



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

Elise M.A. Slob <sup>1,2</sup>, Bronwyn K. Brew<sup>3,4</sup>, Susanne J.H. Vijverberg<sup>1,2</sup>, Chantal J.A.R. Kats<sup>1</sup>, Cristina Longo<sup>1</sup>, Mariëlle W. Pijnenburg<sup>5</sup>, Toos C.E.M. van Beijsterveldt<sup>6</sup>, Conor V. Dolan<sup>6</sup>, Meike Bartels<sup>6</sup>, Patrick Magnusson<sup>3</sup>, Paul Lichtenstein<sup>3</sup>, Tong Gong<sup>3</sup>, Gerard H. Koppelman <sup>7,8</sup>, Catarina Almqvist<sup>3,9</sup>, Dorret I. Boomsma <sup>6</sup> and Anke H. Maitland-van der Zee<sup>1,2</sup>

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**Large twin studies show that antibiotics in early life are 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.** <http://bit.ly/372Qu5A>

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## ABSTRACT

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

**Methods:** We conducted a retrospective cohort study in twins aged 3–10 years from the Netherlands Twin Register (NTR, n=35 365) and a replication study in twins aged 9 years from the Childhood and Adolescent Twin Study in Sweden (CATSS, n=7916). Antibiotic use was recorded at age 0–2 years. Doctor-diagnosed asthma and eczema were reported by parents when children were aged 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 OR 1.45, 95% CI 1.34–1.56) and eczema (NTR OR 1.08, 95% CI 1.03–1.13; CATSS OR 1.07, 95% CI 1.01–1.14) in unmatched analyses. Co-twin analyses in monozygotic and dizygotic twin pairs showed similar results for asthma (NTR OR 1.54, 95% CI 1.20–1.98; CATSS OR 2.00, 95% CI 1.28–3.13), but opposing results for eczema in the NTR (OR 0.99, 95% CI 0.80–1.25) and the CATSS (OR 1.67, 95% CI 1.12–2.49). The risk of asthma increased for antibiotics prescribed for respiratory infections (CATSS OR 1.45, 95% CI 1.34–1.56), but not for antibiotics commonly used for urinary tract/skin infections (CATSS OR 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.

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## Introduction

The worldwide prevalence of atopic diseases increased over the last decades, having social and economic impacts 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 one in four 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 antibiotic use. First, antibiotics perturb the gastrointestinal tract microbiota and promote a shift from a T-helper cell (Th) 1 to a Th2 response [5], increasing the risk of atopic diseases [6]. Antibiotics change the microbiota, with broad-spectrum antibiotics thought to provide more changes than narrow-spectrum 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]. Second, 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 are an indication for antibiotics and might also increase the risk of developing asthma later in life [10–13]. Nevertheless, recent evidence suggests no association between respiratory syncytial virus prevention in late pre-terms 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 factors shared within families (lifestyle, health-seeking behaviour or genetic predisposition to respiratory infection) may have led to a spurious positive 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 2 years of life increased the risk of both asthma (OR 2.18, 95% CI 1.04–4.60; four studies with a total of 8284 patients) and eczema (OR 1.26, 95% CI 1.15–1.37; 22 studies with a total of 394517 patients) [20, 21]. A large population-based study with sibling comparisons suggested that the association between early-life antibiotic 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 on 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) monozygotic and dizygotic twin pairs. A twin study design including twin pairs discordant for eczema/asthma provides a unique opportunity to study the impact of antibiotic use on asthma/eczema development while controlling for shared familial environment and genetic factors.

## 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 twins aged 9 years from the Childhood and Adolescent Twin Study in

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**Affiliations:** <sup>1</sup>Dept of Respiratory Medicine, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, The Netherlands. <sup>2</sup>Dept of Paediatric Pulmonology, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, The Netherlands. <sup>3</sup>Dept of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden. <sup>4</sup>National Perinatal Epidemiology and Statistics Unit, Centre for Big Data Research in Health and Dept of Women's and Children's Health, University of New South Wales, Sydney, Australia. <sup>5</sup>Dept of Paediatrics, Division of Respiratory Medicine and Allergology, ErasmusMC, University Medical Center Rotterdam, Rotterdam, The Netherlands. <sup>6</sup>Netherlands Twin Register, Dept of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands. <sup>7</sup>Dept of Paediatric Pulmonology and Paediatric Allergology, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. <sup>8</sup>Groningen Research Institute for Asthma and COPD (GRIAC), University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. <sup>9</sup>Paediatric Allergy and Pulmonology Unit, Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden.

**Correspondence:** Anke H. Maitland-van der Zee, Dept of Respiratory Medicine, Amsterdam University Medical Center, Meibergdreef 9, 1100 DE Amsterdam, The Netherlands. E-mail: a.h.maitland@amsterdamumc.nl

Sweden (CATSS). Children were excluded if data describing their medication use or asthma/eczema diagnosis were not available. All data were deidentified.

To examine whether early-life antibiotic use was associated with asthma and eczema [23], we undertook 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 with 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. Second, we performed a matched co-twin control analysis in all monozygotic and in same-sex dizygotic twin pairs discordant for either asthma or eczema. In a separate analysis only same-sex dizygotic twin pairs discordant for either asthma or eczema were included. Both analyses adjusted for the contribution of shared familial environment and partially for shared genes. Third, we conducted a matched co-twin control analysis in monozygotic twins discordant for asthma or eczema, which controlled for all shared environmental factors and all confounding by genetic factors. We investigated dizygotic and monozygotic twins separately to compare the role of genetics in the association. If the associations generated by the monozygotic twins are stronger than by the dizygotic twins, it could imply that at least part of the association is driven by genes.

#### **Discovery cohort**

The NTR is a birth cohort which was initiated in 1987 [24]. Twins are registered by their parents after birth, and recruited with the help of a commercial organisation in the Netherlands called Felicitas and the Dutch Association for Parents of Multiple Births. 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 CATSS [25]. Parents were interviewed about their children's health, including questions about asthma and eczema, *via* a telephone questionnaire at age 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, Medical Birth Register and National Patient Register were linked to CATSS information.

#### **Outcomes**

The primary outcomes, *i.e.* parental-reported ever-asthma and ever-eczema diagnosis, were derived at ages 3, 5, 7 and/or 10 years in Dutch twins (supplementary table S1). Asthma or eczema was considered to be present if reported in at least one survey. In the CATSS, parents of twins were asked at age 9 years whether their child ever had asthma and/or eczema (supplementary table S2).

#### **Exposure**

Exposure to antibiotics was defined between 0 and 2 years of age as any parental-reported antibiotics use in Dutch twins and as any prescription claim for antibiotics (Anatomical Therapeutic Chemical (ATC) codes) in the Swedish Prescribed Drug Register for Swedish twins (supplementary table S2).

#### **Confounders and covariates**

A directed acyclic graph was drawn to select covariates (supplementary figure S1). Data describing sex, delivery mode, birthweight, hours of outside childcare, breastfeeding, and educational attainment of the mother and father were derived from the NTR surveys at ages 0, 2, 3, 5, 7 and 9/10 years. In the CATSS, data describing breastfeeding and hours of outside childcare were not available. Perinatal covariates in the CATSS were obtained from the Medical Birth Register, and educational attainment was obtained by questionnaire and divided in four groups:  $\leq 9$  years, 10–12 years,  $< 2$  years tertiary and  $\geq 2$  years tertiary.

#### **Statistical analysis**

We used a logistic regression model with a generalised estimating equation 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 SPSS version 25 (IBM, Armonk, NY, USA).

To investigate the robustness of the findings, we performed sensitivity analyses where we 1) only assessed the parental-reported diagnosis in Dutch twins aged over 5 years (for asthma) and 3 years (for eczema); 2) used validated definitions for asthma and eczema in the CATSS based on medication from the Swedish Prescribed Drug Register and diagnoses from the National Patient Register (supplementary table S2); 3) only investigated CATSS with parental-reported asthma diagnosis after 2 years of age; 4) stratified by antibiotics commonly prescribed for respiratory infections and urinary tract/skin infections in the CATSS adjusting for other antibiotics prescribed in early life (supplementary table S2) [12]; and 5) stratified by broad-spectrum and narrow-spectrum antibiotics in the CATSS adjusting for other antibiotics prescribed in early life (supplementary table S2) [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 the Dutch and Swedish populations are described in table 1 for the asthma analysis and table 2 for the eczema analysis. We included 35 365 individuals in the NTR and 7916 in the CATSS; the total numbers of individuals for the asthma and eczema studies 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 was 15.1% at age 3–10 years in the NTR and 17.5% at age 9 years in the CATSS. For eczema, the prevalences were 19.1% and 21.9%, respectively. The exposure to at least one prescription of any antibiotic between 0 and 2 years was 34.8% in the NTR and 44.9% in the CATSS. The proportion of children born after a gestational age of  $\geq 37$  weeks was higher in the NTR compared with the CATSS (58.6% *versus* 46.1%), and higher in children with asthma born at gestational age  $< 37$  weeks compared with the whole cohort in the NTR (48.4% *versus* 41.4%) and in the CATSS (61.8% *versus* 53.9%). In the NTR, there were more children with asthma not receiving breastfeeding compared with children without asthma (46.3% *versus* 40.2%). In the CATSS we did not identify differences in summary characteristics between children with asthma or eczema and children without the disease. Mothers in the CATSS more often delivered *via* caesarean section compared with mothers in the NTR (41.1% *versus* 28.6%).

### *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 the CATSS (OR 1.45, 95% CI 1.34–1.56) (table 3).

In the dizygotic co-twin control analyses, the risk for developing asthma was associated with exposure to antibiotic 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 3). For monozygotic discordant twins, estimates pointed in the same direction, but did not reach statistical significance (NTR OR 1.52, 95% CI 0.89–2.60; CATSS OR 2.23, 95% CI 0.90–5.53).

### *Sensitivity analyses for asthma*

Sensitivity analyses, in which we 1) only included asthma in those aged 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 tables S3–S5).

### *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 S6a). After restricting to dizygotic twins, the risk of developing asthma remained (OR 1.91, 95% CI 1.12–3.27) (supplementary table S6a). In monozygotic twins, the average effect size remained, but precision was lost (OR 1.82, 95% CI 0.76–2.37) (supplementary table S6a).

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 4). Results for 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 S6b and S10).

### *Narrow-spectrum and broad-spectrum antibiotics and asthma*

Unmatched cohort analyses for use of broad-spectrum and narrow-spectrum antibiotics showed that both were associated with increased risk of asthma, with the highest risk associated with narrow-spectrum

TABLE 1 Summary characteristics of the Netherlands Twin Registry (NTR) and the Childhood and Adolescent Twin Study in Sweden (CATSS) populations in the unmatched cohort study for asthma (step 1)

	NTR			CATSS		
	All individuals	Asthma	No asthma	All individuals	Asthma	No asthma
<b>Subjects n</b>	35365	5141	30224	7906	1381	6525
<b>Sex</b>						
Male	17638 (49.9)	2623 (51.0)	15015 (49.7)	3955 (50.0)	832 (60.2)	3123 (47.9)
Missing	4 (0.0)	1 (0.0)	3 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<b>Delivery mode</b>						
Caesarean section	10116 (28.6)	1488 (28.9)	8628 (28.5)	3252 (41.1)	562 (20.7)	2691 (52.0)
Missing	2908 (8.2)	392 (7.6)	2516 (8.3)	1677 (21.2)	327 (23.6)	1350 (20.7)
<b>Gestational age</b>						
<37 weeks	14645 (41.4)	2489 (48.4)	12156 (40.2)	4259 (53.9)	854 (61.8)	3405 (52.2)
≥37 weeks	20720 (58.6)	2652 (51.6)	18068 (59.8)	3647 (46.1)	527 (38.2)	3120 (47.8)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<b>Birthweight g median (IQR)</b>	2530 (720)	2442 (760)	2540 (710)	2670 (720)	2570 (821)	2685 (710)
<b>Breastfeeding</b>				NA	NA	NA
None	14524 (41.1)	2379 (46.3)	12145 (40.2)			
<2 weeks	3538 (10.0)	504 (9.8)	3034 (10.0)			
2–6 weeks	5502 (15.6)	749 (14.6)	4753 (15.7)			
6 weeks–3 months	4572 (12.9)	618 (12.0)	3954 (13.1)			
3–6 months	3631 (10.3)	464 (9.0)	3167 (10.5)			
>6 months	3459 (9.8)	404 (7.9)	3055 (10.1)			
Missing	139 (0.4)	23 (0.4)	116 (0.4)			
<b>Area type during early life</b>						
Rural area	8938 (25.3)	1246 (24.2)	7692 (25.4)	2385 (30.2)	452 (32.7)	1933 (29.6)
Missing	432 (1.2)	55 (1.1)	377 (1.2)	20 (0.3)	3 (0.2)	17 (0.3)
<b>Older siblings</b>						
None	14709 (41.6)	2260 (44.0)	12449 (54.2)	1391 (17.6)	273 (19.8)	1118 (17.1)
Missing	8278 (23.4)	1006 (19.6)	7272 (24.1)	1668 (21.1)	315 (22.8)	1353 (20.7)
<b>Outside home childcare</b>				NA	NA	NA
None	2404 (8.1)	353 (7.9)	2051 (8.1)			
1–4 h-week <sup>-1</sup>	3804 (12.8)	631 (14.2)	3173 (12.6)			
5–8 h-week <sup>-1</sup>	8049 (27.1)	1273 (28.5)	6776 (26.9)			
9–16 h-week <sup>-1</sup>	6541 (22.0)	987 (22.1)	5554 (22.0)			
17–24 h-week <sup>-1</sup>	5851 (19.7)	792 (17.8)	5059 (20.0)			
>24 h-week <sup>-1</sup>	3039 (10.2)	420 (9.4)	2619 (10.4)			
Missing	5677 (16.1)	685 (13.3)	4992 (16.5)			
<b>Educational attainment mother</b>						
≤9 years	1328 (3.8)	304 (5.9)	1024 (2.9)	166 (2.0)	32 (2.3)	134 (2.1)
10–12 years	8217 (23.2)	1451 (28.2)	6766 (19.2)	1926 (24.4)	379 (27.4)	1547 (23.7)
<2 years tertiary	13348 (62.8)	2110 (41.0)	11238 (31.9)	954 (12.1)	164 (11.9)	790 (12.1)
≥2 years tertiary	8844 (25.0)	1166 (22.7)	7678 (21.8)	4017 (50.8)	680 (49.2)	3337 (51.1)
Missing	3628 (10.3)	90 (1.8)	3538 (10.1)	843 (10.7)	156 (11.3)	717 (11.0)
<b>Educational attainment father</b>						
≤9 years	1777 (5.0)	388 (7.5)	1389 (3.9)	354 (4.5)	74 (5.4)	280 (4.3)
10–12 years	8262 (23.4)	1446 (28.1)	6816 (19.4)	3135 (40.0)	588 (42.6)	2557 (39.2)
<2 years tertiary	11192 (31.6)	1746 (34.0)	9446 (26.8)	845 (10.7)	147 (10.6)	698 (10.7)
≥2 years tertiary	9931 (28.1)	1354 (26.3)	8577 (24.4)	3254 (41.2)	523 (37.9)	2731 (41.9)
Missing	4203 (11.9)	207 (4.0)	3996 (11.4)	318 (4.0)	49 (3.5)	259 (4.0)
<b>Smoking mother</b>				NA	NA	NA
No smoking	26913 (76.1)	3804 (74.0)	23109 (76.5)			
Cigars/pipe	94 (0.3)	10 (0.2)	84 (0.3)			
<10 cigarettes	3705 (10.5)	577 (11.2)	3128 (10.4)			
>10 cigarettes	4194 (11.8)	683 (13.3)	3511 (11.6)			
Do not know	58 (0.2)	5 (0.1)	53 (0.2)			
Always outside	81 (0.2)	13 (0.3)	68 (0.2)			
Missing	320 (0.9)	49 (1.0)	271 (0.9)			
<b>Smoking father</b>				NA	NA	NA
No smoking	24543 (69.4)	3453 (67.2)	21090 (69.8)			
Cigars/pipe	806 (2.3)	137 (2.7)	669 (2.2)			
<10 cigarettes	3638 (10.3)	502 (9.8)	3136 (10.4)			
>10 cigarettes	5572 (15.8)	893 (17.4)	4679 (15.5)			
Do not know	190 (0.5)	56 (1.1)	134 (0.4)			
Always outside	192 (0.5)	19 (0.4)	173 (0.6)			
Missing	424 (1.2)	81 (1.6)	343 (1.1)			

Data are presented as n (%), unless otherwise stated. IQR: interquartile range; NA: not available.

TABLE 2 Summary characteristics of the Netherlands Twin Registry (NTR) and the Childhood and Adolescent Twin Study in Sweden (CATSS) populations in the unmatched cohort study for eczema (step 1)

	NTR			CATSS		
	All individuals	Eczema	No eczema	All individuals	Eczema	No eczema
<b>Subjects n</b>	35 183	6479	28 686	7916	1730	6186
<b>Sex</b>						
Male	17 520 (49.7)	3239 (49.9)	14 281 (49.8)	3962 (50.1)	809 (46.8)	3153 (51.0)
Missing	4 (0.0)	1 (0.0)	3 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<b>Delivery mode</b>						
Caesarean section	10 074 (28.6)	1859 (28.6)	8215 (28.6)	3250 (41.1)	730 (42.2)	2520 (40.7)
Missing	2879 (8.2)	474 (7.3)	2405 (8.4)	1666 (21.0)	340 (19.7)	1326 (21.4)
<b>Gestational age</b>						
<37 weeks	14 511 (41.2)	2702 (41.6)	11 809 (41.2)	4270 (53.9)	949 (54.9)	3321 (53.7)
≥37 weeks	20 672 (58.8)	3795 (58.4)	16 877 (58.8)	3646 (46.1)	781 (45.1)	2865 (46.3)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<b>Birthweight g median (IQR)</b>	2530 (720)	2530 (730)	2530 (720)	2670 (720)	2655 (705)	2670 (725)
<b>Breastfeeding</b>				NA	NA	NA
None	14 410 (40.1)	2589 (39.8)	11 821 (41.2)			
<2 weeks	3541 (10.1)	628 (9.7)	2913 (10.2)			
2–6 weeks	5460 (15.5)	1048 (16.1)	4412 (15.4)			
6 weeks–3 months	4562 (13.0)	859 (13.2)	3703 (12.9)			
3–6 months	3623 (10.3)	682 (10.5)	2941 (10.3)			
>6 months	3451 (9.8)	669 (10.3)	2782 (9.7)			
Missing	136 (0.4)	22 (0.3)	114 (0.5)			
<b>Area type during early life</b>						
Rural area	8897 (25.3)	1520 (23.4)	7377 (25.7)	2392 (30.2)	495 (28.7)	1897 (30.7)
Missing	434 (1.2)	85 (1.3)	349 (1.2)	22 (0.3)	5 (0.3)	17 (0.3)
<b>Older siblings</b>						
None	14 670 (54.5)	3072 (53.8)	11 598 (54.6)	1389 (22.2)	357 (25.6)	1032 (21.2)
Missing	8249 (23.4)	790 (12.2)	7459 (26.0)	1652 (20.9)	335 (19.4)	1317 (21.3)
<b>Outside home childcare</b>				NA	NA	NA
None	2397 (6.8)	394 (6.1)	2003 (6.9)			
1–4 h-week <sup>-1</sup>	3761 (10.7)	689 (10.6)	3072 (10.7)			
5–8 h-week <sup>-1</sup>	7968 (22.6)	1472 (22.7)	6496 (22.6)			
9–16 h-week <sup>-1</sup>	6534 (18.6)	1230 (18.9)	5304 (22.1)			
17–24 h-week <sup>-1</sup>	5857 (16.6)	1142 (17.6)	4715 (18.5)			
>24 h-week <sup>-1</sup>	3017 (8.6)	633 (9.7)	2384 (8.3)			
Missing	5649 (16.1)	937 (14.4)	4712 (16.4)			
<b>Educational attainment mother</b>						
≤9 years	1449 (4.1)	219 (3.4)	1230 (4.3)	165 (2.1)	30 (1.7)	135 (2.2)
10–12 years	8762 (26.0)	1490 (22.9)	7272 (25.4)	1931 (24.4)	408 (23.6)	1523 (24.6)
<2 years tertiary	14 312 (42.1)	2695 (41.5)	11 617 (40.5)	961 (12.1)	225 (13.0)	736 (11.9)
≥2 years tertiary	9395 (27.5)	1889 (29.1)	7506 (26.2)	4013 (50.7)	896 (51.8)	3117 (50.3)
Missing	1264 (3.6)	204 (3.1)	1061 (3.7)	846 (10.7)	171 (9.9)	675 (10.9)
<b>Educational attainment father</b>						
≤9 years	1906 (5.4)	345 (5.3)	1561 (5.4)	354 (4.5)	60 (3.5)	294 (4.8)
10–12 years	8863 (25.2)	1542 (23.7)	7321 (27.1)	3144 (39.7)	657 (38.0)	2487 (40.2)
<2 years tertiary	11 902 (33.8)	2178 (33.5)	9724 (36.2)	848 (10.7)	203 (11.7)	645 (10.4)
≥2 years tertiary	10 645 (30.2)	2140 (32.9)	8505 (30.8)	3248 (41.0)	742 (42.9)	2506 (40.5)
Missing	1867 (5.3)	292 (4.5)	1575 (5.5)	322 (4.1)	68 (3.9)	254 (4.1)
<b>Smoking mother</b>				NA	NA	NA
No smoking	26 792 (76.2)	5093 (78.4)	21 699 (75.6)			
Cigars/pipe	91 (0.3)	17 (0.3)	74 (0.3)			
<10 cigarettes	3673 (10.4)	655 (10.1)	3018 (10.5)			
>10 cigarettes	4170 (11.8)	635 (9.8)	3535 (12.3)			
Do not know	59 (0.2)	9 (0.1)	50 (0.2)			
Always outside	83 (0.2)	25 (0.4)	58 (0.2)			
Missing	315 (0.9)	63 (1.0)	252 (0.9)			
<b>Smoking father</b>				NA	NA	NA
No smoking	24 424 (69.4)	4605 (70.9)	19 819 (69.1)			
Cigars/pipe	796 (2.3)	146 (2.2)	650 (2.2)			
<10 cigarettes	3624 (10.3)	641 (9.9)	2983 (10.4)			
>10 cigarettes	5531 (15.7)	952 (14.6)	4579 (16.0)			
Do not know	184 (0.5)	38 (0.6)	146 (0.5)			
Always outside	193 (0.5)	40 (0.6)	153 (0.6)			
Missing	431 (1.2)	75 (1.2)	356 (1.2)			

Data are presented as n (%), unless otherwise stated. IQR: interquartile range; NA: not available.

TABLE 3 Early-life antibiotic use and subsequent risk of asthma

	AB/asthma	AB/without asthma	Adjusted OR (95% CI)
<b>NTR</b>			
Unmatched	2132/4896 (43.5)	8260/24 988 (33.1)	1.34 [1.28–1.41] <sup>#, ***</sup>
MZ and same-sex DZ	534/1312 (40.7)	482/1312 (36.7)	1.54 [1.20–1.98] <sup>¶, **</sup>
Same-sex DZ	383/924 (41.5)	342/924 (37.0)	1.60 [1.18–2.16] <sup>¶, **</sup>
MZ	151/388 (38.9)	140/388 (36.1)	1.52 [0.89–2.60] <sup>¶</sup>
<b>CATSS</b>			
Unmatched	768/1381 (55.6)	2455/5825 (42.1)	1.45 [1.34–1.56] <sup>*, ***</sup>
MZ and same-sex DZ	206/380 (54.2)	174/380 (45.7)	2.00 [1.28–3.13] <sup>¶, **</sup>
Same-sex DZ	141/275 (51.3)	122/275 (44.4)	1.86 [1.11–3.18] <sup>¶, *</sup>
MZ	65/105 (61.9)	55/105 (52.4)	2.23 [0.90–5.53] <sup>¶</sup>

Data are presented as n/n (%), unless otherwise stated. AB: users of any antibiotics; NTR: Netherlands Twin Registry; MZ: monozygotic twin pair level; DZ: dizygotic twin pair level; CATSS: Childhood and Adolescent Twin Study in Sweden. <sup>#</sup>: adjusted for socioeconomic status, hours of outside childcare, breastfeeding, delivery mode, sex and birthweight; <sup>¶</sup>: adjusted for birthweight; <sup>\*</sup>: adjusted for educational attainment, delivery mode, sex and birthweight. <sup>\*</sup>: p<0.05; <sup>\*\*</sup>: p<0.01; <sup>\*\*\*</sup>: p<0.001.

antibiotics (narrow-spectrum antibiotics OR 1.83, 95% CI 1.50–2.24; broad-spectrum antibiotics OR 1.24, 95% CI 1.11–1.38). For narrow-spectrum antibiotics, the direction of the association remained after controlling for shared familial environment factors (OR 1.95, 95% CI 1.22–3.14) when studying monozygotic and dizygotic twins and when exposure to broad-spectrum 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 monozygotic twins for use of narrow-spectrum antibiotics (OR 1.92, 95% CI 0.73–4.99) and for use of broad-spectrum antibiotics (OR 3.13, 95% CI 0.79–12.47) (supplementary tables S7a and b).

**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 the CATSS (OR 1.19, 95% CI 1.03–1.38) (table 5). However, this risk attenuated after controlling for shared familial environment factors in the NTR including same-sex dizygotic pairs (OR 0.99, 95% CI 0.80–1.25), but not in the CATSS (OR 1.67, 95% CI 1.12–2.49). In monozygotic pairs, the association attenuated in the NTR (OR 1.02, 95% CI 0.69–1.52), but increased odds were found in the CATSS (OR 1.76, 95% CI 0.98–3.14).

**Sensitivity analyses for eczema**

The results of our sensitivity analyses in which we 1) only included eczema in those aged over 3 years and 2) re-defined eczema based on medication and diagnosis (CATSS) showed consistent results in the NTR.

TABLE 4 Early-life antibiotic use commonly prescribed for urinary tract/skin infections and subsequent risk of asthma (with correction for respiratory antibiotics)

	AB/asthma	AB/without asthma	Adjusted OR (95% CI)
<b>CATSS</b>			
Unmatched	72/1314 (5.5)	263/5584 (4.7)	1.02 [0.88–1.17] <sup>#</sup>
MZ and same-sex DZ	16/380 (4.2)	18/380 (4.7)	0.76 [0.34–1.66] <sup>¶</sup>
Same-sex DZ	12/275 (4.4)	13/275 (4.7)	0.77 [0.30–1.95] <sup>¶</sup>
MZ	4/105 (3.8)	5/105 (4.8)	0.81 [0.18–3.71] <sup>¶</sup>

Data are presented as n/n (%), unless otherwise stated. AB: users of antibiotics commonly prescribed for urinary tract/skin infections (pivmecillinam, trimethoprim, sulphonamide, ciprofloxacin, norfloxacin, nitrofurantoin, cloxacillin, flucloxacillin and dicloxacillin); CATSS: Childhood and Adolescent Twin Study in Sweden; MZ: monozygotic twin pair level; DZ: dizygotic twin pair level. <sup>#</sup>: adjusted for: antibiotics commonly prescribed for respiratory infections (amoxicillin, penicillin, cephalosporin and macrolides), educational attainment, delivery mode, sex and birthweight; <sup>¶</sup>: adjusted for respiratory antibiotics and birthweight.

TABLE 5 Early-life antibiotic use and subsequent risk of eczema

	AB/eczema	AB/without eczema	Adjusted OR (95% CI)
<b>NTR</b>			
Unmatched	2286/5918 (38.6)	7763/22983 (33.8)	1.08 (1.03–1.13) <sup>#</sup>
MZ and same-sex DZ	687/1837 (37.4)	684/1837 (37.2)	0.99 (0.80–1.25) <sup>¶</sup>
Same-sex DZ	441/1191 (37.0)	441/1191 (37.0)	0.96 (0.73–1.26) <sup>¶</sup>
MZ	243/646 (37.6)	243/646 (37.6)	1.02 (0.69–1.52) <sup>¶</sup>
<b>CATSS</b>			
Unmatched	819/1730 (47.3)	2278/5114 (44.5)	1.19 (1.03–1.38) <sup>*,*</sup>
MZ and same-sex DZ	296/624 (47.4)	255/624 (40.9)	1.67 (1.12–2.49) <sup>¶,*</sup>
Same-sex DZ	187/411 (45.5)	160/411 (38.9)	1.71 (1.10–2.68) <sup>¶,*</sup>
MZ	109/213 (51.2)	95/213 (44.6)	1.76 (0.98–3.14) <sup>¶</sup>

Data are presented as n/n (%), unless otherwise stated. AB: users of any antibiotics; NTR: Netherlands Twin Registry; MZ: monozygotic twin pair level; DZ: dizygotic twin pair level; CATSS: Childhood and Adolescent Twin Study in Sweden. <sup>#</sup>: adjusted for socioeconomic status, hours of outside childcare, breastfeeding, delivery mode, sex, birthweight and asthma; <sup>¶</sup>: adjusted for birthweight and asthma; <sup>\*</sup>: adjusted for educational attainment, delivery mode, sex, birthweight and asthma. \*: p<0.05.

In the CATSS the risk estimates for same-sex dizygotic and monozygotic pairs moved towards the null compared with unmatched analyses (supplementary tables S8 and S9).

## 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 factors in same-sex dizygotic discordant twin pairs. After correction for shared familial environment and genetic factors in monozygotic 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 genetic factors.

This study is the first using a co-twin control analysis assessing the impact of confounding by shared familial environment and genetic factors in the association of early-life antibiotic 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 the CATSS aligns with previous population-based cohort studies suggesting that populations of twins may be representative of the general paediatric population [12, 16, 21]. 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) and Sweden (26–61% depending on the region in 2010); these percentages tend to be among the lowest in the world [30, 31].

In our study, after analysing matched discordant twins (both monozygotic and dizygotic) 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: increased risk of asthma was observed in siblings treated with antibiotics commonly prescribed for respiratory tract infections in early life, but not for urinary tract/skin infections [12]. In contrast, a Finnish nested case-control study (n=6690 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 narrow-spectrum and broad-spectrum antibiotics use was associated with increased risk of asthma, even after controlling for shared familial environment and genetic factors, which was consistent with other studies. A birth cohort study of 13 116 Canadian children found increased risk of asthma at age 7 years for children exposed during the first year of life to broad-spectrum antibiotics (OR 1.50, 95% CI 1.16–1.93) and narrow-spectrum antibiotics (OR 1.35, 95% CI 0.29–6.23), although the broad-spectrum and narrow-spectrum study suffered from reduced power [33]. 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 broad-spectrum antibiotics in our analysis [32]. However, to the best of our knowledge, we are the first to investigate the effects of antibiotic spectra in a twin design. Prescription of narrow-spectrum antibiotics was more



common in our population compared with broad-spectrum 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 2 years. We would have expected to observe a similar effect of narrow-spectrum and broad-spectrum antibiotics, or broad-spectrum 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 genetic factors. In the CATSS, the association between early-life antibiotic use and eczema also attenuated when the definition for eczema was based on medication and diagnoses after 2 years, rather than parental report for ever-eczema. Since eczema is common in children aged under 2 years, the parental-report definition is open to reverse causation bias, and therefore the validated definition using medication and diagnosis in those aged over 2 years is a more robust measure. Our results in the unmatched analyses are similar to previous studies, which did not assess confounding by shared genetic or familial factors [17, 20]. Considering the aforementioned, we hypothesise 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 genome-wide association study of a multidisease 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 [34], 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 type 1 immune system development, and therefore protects against asthma [35, 36]. 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 the severity of underlying infections rather than the antibiotic itself. Respiratory viral infections, in particular, have been shown to initiate a cascade of host immune responses altering microbial growth in the respiratory tract and gut [37]. 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 *versus* 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 urinary tract infection 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 8000 Swedish twins. We found similar results for asthma in the CATSS compared with the NTR, meaning that our results are generalisable to Western European children. Our twin design controls for shared familial environment and genetic factors. We addressed recall bias, reverse causation for asthma, and validated our asthma and eczema definition using International Classification of Diseases codes.

This study also had some limitations. First, our discovery cohort only consisted of parental-reported antibiotic use. Nevertheless, antibiotic use between 0 and 2 years was ascertained in the NTR at age 2 years, minimising recall bias. Moreover, after defining use of antibiotics with medication prescription claims in the CATSS, results were similar. Second, our subgroup analyses had reduced power because ATC codes were only available in the CATSS (supplementary material). Third, outcome misclassification may have occurred due to parental-reported measures for asthma and eczema. Fourth, more children in the CATSS were delivered *via* caesarean section compared with 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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