Children exposed to early-life antibiotic use, particularly prescribed for respiratory infections, may be at higher risk of asthma. This risk can still be observed, when correcting for genetic and environmental factors. Our results could not elucidate whether the relationship between early-life antibiotic use and eczema is confounded by familial and genetic factors.

Introduction

The worldwide prevalence of atopic diseases increased over the last decades, having social and economic impact on society [1,2]. The atopic disease epidemic parallels increased antibiotics use [3]. A recent study reported that antibiotics are prescribed to Dutch children for 1 in 4 infectious episodes diagnosed by a general practitioner (714 prescriptions per 1000 patient-years in 1 year-olds) [4].

Different hypotheses have been put forward to explain the relationship between asthma/eczema development and early-life antibiotics use. Firstly, antibiotics perturb gastrointestinal tract microbiota and promote a shift from T-helper cell (Th) 1 to Th2 response [5], increasing risk of atopic diseases [6]. Antibiotics change microbiota, with broad spectrum (BS) antibiotics thought to provide more changes than narrow-spectrum (NS) antibiotics and subsequently increasing the susceptibility of developing asthma. Early studies investigating microbiota differences in children with asthma and eczema support this hypothesis [7-9]. Secondly, undiagnosed asthma may be associated with increased susceptibility to infections and subsequent antibiotic treatment, or early-onset symptoms of asthma may have been misdiagnosed and treated with antibiotics – a phenomenon known as reverse causation [10]. Furthermore, the association between antibiotics and asthma may be confounded by a third factor such as respiratory tract infections, which is both an indication for antibiotics and might increase the risk of developing asthma later in life [10-13]. Nevertheless, recent evidence suggests no association between respiratory syncytial virus (RSV) prevention in late preterms and incident asthma at school age [14]. Conflicting results have been published from epidemiological studies attempting to investigate the relationship between asthma and respiratory infections [15]. Lastly, unmeasured confounding factors such as environmental and genetic confounding factors shared within families (life style, health-seeking behaviour or genetic predisposition to respiratory infection) may have led to a positive spurious relationship between antibiotics and asthma.

Previous studies suggested that early-life antibiotic use is associated with increased risk of asthma or eczema, but results were inconsistent [16-19]. Two meta-analyses concluded that exposure to antibiotics in the first two years of life increased risk of both asthma (odds ratio (OR) 2.18, 95% confidence interval (95%CI): 1.04-4.60; including four studies with in total 8,284 patients) and eczema (OR 1.26, 95%CI: 1.15-1.37; including 22 studies with 394,517 patients) [20,21]. A large population-based study with sibling comparisons suggested that the association between early-life antibiotics exposure and subsequent childhood asthma may be confounded by shared familial factors, in addition to confounding by indication [12]. While the strength of the sibling design is the inherent control for shared familial factors some additional genetic confounding may remain as siblings only share half of their genes. Monozygotic twins share the same genes and environment and therefore, a twin study is an improvement to the sibling design. Furthermore, the role of shared familial factors is unknown for the association between antibiotics and eczema [22].

We aimed to assess whether early-life antibiotic use is associated with asthma and eczema in Dutch (discovery cohort) and Swedish (replication cohort) mono- and dizygotic twin pairs. A twin study design including twin pairs discordant for eczema/asthma provides a unique opportunity to study the impact of antibiotics use on asthma/eczema development while controlling for shared familial environment and genetics.

Methods

Study design and populations

We conducted a retrospective cohort study in twins aged 3-12 years from the Netherlands Twin Registry (NTR) and replicated our results in 9-year old twins from the Childhood and Adolescent Twin Study in Sweden (CATSS). Children were excluded if data describing their medication use or asthma/eczema diagnosis were not available. All data were de-identified.

To examine whether early-life antibiotic use was associated with asthma and eczema [23], we have

undertaken three steps: first, we conducted an unmatched cohort analysis to assess whether an association between early-life antibiotic use and asthma or eczema was found. Children with asthma/eczema were compared to healthy control individuals. Controls from twin pairs discordant for asthma or eczema were excluded from this first step, to adjust for twin-relatedness. These analyses establish the presence of an association at the population level, without accounting for genetic and/or other shared familial factors.

Step two was a matched co-twin control analysis in all monozygotic (MZ) and in same-sex dizygotic (DZ) twin pairs discordant for either asthma or eczema. In a separate analysis only same sex DZ twin pairs discordant for either asthma or eczema were included. Both analyses adjust for contribution of shared familial environment and partially for shared genes. Third, we conducted a matched co-twin control analysis in monozygotic (MZ) twins discordant for asthma or eczema, which controls for all shared environmental and all confounding by genetic factors. We investigate DZ and MZ separately to compare the role of genetics in the association. If the associations generated by the MZ twins are stronger than the DZ twins, it could imply that at least part of the association is driven by genes.

Discovery cohort

The NTR is a birth cohort [24] which was initiated in 1987. Twins are registered by their parents after birth, and recruited with the help of a commercial organization in the Netherlands called Felicitas and the association for parents of multiples. Data are collected by sending surveys at ages 0, 2, 3, 5, 7, 9/10 and 12 years. The response rate varied from 40% to 75%. For the current study, data collected between 1989 and 2016 were used.

Replication cohort

The replication cohort consisted of Swedish twins born between January 2005 and February 2010 in the Childhood and Adolescent Twin Study in Sweden (CATSS) [25]. Parents were interviewed about

their children's health, including questions about asthma and eczema, via a telephone questionnaire at 9 years. Twins were identified via the Swedish Twin Registry, including all Swedish twins. The participation rate was 79.5%. Data were linked to Swedish national health registers by personal identity number via the National Board of Health and Welfare and Statistics in Sweden [26]. The Swedish Prescribed Drug Register (SPDR), Medical Birth Register (MBR) and National Patient Register (NPR) were linked to CATSS information.

Outcomes

The primary outcomes, parental-reported ever-asthma and -eczema diagnosis, were derived at ages 3, 5, 7 and/or 10 in Dutch twins (Supplementary table 1). Asthma or eczema was considered to be present if reported in at least one survey. In CATSS, parents of twins were asked at age 9 whether their child ever had asthma, and/or eczema (Supplementary table 2).

Exposure

Exposure to antibiotics was defined between 0-2 years of age as any parental-reported antibiotics use in Dutch twins and as any prescription claim for antibiotics (ATC codes) in the SPDR for Swedish twins (Supplementary table 2).

Confounders and covariates

A directed acyclic graph (DAG) was drawn to select covariates (Supplementary Figure 1). Data describing gender, delivery mode, birth weight, hours of outside childcare, breastfeeding and educational attainment of mother and father were derived from NTR surveys 0, 2, 3, 5, 7 and 9/10. In CATSS, data describing breastfeeding and hours of outside childcare were not available. Perinatal covariates in CATSS were obtained from the MBR and educational attainment was obtained by questionnaire and divided in four groups: ≤ 9 years, 10-12 years, < 2 years tertiary, ≥2 years tertiary.

Statistical analysis

We used a logistic regression model with a Generalized Estimating Equation (GEE) approach for unmatched analysis to account for twin relatedness adjusted for all measured covariates. In the co-twin matched analyses, conditional logistic regression models were used within each discordant twin pair to account for shared genetic and/or environmental factors. Analyses were performed using Statistical Packages of Social Sciences (SPSS) version 25 (IBM, New York, USA).

To investigate robustness of findings, we performed sensitivity analyses where we 1) only assessed the parental-reported diagnosis in Dutch twins aged over five years (for asthma) and three years (for eczema); 2) used validated definitions for asthma and eczema in CATSS based on medication from the SPDR, and diagnoses from the NPR (Supplementary table 2); 3) only investigated CATSS with parental-reported asthma diagnosis after 2 years of age; 4) stratified by antibiotics commonly prescribed for respiratory, and urinary tract and/or skin infections in CATSS adjusting for other antibiotics prescribed in early-life (Supplementary Table 2) [12]; 5) stratified by BS and NS antibiotics in CATSS adjusting for other antibiotics prescribed in early-life (Supplementary Table 2) [27].

A sample size of 2480 and 2220 children for asthma and eczema, respectively, would be needed to detect an OR of 1.30, assuming a prevalence of 35% for antibiotic use in early life, 25% incidence of asthma, and 31% incidence of eczema (based on our dataset) [28,29].

Results

Summary characteristics of Dutch and Swedish populations are described in Table 1A for the asthma and table 1B for the eczema analysis. We included 34,352 individuals in the NTR and 7,916 in CATSS, the total numbers of individuals for the asthma and eczema study slightly differ based on the number of responses to the questions about the asthma and eczema diagnoses. The parental reported prevalence of an ever-diagnosis of asthma from ages 3-10 was 15.1% in the NTR and 17.5% at age 9

in CATSS. For eczema, the prevalences were 19.1% and 21.9%, respectively. The exposure to at least one prescription of any antibiotic between 0-2 years was 34.8% in the NTR and 44.9% in CATSS. The proportion of children born after a gestational age (GA) of >37 weeks was higher in the NTR compared to CATSS: 58.6% vs. 46.1% and higher in children with asthma compared to the whole cohort in the NTR (48.8% vs. 41.4%) and in CATSS (61.8% vs. 53.9%). In the NTR, there were more children with asthma not receiving breastfeeding compared to the children without asthma (46.5% vs. 40.3%). In CATSS we did not identify differences in summary characteristics between children with asthma or eczema and children without the disease. Mothers in CATSS more often delivered via caesarean section compared to the NTR (52.2% vs. 31.2%).

Antibiotics and asthma

Use of any antibiotic was associated with an increased risk of asthma in the NTR (OR 1.34, 95%CI 1.28-1.41) and CATSS (OR 1.45, 95%CI 1.34-1.56; table 2).

In the DZ co-twin control analyses, the risk for developing asthma was associated with exposure to antibiotics use at age 0-2 years in both cohorts (NTR OR 1.60, 95%CI 1.18-2.16; CATSS OR 1.86, 95%CI 1.11-3.18) (Table 2). For MZ discordant twins, estimates pointed in the same direction but did not reach statistical significance (NTR OR 1.52, 95%CI 0.89-2.60 and CATSS OR 2.23, 95%CI 0.90-5.53).

Sensitivity analyses for asthma

Sensitivity analyses, in which we 1) only included asthma over 5 years (NTR), 2) used a validated definition for asthma and eczema based on medication and diagnosis (CATSS) and 3) only investigated twins with parental answers to questions on any given asthma diagnosis from 2 years of age (CATSS), demonstrated similar findings (Supplementary table 3, 4 and 5).

Antibiotics commonly prescribed for respiratory and urinary tract/skin infections and asthma

Subgroup analyses showed that asthma risk was associated with early-life exposure to antibiotics commonly prescribed for respiratory tract infections (OR 1.45, 95%CI 1.34-1.56; Supplementary table 6a). After restricting to DZ twins, the risk of developing asthma remained (OR 1.91, 95%CI 1.12-3.27; Supplementary table 6a). In MZ twins, the average effect size remained, but precision was lost: (OR 1.82, 95%CI 0.76-2.37; Supplementary table 6a).

Early-life exposure to antibiotics commonly prescribed for urinary tract/skin infections was however not associated with asthma in the unmatched analysis (OR 1.02, 95%CI 0.88-1.17; Table 3). Results of children with antibiotic use commonly prescribed for urinary tract/skin infections, after controlling for antibiotics commonly prescribed for respiratory tract infections during infancy could not be interpreted due to insufficient power (Supplementary table 6b and 10).

Narrow and broad spectrum antibiotics and asthma

Unmatched cohort analyses for use of BS and NS antibiotics showed that both were associated with increased risk of asthma, with the highest risk associated with NS antibiotics (OR for NS antibiotics 1.83, 95%CI 1.50-2.24; OR for BS antibiotics 1.24, 95%CI 1.11-1.38). In NS antibiotics, the direction of the association remained after controlling for shared familial environment (OR 1.95, 95%CI 1.22-3.14) when studying MZ and DZ twins and when exposure to BS antibiotics was studied (OR 1.38, 95%CI 0.63-3.03) with some loss in precision. Point estimates were similar, but confidence intervals included the null when restricted to MZ twins for use of NS antibiotics (OR 1.92, 95%CI 0.73-4.99) and for use of BS antibiotics (OR 3.13, 95%CI 0.79-12.47) (Supplementary tables 7a and 7b).

Antibiotics and eczema

Increased risk of eczema was observed after early-life antibiotic use in the unmatched cohort analysis in the NTR (OR 1.08, 95%CI 1.03-1.13) and CATSS (OR 1.19, 95%CI 1.03-1.38) (Table 4). However, this

risk attenuated after controlling for shared familial environmental factors in the NTR including same sex DZ (OR 1.01, 95%CI 0.78-1.30), but not in CATSS (OR 1.86, 95%CI 1.20-2.89). In MZ pairs, the association attenuated in the NTR (OR 1.04, 95%CI 0.71 to 1.54), but increased odds were found in CATSS (OR 1.77, 95%CI 1.00-3.17).

Sensitivity analyses for eczema

The results of our sensitivity analyses in which we 1) only included eczema over three years and 2) re-defined eczema based on medication and diagnosis (CATSS), showed consistent results in the NTR. In CATSS the risk estimates for same sex DZ and MZ moved towards the null compared to unmatched analyses (Supplementary tables 8 and 9).

Discussion

In this large twin study, early-life exposure to antibiotics was associated with the development of asthma, even after correcting for shared familial environment in DZ same sex discordant twin pairs. After correction for shared familial environment and genetics in MZ discordant twin pairs, there was reduced power, but the point estimates were in the same direction. The association between early-life antibiotic exposure and risk of eczema was potentially confounded by shared familial environment and genetics.

This study is the first using a co-twin control analysis assessing the impact of confounding by shared familial environment and genetics in the association of early-life antibiotics use with asthma and eczema. Our finding that early-life exposure to antibiotics was associated with an increased risk of asthma in the unmatched cohort study in the NTR and CATSS aligns with previous population-based cohort studies [12,16,21] suggesting that population of twins may be representative of the general paediatric population. Note that the overall rate of antibiotic exposure in our population is consistent with national data describing antibiotic use in young children in the Netherlands (31% in 2019)[30] and

Sweden (26-61% depending on the region in 2010)[31], these percentages tend to be among the lowest in the world.

In our study, after analysing matched discordant twins (both MZ and DZ) analyses, the risk of asthma remained for antibiotics commonly prescribed for respiratory infections, but not for antibiotics commonly prescribed for urinary tract/skin infections. These results correspond with findings in the Swedish sibling study [12]: increased risk of asthma was observed in siblings treated with antibiotics in early-life commonly prescribed for respiratory tract infections, but not for urinary tract/skin infections. In contrast, a Finnish nested case-control study (n=6,690 case-control pairs) found an increase in risk (OR 1.60, 95%CI 1.48–1.73) of asthma in 23 children treated with antibiotics commonly prescribed for urinary tract infections (defined as trimethoprim and its combinations with sulphonamides) in early-life, which differed from our definition of antibiotics prescribed for urinary tract/skin infections [32]. A relatively small sample size and differences in selected antibiotics may explain this discordance in findings.

Our study also shows that NS and BS antibiotics use was associated with increased risk of asthma, even after controlling for shared familial environment and genetics, which was consistent with other studies. A birth cohort of 13,116 Canadian children found increased risk of asthma at age 7 for children exposed during the first year of life to BS (OR 1.50; 95%CI, 1.16 to 1.93) and NS antibiotics (OR 1.35; 95%CI, 0.29 to 6.23), although the BS and NS study suffered from reduced power. Moreover, a Finnish nested case-control study also showed a positive association in 41 children treated with antibiotics against gram-positive bacterial infections and urinary tract infections, overlapping with the BS antibiotics in our analysis [32]. However, to our knowledge we are the first investigating the effects of antibiotic spectra in a twin design. Prescription of NS antibiotics was more common in our population compared to BS antibiotics in both the group with and the group without asthma, when adjusted for overlap of use of both types of antibiotics groups within the time frame of

two years. We would have expected to observe a similar effect of NS and BS antibiotics, or BS antibiotics should have even shown a larger risk of asthma, regarding the hypothesis of its influence on the microbiota. If the respiratory infection is driving the asthma, this could be a potential explanation. However, we lack information regarding respiratory infections and cannot conclude this with the current data.

In the NTR, the association between early-life antibiotic use and eczema attenuated in the co-twin control analysis, suggesting possible confounding by shared familial environment and genetics. In CATSS, the association between early-life antibiotic use and eczema also attenuated when the definition for eczema was based on medication and diagnoses after two years, rather than parent-report for eczema ever. Since eczema is common in children under 2 years, the parent-report definition is open to reverse causation bias, therefore the validated definition using medication and diagnosis over two years is a more robust measure. Our results in the unmatched analyses are similar to previous studies, which did not assess confounding by shared genetics or familial factors [17,20]. Considering the above, we hypothesize that the association between early-life antibiotic use and eczema may be driven by confounding from shared environmental factors, whereas this may not be the case for asthma. We expected to find similar findings for asthma and eczema as the causal pathways of both diseases are assumed to be similar. A large GWAS of a multi-disease phenotype based on information from three genetically correlated diseases, asthma, hay fever and eczema, also identified six variants with disease-specific effects besides 130 overlapping variants[33], showing that the three diseases share some but not all characteristics. Confounding factors may also be disease-specific and cannot be unravelled in the current study design.

The positive association between all antibiotics and risk of asthma can possibly be elucidated by the microbial diversity hypothesis. It states that early-life exposure to microorganisms increases variety in gut microflora and T1 immune system development, and therefore protects against asthma [34,35]. Antibiotic therapy affects microbiome variety and thus may increase the risk of atopic diseases.

Another possible underlying reason may be that the associations observed were due to severity of underlying infections rather than the antibiotic itself. Respiratory viral infections in particular have shown to initiate a cascade of host immune responses altering microbial growth in the respiratory tract and gut [36]. In our analysis, we attempted to assess the impact of confounding by indication due to respiratory infections using antibiotic subgroups as proxies for infections (respiratory vs urinary tract/skin infections). The analysis for urinary tract infection antibiotics showed no associations and therefore could suggest that the association between antibiotics for respiratory infections and asthma is due to confounding by indication, but there are some limitations to this analysis. Infections that do not warrant antibiotics are not captured in our databases and, as such, we could not assess whether observed associations were due to specific infections or antibiotics, as they occur simultaneously. Moreover, the small sample size in the UTI subgroup may not have allowed us to detect moderate associations in this subgroup. Therefore, we hesitate to conclude that confounding by indication is driving the results.

The strength of our study is the large discovery cohort including almost 35,000 Dutch twins and replication in almost 8,000 Swedish twins. We found similar results for asthma in CATSS compared to the NTR, meaning that our results are generalisable to Western European children. Our twin design controls for shared familial environment and genetics. We addressed recall bias, reverse causation for asthma, and validated our asthma and eczema definition using ICD-codes.

This study also had some limitations. Firstly, our discovery cohort only consisted of parental-reported antibiotic use. Nevertheless, antibiotic use between 0-2 years was ascertained in the NTR at 2 years, minimizing recall bias. Moreover, after defining use of antibiotics with medication dispenses in CATSS, results were similar. Secondly, our subgroup analyses had reduced power because ATC-codes were only available in CATSS (Supplements). Thirdly, outcome misclassification may have occurred

due to parental-reported measures for asthma and eczema. Fourth, more children in CATSS were delivered via caesarean section compared to the NTR and this may result in differences in risks of asthma or eczema development.

To conclude, despite familial and genetic factors, children exposed to early-life antibiotic use, particularly antibiotics commonly prescribed for respiratory tract infections, may be at a higher risk of asthma. Antibiotic use was also associated with increased risk of eczema, however it is likely that the relationship between early-life antibiotic use and eczema is confounded by shared familial environment and genetic factors. Given our findings, physicians should ensure that antibiotics are prescribed for bacterial infections only. Future studies should have a prospective design monitoring timing of events, type of infection, and number of antibiotics used in early-life.

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Conflict of Interest

A.H. Maitland-van der Zee received unrestricted research funding from GSK and Boehringer Ingelheim. She participated in advisory boards of Astra Zeneca and Boehringer Ingelheim. G.H. Koppelman reports grants from Lung Foundation of the Netherlands, TEVA the Netherlands, UBBO EMMIUS Foundation, TETRI Foundation, GSK, and VERTEX, outside the submitted work; and GHK partipated in a global advisory board on pediatric asthma for GSK. C. Longo, S.J.H. Vijverberg, M.W. Pijnenburg, B.K. Brew, D.I. Boomsma, C. Almqvist, C.J.A.R. Kats, C.E.M. van Beijsterveldt, M. Bartels, P. Magnusson, T. Gong, C. Dolan, P. Lichtenstein and E.M.A. Slob report no COI for this study.

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Large twin studies show that antibiotics in early life is associated with risk of asthma, regardless of familial environment and genetics and possibly due to early infections. Risks and benefits of antibiotics use in infants should be considered.

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Table 1A. Summary characteristics of the NTR and CATSS populations in the unmatched cohort study for asthma (step 1).

	All individuals NTR (n=34,352)	Asthma NTR (n=5,181)	No asthma NTR (n=29,171)	All individuals CATSS (n=7,906)	Asthma CATSS (n=1,381)	No asthma CATSS (n=6,525)
Gender						
Male	17,638 (49.9%)	2,623 (51.0%)	15,015 (49.7%)	3,955 (50.0%)	832 (60.2%)	3,123 (47.9%)
Delivery mode						
Caesarean section	10,116 (31.2%)	1,488 (31.3%)	8,628 (31.1%)	3,252 (52.2%)	562 (52.7%)	2,691 (52.0%)
Gestational age						
< 37 wk	14,645 (41.4%)	2,489 (48.4%)	12,156 (40.2%)	4,259 (53.9%)	854 (61.8%)	3,405 (52.2%)
≥ 37 wk	20,720 (58.6%)	2,652 (51.6%)	18,068 (59.8%)	3,647 (46.1%)	527 (38.2%)	3,120 (47.8%)
Birth weight (g)	2,530 (720)	2,442 (760)	2,540 (710)	2,670 (720)	2,570 (821)	2,685 (710)
Breast feeding				NA	NA	NA
None	14,524 (41.2%)	2,379 (46.5%)	12,145 (40.3%)			
< 2 wk	3,538 (10.0%)	504 (9.8%)	3,034 (10.1%)			
2-6 wk	5,502 (15.6%)	749 (14.6%)	4,753 (15.8%)			
6 wk– 3 mths	4,572 (13.0%)	618 (12.1%)	3,954 (13.1%)			
3-6 mths	3,631 (10.3%)	464 (9.1%)	3,167 (10.5%)			
> 6 mths	3,459 (9.8%)	404 (7.9%)	3,055 (10.1%)			
Area type during early-life						
Rural area	8,938 (25.6%)	1,246 (24.5%)	7,692 (25.8%)	2,385 (30.2%)	452 (32.8%)	1,933 (29.7%)
Older siblings						
None	14,709 (54.3%)	2,260 (54.7%)	12,449 (54.2%)	1,391 (22.3%)	273 (25.6%)	1118 (21.6%)
Outside home child care				NA	NA	NA
None	2,404 (8.1%)	353 (7.9%)	2,051 (8.1%)			
1-4 h/wk	3,804 (12.8%)	631 (14.2%)	3,173 (12.6%)			
5-8 h/wk	8,049 (27.1%)	1,273 (28.5%)	6,776 (26.9%)			
9-16 h/wk	6,541 (22.0%)	987 (22.1%)	5,554 (22.0%)			
17-24 h/wk	5,851 (19.7%)	792 (17.8%)	5,059 (20.0%)			
>24 h/wk	3,039 (10.2%)	420 (9.4%)	2,619 (10.4%)			
educational attainment mother						
≤ 9 years	1,328 (4.2%)	304 (6.0%)	1,024 (3.8%)	166 (2.4%)	32 (2.5%)	134 (2.3%)
10-12 years	8,217 (25.9%)	1,451 (28.8%)	6,766 (25.3%)	1,926 (27.3%)	379 (30.2%)	1,547 (26.6%)
< 2 years tertiary	13,348 (42.1%)	2,110 (41.9%)	11,238 (42.1%)	954 (13.5%)	164 (13.1%)	790 (13.6%)
≥ 2 years tertiary	8,844 (27.9%)	1,166 (23.2%)	7,678 (28.8%)	4,017 (56.9%)	680 (54.2%)	3,337 (57.5%)
educational attainment father						
≤ 9 years	1,777 (5.7%)	388 (7.9%)	1,389 5.3%)	354 (4.7%)	74 (5.6%)	280 (4.5%)
10-12 years	8,262 (26.5%)	1,446 (29.3%)	6,816 (26.0%)	3,135 (41.3%)	588 (44.1%)	2,557 (40.7%)
< 2 years tertiary	11,192 (35.9%)	1,746 (35.3%)	9,446 (36.0%)	845 (11.1%)	147 (11.0%)	698 (11.1%)
≥ 2 years tertiary	9,931 (31.9%)	1,354 (27.4%)	8,577 (32.7%)	3,254 (42.9%)	523 (39.3%)	2,731 (43.7%)
Smoking mother				NA	NA	NA
No smoking	25,149 (77.0%)	3,804 (74.7%)	21,345 (77.5%)			
cigars/pipe	89 (0.3%)	10 (0.2%)	79 (0.3%)			
< 10 cigarettes	3,430 (10.5%)	577 (11.3%)	2,853 (10.4%)			
> 10 cigarettes	3,844 (11.8%)	683 (13.4%)	3,161 (11.5%)			
Do not know	57 (0.2%)	5 (0.1%)	52 (0.2%)			
Always outside	78 (0.4%)	13 (0.3%)	65 (0.2%)			
				NA	NA	NA
Smoking father						
No smoking	19,490 (70.9%)	3,453 (68.2%)	22,943 (70.5%)			
cigars/pipe	621 (2.3%)	137 (2.7%)	758 (2.3%)			
< 10 cigarettes	2,878 (10.5%)	502 (9.9%)	3,380 (10.4%)			
> 10 cigarettes	4,219 (15.3%)	893 (17.6%)	5,112 (15.7%)			
Do not know	117 (0.4%)	56 (1.1%)	173 (0.5%)			
Always outside	167 (0.6%)	19 (0.4%)	186 (0.6%)			

Categorical characteristics are denoted as frequencies (proportions). Continuous characteristics are denoted as median (inter quartile range). NA=not available

Table 1B. Summary characteristics of the NTR and CATSS populations in the unmatched cohort study for eczema (step 1).

	All individuals NTR (n=32,777)	Eczema NTR (n=6,251)	No eczema NTR (n=26,526)	All individuals CATSS (n=7,916)	Eczema STR (n=1,730)	No eczema CATSS (n=6,186)
Gender						
Male	17,520 (61.1%)	3239 (49.9%)	14,281 (49.8%)	3,962 (50.1%)	809 (46.8%)	3,153 (51.0%)
Delivery mode						
Caesarean section	10,074 (31.2%)	1,859 (30.9%)	8,215 (31.3%)	3,250 (52.0%)	730 (52.5%)	2,520 (51.9%)
Gestational age						
< 37 wk	14,511 (41.2%)	2,702 (41.6%)	11,809 (41.2%)	4,270 (53.9%)	949 (54.9%)	3,321 (53.7%)
≥ 37 wk	20,672 (58.8%)	3,795 (58.4%)	16,877 (58.8%)	3,646 (46.1%)	781 (45.1%)	2,865 (46.3%)
Birth weight (g)	2,530 (720)	2,530 (730)	2,530 (720)	2,670 (720)	2,655 (705)	
Breast feeding				NA	NA	NA
None	14,410 (41.1%)	2,589 (40.0%)	11,821 (41.4%)			
< 2 wk	3,541 (10.1%)	628 (9.7%)	2,913 (10.2%)			
2-6 wk	5,460 (15.6%)	1,048 (16.2%)	4,412 (15.4%)			
6 wk– 3 mths	4,562 (13.0%)	859 (13.3%)	3,703 (13.0%)			
3-6 mths	3,623 (10.3%)	682 (10.5%)	2,941 (10.3%)			
> 6 mths	3,451 (9.8%)	669 (10.3%)	2,782 (9.7%)			
Area type during early-life						
Rural area	8,897 (25.6%)	1,520 (23.7%)	7,377 (26.0%)	2,392 (30.3%)	495 (28.7%)	1,897 (30.7%)
Older siblings						
None	14,670 (54.5%)	3,072 (53.8%)	11,598 (54.6%)	1,389 (22.2%)	357 (25.6%)	1,032 (21.2%)
Outside home child care				NA	NA	NA
None	2,397 (8.1%)	394 (7.1%)	2,003 (8.4%)			
1-4 h/wk	3,761 (12.7%)	689 (12.4%)	3,072 (12.8%)			
5-8 h/wk	7,968 (27.0%)	1,472 (26.5%)	6,496 (27.1%)			
9-16 h/wk	6,534 (22.1%)	1,230 (22.1%)	5,304 (22.1%)			
17-24 h/wk	5,857 (19.8%)	1,142 (20.5%)	4,715 (19.7%)			
>24 h/wk	3,017 (10.2%)	633 (11.4%)	2,384 (9.9%)			
educational attainment mother						
≤ 9 years	1,331 (4.3%)	219 (3.5%)	1,112 (4.6%)	165 (2.3%)	30 (1.9%)	135 (2.4%)
10-12 years	7,971 (26.0%)	1,490 (23.7%)	6,481 (26.6%)	1,931 (27.3%)	408 (26.2%)	1,523 (27.6%)
< 2 years tertiary	12,900 (42.1%)	2,695 (42.8%)	10,205 (41.9%)	961 (13.6%)	225 (14.4%)	736 (13.4%)
≥ 2 years tertiary	8,433 (27.5%)	1,889 (30.0%)	6,544 (26.9%)	4,013 (56.8%)	896 (57.5%)	3,117 (56.6%)
educational attainment father						
≤ 9 years	1,730 (5.8%)	345 (5.6%)	1,385 (0.6%)	354 (4.7%)	60 (3.6%)	294 (5.0%)
10-12 years	8,033 (26.7%)	1,542 (24.9%)	6,491 (27.1%)	3,144 (41.4%)	657 (39.5%)	2,487 (41.9%)
< 2 years tertiary	10,815 (36.0%)	2,178 (35.1%)	8,637 (36.2%)	848 (11.2%)	203 (12.2%)	645 (10.9%)
≥ 2 years tertiary	9,501 (31.6%)	2,140 (34.5%)	7,361 (30.8%)	3,248 (42.8%)	742 (44.6%)	2,506 (42.2%)
Smoking mother				NA	NA	NA
No smoking	24,156 (76.7%)	5,093 (79.1%)	19,063 (76.0%)			
cigars/pipe	82 (0.3%)	17 (0.3%)	65 (0.3%)			
< 10 cigarettes	3,331 (10.6%)	655 (10.2%)	2,676 (10.7%)			
> 10 cigarettes	3,803 (12.1%)	635 (9.9%)	3,168 (12.6%)			
Do not know	54 (0.2%)	9 (0.1%)	45 (0.2%)			
Always outside	76 (0.2%)	25 (0.4%)	51 (0.2%)			
Smoking father				NA	NA	NA
No smoking	17,412 (69.7%)	4,605 (71.7%)	22,017 (70.1%)			
cigars/pipe	575 (2.3%)	146 (2.3%)	721 (2.3%)			
< 10 cigarettes	2,681 (10.7%)	641 (10.0%)	3,322 (10.6%)			
> 10 cigarettes	4,047 (16.2%)	952 (14.8%)	4,999 (15.9%)			
Do not know	128 (0.5%)	38 (0.6%)	166 (0.5%)			
Always outside	135 (0.5%)	40 (0.6%)	175 (0.6%)			

Categorical characteristics are denoted as frequencies (proportions). Continuous characteristics are denoted as median (inter quartile range). NA=not available

Table 2. Early-life antibiotics use and subsequent risk of asthma

		n AB/asthma (%)	n AB/without asthma (%)	OR adjusted (95% CI)
NTR	**Unmatched**	2,132/4,896 (43.5%)	8,260/24,988 (33.1%)	1.34 (1.28-1.41)***,#
	MZ and same sex DZ	534/1,312 (40.7%)	482/1,312 (36.7%)	1.54 (1.20-1.98)**,§
	Same sex DZ	383/924 (41.5%)	342/924 (37.0%)	1.60 (1.18-2.16)**,§
	MZ	151/388 (38.9%)	140/388 (36.1%)	1.52 (0.89-2.60) §
CATSS	**Unmatched**	768/1,381 (55.6%)	2,455/5,825 (42.1%)	1.45 (1.34-1.56)***,£
	MZ and same sex DZ	206/380 (54.2%)	174/380 (45.7%)	2.00 (1.28-3.13)**, §
	Same sex DZ	141/275 (51.3%)	122/275 (44.4%)	1.86 (1.11-3.18)*,§
	MZ	65/105 (61.9%)	55/105 (52.4%)	2.23 (0.90-5.53)§

AB: users of any antibiotics, MZ: monozygotic twin pair level, DZ: dizygotic twin pair level, OR: odds ratio, CI: confidence interval, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, # adjusted for: socioeconomic status, hours of outside childcare, breastfeeding, delivery mode, gender, birth weight, § adjusted for birth weight, £ adjusted for: educational attainment, delivery mode, gender, birth weight.

Table 3. Early-life antibiotics use commonly prescribed for urinary tract/skin infections and subsequent risk of asthma (with correction for respiratory antibiotics)

		n AB/asthma (%)	n AB/without asthma (%)	OR adjusted (95% CI)
CATSS	**Unmatched**	72/1,314 (5.5%)	263/5,584 (4.7%)	1.02 (0.88-1.17)[£]
	MZ and same sex DZ	16/380 (4.2%)	18/380 (4.7%)	0.76 (0.34-1.66)[§]
	Same sex DZ	12/275 (4.4%)	13/275 (4.7%)	0.77 (0.30-1.95)[§]
	MZ	4/105 (3.8%)	5/105 (4.8%)	0.81 (0.18-3.71)[§]

AB: users of antibiotics commonly prescribed for urinary tract/skin infections (pivmecillinam, trimethoprim, sulphonamide, ciprofloxacin, norfloxacin, nitrofurantoin, cloxacillin, flucloxacillin, and dicloxacillin), MZ: monozygotic twin pair level, DZ: dizygotic twin pair level, OR: odds ratio, CI: confidence interval, § adjusted for respiratory antibiotics, birth weight, £ adjusted for: antibiotics commonly prescribed for respiratory infections (amoxicillin, penicillin, cephalosporin, and macrolides), educational attainment, delivery mode, gender, birth weight.

Table 4. Early-life antibiotics use and subsequent risk of eczema

		n AB/eczema (%)	n AB/without eczema (%)	OR adjusted (95% CI)
NTR	**Unmatched**	2,286/5,918 (38.6%)	7,763/22,983 (33.8%)	1.08 (1.03-1.13)[#]
	MZ and same sex DZ	687/1,837 (37.4%)	684/1,837 (37.2%)	0.99 (0.80-1.25)[§]
	Same sex DZ	441/1,191 (37.0%)	441/1,191 (37.0%)	0.96 (0.73-1.26)[§]
	MZ	243/646 (37.6%)	243/646 (37.6%)	1.02 (0.69-1.52)[§]
CATSS	**Unmatched**	819/1,730 (47.3%)	2,278/5,114 (44.5%)	1.19 (1.03-1.38)*[£]
	MZ and same sex DZ	296/624 (47.4%)	255/624 (40.9%)	1.67 (1.12-2.49)*[§]
	Same sex DZ	187/411 (45.5%)	160/411 (38.9%)	1.71 (1.10-2.68)*[§]
	MZ	109/213 (51.2%)	95/213 (44.6%)	1.76 (0.98-3.14)[§]

AB: users of any antibiotics, MZ: monozygotic twin pair level, DZ: dizygotic twin pair level, OR: odds ratio, CI: confidence interval, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, # adjusted for: socioeconomic status, hours of outside childcare, breastfeeding, delivery mode, gender, birth weight, asthma § adjusted for birth weight, asthma £ adjusted for: educational attainment, delivery mode, gender, birth weight, asthma.

Supplementary Information

Supplementary table 1. Overview NTR survey questions and outcome definitions regarding early-life antibiotic use, asthma and eczema.

SURVEY ITEM	INTENDED AGE	QUESTION	OUTCOME DEFINITION
ANTIBIOTIC USE	2 years old	“Did the twins ever use drugs?”	Specified drug name of an antibiotic‡ or collective name 'antibiotics'.
ASTHMA	3, 7 and 10 years old	"Could you indicate for each of the following conditions, whether it applies?"	Checked box for 'asthma, chronic bronchitis or CARA’.
	5 years old	“Did a doctor ever diagnose the twin with: ”	Checked box for 'asthma'.
ECZEMA	3, 7 and 10 years old	"Could you indicate for each of the following conditions, whether it applies?"	Checked box for 'severe skin disease or eczema'.
	5 years old	“Did a doctor ever diagnose the twin with: ”	Checked box for ‘eczema’.

CARA: chronic non-specific lung diseases. ‡: antibacterials for systemic use according to the Anatomical Therapeutic Chemical classification, defined by codes starting with 'J01'.

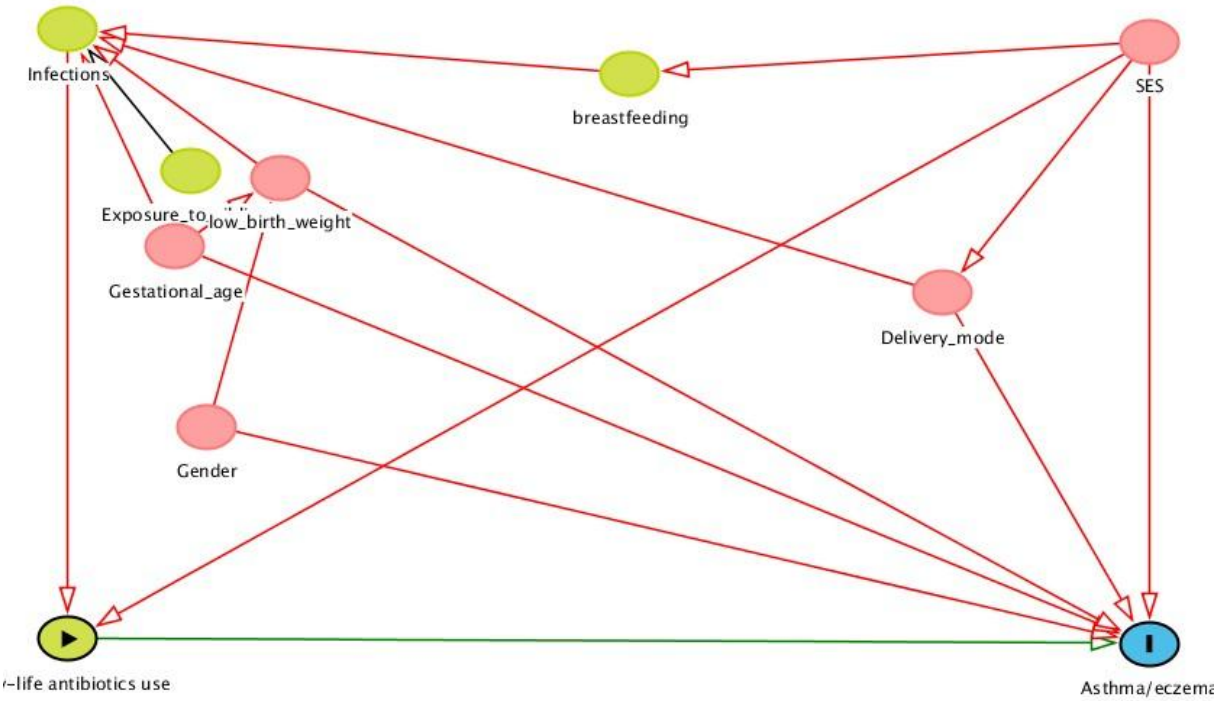
Supplementary table 2. Overview CATSS survey questions, medication, diagnoses and outcome definitions regarding early-life antibiotic use, asthma and eczema.

SURVEY ITEM	INTENDED AGE	OUTCOME DEFINITION	CODES
ANTIBIOTIC USE	0-2 years	Use of antibiotics	ATC code J01
RESPIRATORY ANTIBIOTIC USE	0-2 years	Use of respiratory antibiotics: amoxicillin, penicillin, cephalosporin, and macrolides	ATC codes J01CA04, J01C, J01D, J01FA.
URINARY TRACT ANTIBIOTIC USE	0-2 years	Use of urinary tract antibiotics: pivmecillinam, trimethoprim, sulphonamide, ciprofloxacin, norfloxacin, nitrofurantoin, cloxacillin, flucloxacillin, and dicloxacillin.	ATC codes: J01CA02, J01EA01, J01EB, J01MA02, J01MA06, J01XE01, J01CF02, J01CF05, J01CF01.
ASTHMA	9 years	Parent reported outcome diagnosis: Does your child ever had asthma? Question	
	>= 2 years	Asthma diagnosis in the NPR	ICD-10 code J45 and J46 in the NPR
	< 4.5 years Sensitivity analysis	two or more prescriptions for preventer medications (ICS, LRTA, ICS combinations) OR 3 SABA prescriptions OR 2 SABA and 1 preventer medication. Children under 4.5 years were required to fulfil both diagnosis AND medication criteria in order be more certain that children had asthma rather than preschool wheeze in this age group.	ATC codes for respiratory drugs in SPDR: R03BA, R03DC03, R03AK, R03AC. ICD-10 code J45 and J46 in the NPR
	> 4.5 years Sensitivity analysis	a) two or more dispenses of preventer medications since 2005, that is either; inhaled corticosteroids (ICS, ATC code R03BA), leukotriene receptor agonists (LTRA, ATC code R03DC03) or fixed combinations of β2-agonists and corticosteroids (β2-ICS, ATC code R03AK) ; b) two dispenses of β2-adrenoreceptor agonists (ATC code R03AC), and either a third dispense of a β2- adrenoreceptor agonist or of a preventer medication (ICS, LTRA, β2-ICS) in any 12 month period; c) an asthma diagnosis in the NPR after the age of 4.5 years.	ATC codes for respiratory drugs in SPDR: R03BA, R03DC03, R03AK06, R03AK07, R03AC. ICD-10 code J45 and J46 in the NPR
ECZEMA	9 years	Parent reported outcome diagnosis: Does your child ever have eczema? Question	
	3-12 years	Children with atopic dermatitis fulfilled either criterion 1 – 3.	
		CRITERIA 1 (ICD-10):	
		≥1 hospital contact for: atopic dermatitis and/or winter feet.	L20 “atopic dermatitis” L308C “winter feet”

		CRITERIA 2 (based on ATC):	
		≥1 filled prescription of: “agents for dermatitis: tacrolimus, pimecrolimus” without any of the exclusion criteria specified below	D11AH
		CRITERIA 3 (based on ATC):	
		≥ 2 filled prescriptions of: “corticosteroids for topical use” within 12 months without any of the exclusion criteria specified below, without co-occurring hospital contacts and/ or combination of filled prescriptions below (exclusions criteria): Children WITHOUT atopic dermatitis with a diagnosis of seborrheic dermatitis, diaper dermatitis, allergic contact dermatitis, irritant contact dermatitis, unspecified contact dermatitis, exfoliative dermatitis, dermatitis due to substances taken internally, lichen simplex chronicus and prurigo, prutitis, other dermatitis, papulosquamous disorders, other erythematosus conditions, sunburn, other acute skin changes due to ultraviolet radiation, vitiligo, atrophic disorders of the skin, lupus erythematosus.	D07 “corticosteroids for topical use” L20 “atopic dermatitis” L21 “seborrhoeic dermatitis” L22 “diaper dermatitis” L23 “allergic contact dermatitis” L24 “irritant contact dermatitis” L25 “unspecified contact dermatitis” L26 “exfoliative dermatitis” L27 “dermatitis due to substances taken internally” L28 “lichen simplex chronicus and prurigo” L29 “pruritus” L30 “other dermatitis” (except L308C) L40 – L45 “papulosquamous disorders” L53 “other erythematosus conditions” L55 “sunburn” L56 “other acute skin changes due to ultraviolet radiation” L80 “vitiligo” L90 “atrophic disorders of the skin” L93 “lupus erythematosus”
		Exclusion medication criteria: ≥1 filled prescription of either: antipsoriasics or salicylates for dermatological use or corticosteroids moderate or potent other combinations or corticosteroids (group IV) clobetasol or clobetasol and antibiotics and antifungals. *For prescription of corticosteroid group IV, a filled prescription of group I-III should also be used (as atopic dermatitisis never treated alone with group IV)	D05 “antipsoriasics” D02AF “salicylates for dermatological use” D07XB “corticosteroids moderate or potent other combinations“ D07XC “corticosteroids moderate or potent other combinations“ D07AD01 “corticosteroids (group IV) clobetasol” D07CD01 “clobetasol and

			antibiotics") D01 "antifungals" (implies corticosteroid (group IV) use for vaginal fungal infection)
NARROW SPECTRUM ANTIBIOTICS	0-2 years	Use of the following antibiotics: vancomycin (oral), benzylpenicillin, phenoxymethylpenicillin, dicloxacillin, flucloxacillin, cephalexine, aztreonam, trimethoprim, sulfamethizole, erythromycin, roxithromycin, clarithromycin, azithromycin, clindamycin, vancomycin, teicoplanin, fusic acid, metronidazole (intravenous), fusidic acid, metronidazole, nitrofurantoin, linezolide, daptomycin, rifampicin, rifabutin, metronidazole. (30)	ATC code: A07AA09, J01CE01, J01CE02, J01CF01, J01CF05, J01DB01, J01DF01, J01EA0, J01EB02, J01FA01, J01FA06, J01FA09, J01FA10, J01FF01, J01XA01, J01XA02, J01XC01, J01XD01, J01XE01, J01XX08, J01XX09, J04AB02, J04AB04, P01AB01.
BROAD SPECTRUM ANTIBIOTICS	0-2 years	Use of the following antibiotics: doxycycline, lymecline, tetracyclines, tigecycline, ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, cefataxime, ceftazidime, ceftriaxone, meropenem, ertapenem, sulfamethoxazole-trimethoprim, tobramycin, gentamycin, ofloxacin, ciprofloxacin, moxifloxacin, colistinmethatnatrium, (30)	ATC code: J01AA01, J01AA04, J01AA07, J01AA12, J01CA01, J01CA02, J01CA04, J01CA08, J01CA11, J01CR02, J01CR05, J01DC02, J01DD01, J01DD02, J01DD04, J01DH02, J01DH03, J01EE01, J01GB01, J01GB03, J01MA01, J01MA14, J01XB01.

Supplementary Figure 1. DAG for early-life antibiotics use and increased risk of asthma and eczema



Supplementary table 3. Sensitivity analysis on increased risk of asthma reported at 5-10 years in the NTR

		n AB/asthma (%)	n AB/without asthma (%)	OR adjusted (95% CI)
NTR	**Unmatched**	1,598/3,706 (43.1%)	8,545/25,271 (33.8%)	1.34 (1.26-1.43)*** #
	MZ and same sex DZ	451/1,113 (40.5%)	424/1,113 (38.1%)	1.41 (1.07-1.86)* §
	Same sex DZ	311/760 (40.9%)	293/760 (38.6%)	1.26 (0.90-1.74) §
	MZ	140/353 (39.7%)	131/353 (37.1%)	1.31 (0.98-1.74) §

AB: users of any antibiotics, MZ: monozygotic twin pair level, DZ: dizygotic twin pair level, OR: odds ratio, CI: confidence interval, * p < 0.05, ** p < 0.01, *** p < 0.001, # adjusted for: educational attainment, hours of outside childcare, breastfeeding, delivery mode, gender, birth weight, § adjusted for: birth weight.

Supplementary table 4. Asthma sensitivity analysis using medication and diagnosis data at age 3-9 years in CATSS

		n AB/asthma (%)	n AB/without asthma (%)	OR adjusted (95% CI)
CATSS	**Unmatched**	568/785 (74.5%)	2,714/6,700 (40.5%)	1.60 (1.43-1.78)***, £
	MZ and same sex DZ	193/276 (69.9%)	167/276 (60.5%)	2.05 (1.27-3.31)* §
	Same sex DZ	126/184 (65.8%)	110/184 (59.8%)	2.08 (1.13-3.80)* §
	MZ	67/92 (72.8%)	57/92 (61.9%)	2.15 (0.94-4.92) §

AB: users of any antibiotics, MZ: monozygotic twin pair level, DZ: dizygotic twin pair level, OR: odds ratio, CI: confidence interval, * p < 0.05, ** p < 0.01, *** p < 0.001, § adjusted for birth weight, £ adjusted for: respiratory antibiotics (amoxicillin, penicillin, cephalosporin, and macrolides), educational attainment, delivery mode, gender, birth weight.

Supplementary table 5. Asthma sensitivity analysis using parental-reported asthma diagnosis after two years of age in CATSS

		n AB/asthma (%)	n AB/without asthma (%)	OR adjusted (95% CI)
STR	**Unmatched**	54/108 (50.0%)	3,386/7,598 (44.6%)	2.07 (1.19-3.61)*, £
	MZ and same sex DZ	73/147 (49.7%)	63/147 (42.9%)	1.78 (0.90-3.53) §
	Same sex DZ	48/96 (50.0%)	42/96 (43.7%)	1.60 (0.72-3.52) §
	MZ	25/51 (49.0%)	21/51 (41.1%)	2.97 (0.69-12.80) §

AB: users of antibiotics, MZ: monozygotic twin pair level, DZ: dizygotic twin pair level, OR: odds ratio, CI: confidence interval, , * p < 0.05, ** p < 0.01, *** p < 0.001, £ adjusted for: educational attainment, delivery mode, gender, birth weight. § adjusted for birth weight.

Supplementary table 6a. Sensitivity analysis using antibiotics commonly prescribed for respiratory infections In CATSS

		n AB/asthma (%)	n AB/without asthma (%)	OR adjusted (95% CI)
CATSS	**Unmatched**	775/1381 (54.7%)	2,426/5,835 (41.6%)	1.45 (1.34-1.56)***£
	MZ and same sex DZ	203/380 (53.4%)	177/380 (46.6%)	1.93 (1.23-3.02)**§
	Same sex DZ	140/275 (50.9%)	121/275 (44.0%)	1.91 (1.12-3.27)§
	MZ	63/105 (42.0%)	55/105 (52.4%)	1.82 (0.76-2.37)§

AB: users of antibiotics commonly prescribed for respiratory infections (amoxicillin, penicillin, cephalosporin, and macrolides), MZ: monozygotic twin pair level, DZ: dizygotic twin pair level, OR: odds ratio, CI: confidence interval, * p < 0.05, ** p < 0.01, *** p < 0.001, § adjusted for antibiotics commonly prescribed for urinary tract/skin infections (pivmecillinam, trimethoprim, sulphonamide, ciprofloxacin, norfloxacin, nitrofurantoin, cloxacillin, flucloxacillin, and dicloxacillin), birth weight, £ adjusted for:

antibiotics commonly prescribed for urinary tract/skin infections (pivmecillinam, trimethoprim, sulphonamide, ciprofloxacin, norfloxacin, nitrofurantoin, cloxacillin, flucloxacillin, and dicloxacillin), educational attainment, delivery mode, gender, birth weight.

Supplementary table 6b. Sensitivity analysis using antibiotics commonly prescribed for urinary tract and skin infections (without respiratory infections) in CATSS

		n AB/asthma (%)	n AB/without asthma (%)	OR adjusted (95% CI)
CATSS	**Unmatched**	5/1,314 (0.40%)	12/5,584 (0.21%)	1.66 (0.96-2.88)[£]
	MZ and same sex DZ	1/363 (0.27%)	1/363 (0.27%)	Insufficient power
	Same sex DZ	0/263 (0.0%)	1/263 (0.38%)	Insufficient power
	MZ	1/100 (1.0%)	0/100 (0.0%)	Insufficient power

AB: antibiotics commonly prescribed for urinary tract/skin infections (pivmecillinam, trimethoprim, sulphonamide, ciprofloxacin, norfloxacin, nitrofurantoin, cloxacillin, flucloxacillin, and dicloxacillin), MZ: monozygotic twin pair level, DZ: dizygotic twin pair level, OR: odds ratio, CI: confidence interval, * p < 0.05, ** p < 0.01, *** p < 0.001, £ adjusted for: educational attainment, delivery mode, gender, birth weight.

Supplementary table 7a. Sensitivity analysis using narrow spectrum antibiotics in CATSS

		n AB/asthma (%)	n AB/without asthma (%)	OR adjusted for broad spectrum (95%CI)
CATSS	**Unmatched**	775/1381 (54.7%)	2,426/5,835 (41.6%)	1.83 (1.50-2.24)***[≠]
	MZ and same sex DZ	203/380 (53.4%)	177/380 (46.6%)	1.95 (1.22-3.14)**[,×]
	Same sex DZ	140/275 (50.9%)	121/275 (44.0%)	1.92 (1.10-3.36)*[,×]
	MZ	63/105 (60.0%)	55/105 (52.4%)	1.92 (0.73-4.99)[×]

AB: users of narrow spectrum antibiotics (vancomycin (oral), benzylpenicillin, phenoxymethylpenicillin, dicloxacillin, flucloxacillin, cephalexine, aztreonam, trimethoprim, sulfamethizole, erythromycin, roxithromycin, clarithromycin, azithromycin, clindamycin, vancomycin, teicoplanin, fusic acid, metronidazole (intravenous), fusidic acid, metronidazole, nitrofurantoin, linezolide, daptomycin, rifampicin, rifabutin, metronidazole), MZ: monozygotic twin pair level, DZ: dizygotic twin pair level, OR: odds ratio, CI: confidence interval, * p < 0.05, ** p < 0.01, *** p < 0.001, § adjusted for birth weight £ adjusted for educational attainment, delivery mode, gender, birth weight, ≠ adjusted for: broad spectrum antibiotics (doxycycline, lymecline, tetracyclines, tigecycline, ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, cefataxime, ceftazidime, ceftriaxone, meropenem, ertapenem, sulfamethoxazole-trimethoprim, tobramycin, gentamycin, ofloxacin, ciprofloxacin, moxifloxacin, colistinmethatnatrium), socioeconomic status, delivery mode, gender, birth weight, × adjusted for: birth weight, broad spectrum antibiotics (doxycycline, lymecline, tetracyclines, tigecycline, ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, cefataxime, ceftazidime, ceftriaxone, meropenem, ertapenem, sulfamethoxazole-trimethoprim, tobramycin, gentamycin, ofloxacin, ciprofloxacin, moxifloxacin, colistinmethatnatrium).

Supplementary table 7b. Sensitivity analysis using broad spectrum antibiotics in CATSS

		n AB/asthma (%)	n AB/without asthma (%)	OR adjusted for narrow spectrum (95% CI)
CATSS	**Unmatched**	371/1,374 (27.0%)	1,010/5,842 (17.3%)	1.24 (1.11-1.38)***[£]
	MZ and same sex DZ	93/380 (24.5%)	87/380 (22.9%)	1.38 (0.63-3.03)[§]
	Same sex DZ	63/275 (22.9%)	59/275 (21.5%)	0.97 (0.48-1.98)[§]
	MZ	30/105 (28.6%)	28/105 (26.7%)	3.13 (0.79-12.47)[§]

AB: users of broad spectrum antibiotics (doxycycline, lymecline, tetracyclines, tigecycline, ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, cefataxime, ceftazidime, ceftriaxone, meropenem, ertapenem, sulfamethoxazole-trimethoprim, tobramycin, gentamycin, ofloxacin, ciprofloxacin, moxifloxacin, colistinmethatnatrium), MZ: monozygotic twin pair level, DZ: dizygotic twin pair level, OR: odds ratio, CI: confidence interval, * p

< 0.05, ** p < 0.01, *** p < 0.001, § adjusted for : narrow spectrum antibiotics(vancomycin (oral), benzylpenicillin, phenoxymethylpenicillin, dicloxacillin, flucloxacillin, cephalexine, aztreonam, trimethoprim, sulfamethizole, erythromycin, roxithromycin, clarithromycin, azithromycin, clindamycin, vancomycin, teicoplanin, fusic acid, metronidazole (intravenous), fusidic acid, metronidazole, nitrofurantoin, linezolide, daptomycin, rifampicin, rifabutin, metronidazole), birth weight, £ adjusted for: educational attainment, delivery mode, gender, birth weight, narrow spectrum antibiotics (vancomycin (oral), benzylpenicillin, phenoxymethylpenicillin, dicloxacillin, flucloxacillin, cephalexine, aztreonam, trimethoprim, sulfamethizole, erythromycin, roxithromycin, clarithromycin, azithromycin, clindamycin, vancomycin, teicoplanin, fusic acid, metronidazole (intravenous), fusidic acid, metronidazole, nitrofurantoin, linezolide, daptomycin, rifampicin, rifabutin, metronidazole).

Supplementary table 8. Sensitivity analysis eczema after age 3 in NTR

		n AB/eczema (%)	n AB/without eczema (%)	OR adjusted (95% CI)
NTR	**Unmatched**	892/2,236 (39.9%)	6,467/19,285 (33.5%)	1.11 (1.00-1.24)*,#
	MZ and same sex DZ	289/736 (39.3%)	297/736 (40.4%)	0.85 (0.60-1.20)§
	Same sex DZ	178/468 (38.0%)	185/468 (39.5%)	0.82 (0.54-1.23) §
	MZ	111/268 (41.4%)	112/268 (41.8%)	0.92 (0.48-1.78) §

AB: users of antibiotics, MZ: monozygotic twin pair level, DZ: dizygotic twin pair level, OR: odds ratio, CI: confidence interval, , * p < 0.05, ** p < 0.01, *** p < 0.001, # adjusted for: educational attainment, hours of outside childcare, breastfeeding, delivery mode, gender, birth weight, asthma § adjusted for birth weight, asthma

Supplementary table 9. Eczema sensitivity analysis using medication and diagnosis since 2 years of age in CATSS

		n AB/eczema (%)	n AB/without eczema (%)	OR adjusted (95% CI)
STR	**Unmatched**	256/419 (61.1%)	3,184/7,287 (43.7%)	2.39 (1.20-4.74)*,£
	MZ and same sex DZ	133/236 (56.4%)	122/236 (51.7%)	1.33 (0.83-2.14) §
	Same sex DZ	75/139 (54.0%)	69/139 (49.6%)	1.26 (0.69-2.31) §
	MZ	58/97 (59.8%)	53/97 (54.6%)	1.49 (0.69-3.26) §

AB: users of antibiotics, MZ: monozygotic twin pair level, DZ: dizygotic twin pair level, OR: odds ratio, CI: confidence interval, , * p < 0.05, ** p < 0.01, *** p < 0.001, £ adjusted for: educational attainment, delivery mode, gender, birth weight, and asthma. § adjusted for birth weight and asthma.

Supplementary information: power calculations for sensitivity analyses

To provide more insights in the power issue discussed in our discussion, we show here the power estimations for the different analyses (Supplementary table 7). As shown, the power is decreased in all analyses and makes it difficult to draw conclusions, particularly in the discordant twin analyses with narrow spectrum antibiotics.

Supplementary table 10. Post-hoc power calculations of different sensitivity analyses in our study

Analysis	*Power in current study*
Unmatched analysis, antibiotics commonly prescribed for urinary tract/skin infections and subsequent risk of asthma (with correction for respiratory antibiotics, Table 3)	0.548
MZ and same sex DZ twins, Narrow spectrum antibiotics and asthma (Table 6a)	0.291
Same sex DZ twins, Narrow spectrum antibiotics and asthma (Table 6a)	0.218
MZ twins, Narrow spectrum antibiotics and asthma (Table 6a)	0.115

The algorithms used for this power calculation are described in earlier published research (28, 29)