

## Supplementary Information

**Supplementary table S1. Overview NTR survey questions and outcome definitions regarding early-life antibiotic use, asthma and eczema.**

<b>SURVEY ITEM</b>	<b>INTENDED AGE</b>	<b>QUESTION</b>	<b>OUTCOME DEFINITION</b>
<b>ANTIBIOTIC USE</b>	2 years old	"Did the twins ever use drugs?"	Specified drug name of an antibiotic‡ or collective name 'antibiotics'.
<b>ASTHMA</b>	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'.
<b>ECZEMA</b>	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 S2. Overview CATSS survey questions, medication, diagnoses and outcome definitions regarding early-life antibiotic use, asthma and eczema.**

SURVEY ITEM	INTENDED AGE	OUTCOME DEFINITION	CODES
<b>ANTIBIOTIC USE</b>	0-2 years	Use of antibiotics	ATC code J01
<b>RESPIRATORY ANTIBIOTIC USE</b>	0-2 years	Use of respiratory antibiotics: amoxicillin, penicillin, cephalosporin, and macrolides	ATC codes J01CA04, J01C, J01D, J01FA.
<b>URINARY TRACT ANTIBIOTIC USE</b>	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.
<b>ASTHMA</b>	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 $\beta$ 2-agonists and corticosteroids ( $\beta$ 2-ICS, ATC code R03AK) ; b) two dispenses of $\beta$ 2-adrenoreceptor agonists (ATC code R03AC), and either a third dispense of a $\beta$ 2- adrenoreceptor agonist or of a preventer medication (ICS, LTRA, $\beta$ 2-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	
<b>ECZEMA</b>	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. <b>CRITERIA 1 (ICD-10):</b> $\geq$ 1 hospital contact for: atopic dermatitis and/or winter	L20 "atopic dermatitis"

feet.

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, pruritis, other dermatitis, papulosquamous disorders, other erythematous 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 "seborrheic 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 erythematous 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: antipsoriasisics or salicylates for dermatological use or corticosteroids moderate or potent other combinations or corticosteroids (group IV) clobetasol or clobetasol and antibiotics and antifungals.

D05 "antipsoriasisics"

D02AF "salicylates for dermatological use"

D07XB "corticosteroids moderate or potent other combinations"

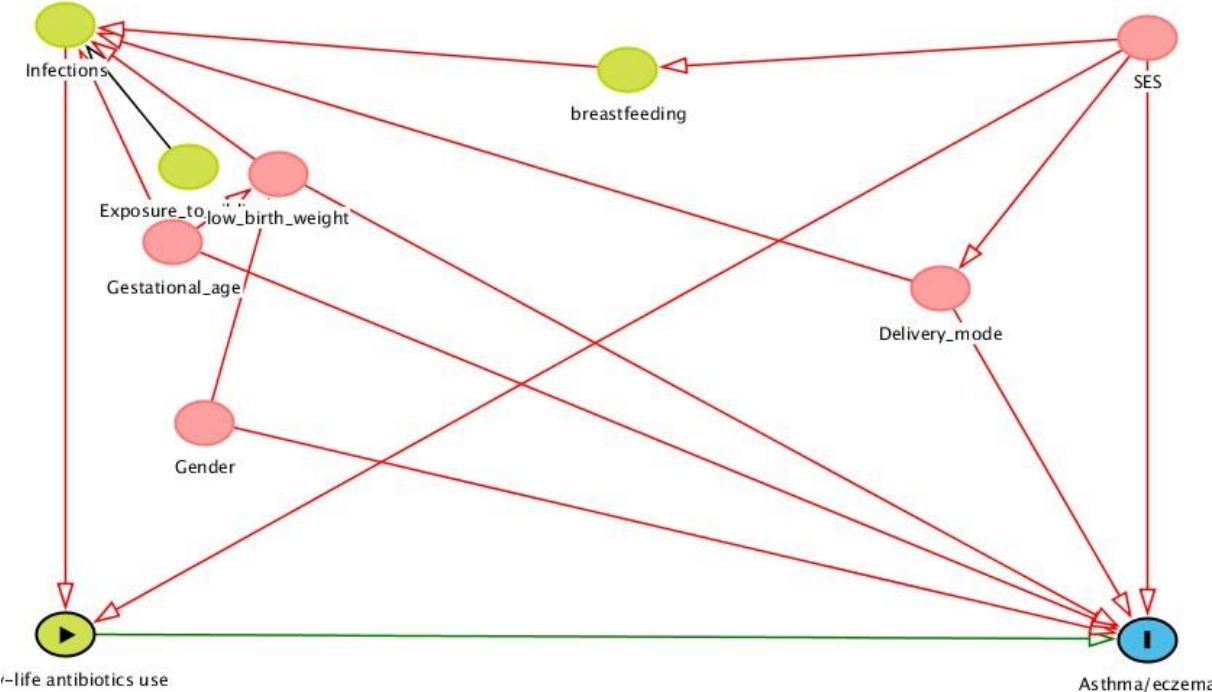
\*For prescription of corticosteroid group IV, a filled prescription of group I-III should also be used (as atopic dermatitis never treated alone with group IV)

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)
<b>NARROW SPECTRUM ANTIBIOTICS</b>	0-2 years	Use of the following antibiotics: vancomycin (oral), benzylpenicillin, phenoxymethylpenicillin, dicloxacillin, flucloxacillin, cephalaxine, aztreonam, trimethoprim, sulfamethizole, erythromycin, roxithromycin, clarithromycin, azithromycin, clindamycin, vancomycin, teicoplanin, fusidic acid, metronidazole (intravenous), fusidic acid, metronidazole, nitrofurantoin, linezolid, 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.
<b>BROAD SPECTRUM ANTIBIOTICS</b>	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 S1. DAG for early-life antibiotics use and increased risk of asthma and eczema



**Supplementary table S3. 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)
<b>NTR</b>	<b>Unmatched</b>	1,598/3,706 (43.1%)	8,545/25,271 (33.8%)	1.34 (1.26-1.43)***#
	<b>MZ and same sex DZ</b>	451/1,113 (40.5%)	424/1,113 (38.1%)	1.41 (1.07-1.86)*§
	<b>Same sex DZ</b>	311/760 (40.9%)	293/760 (38.6%)	1.26 (0.90-1.74)§
	<b>MZ</b>	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 S4. 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)
<b>CATSS</b>	<b>Unmatched</b>	568/785 (74.5%)	2,714/6,700 (40.5%)	1.60 (1.43-1.78)***, £
	<b>MZ and same sex DZ</b>	193/276 (69.9%)	167/276 (60.5%)	2.05 (1.27-3.31)* §
	<b>Same sex DZ</b>	126/184 (65.8%)	110/184 (59.8%)	2.08 (1.13-3.80)* §
	<b>MZ</b>	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 S5. 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)
<b>STR</b>	<b>Unmatched</b>	54/108 (50.0%)	3,386/7,598 (44.6%)	2.07 (1.19-3.61)*, £
	<b>MZ and same sex DZ</b>	73/147 (49.7%)	63/147 (42.9%)	1.78 (0.90-3.53) §
	<b>Same sex DZ</b>	48/96 (50.0%)	42/96 (43.7%)	1.60 (0.72-3.52) §
	<b>MZ</b>	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 S6a. Sensitivity analysis using antibiotics commonly prescribed for respiratory infections in CATSS**

		n AB/asthma (%)	n AB/without asthma (%)	OR adjusted (95% CI)
<b>CATSS</b>	<b>Unmatched</b>	775/1381 (54.7%)	2,426/5,835 (41.6%)	1.45 (1.34-1.56)***£
	<b>MZ and same sex DZ</b>	203/380 (53.4%)	177/380 (46.6%)	1.93 (1.23-3.02)**§
	<b>Same sex DZ</b>	140/275 (50.9%)	121/275 (44.0%)	1.91 (1.12-3.27)§
	<b>MZ</b>	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 S6b. 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)
<b>CATSS</b>	<b>Unmatched</b>	5/1,314 (0.40%)	12/5,584 (0.21%)	1.66 (0.96-2.88) <sup>£</sup>
	<b>MZ and same sex</b>	1/363 (0.27%)	1/363 (0.27%)	
	<b>DZ</b>			Insufficient power
	<b>Same sex DZ</b>	0/263 (0.0%)	1/263 (0.38%)	Insufficient power
	<b>MZ</b>	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 S7a. Sensitivity analysis using narrow spectrum antibiotics in CATSS**

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

AB: users of narrow spectrum antibiotics (vancomycin (oral), benzylpenicillin, phenoxymethylpenicillin, dicloxacillin, flucloxacillin, cephalaxine, aztreonam, trimethoprim, sulfamethizole, erythromycin, roxithromycin, clarithromycin, azithromycin, clindamycin, vancomycin, teicoplanin, fusidic acid, metronidazole (intravenous), fusidic acid, metronidazole, nitrofurantoin, linezolid, 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, x adjusted for: birth weight, broad spectrum antibiotics (doxycycline, lymecline, tetracyclines, tigecycline, ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, ceftazidime, ceftriaxone, meropenem, ertapenem, sulfamethoxazole-trimethoprim, tobramycin, gentamycin, ofloxacin, ciprofloxacin, moxifloxacin, colistinmethatnatrium).

**Supplementary table S7b. Sensitivity analysis using broad spectrum antibiotics in CATSS**

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

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, cephalaxine, aztreonam, trimethoprim, sulfamethizole, erythromycin, roxithromycin, clarithromycin, azithromycin, clindamycin, vancomycin, teicoplanin, fusidic acid, metronidazole (intravenous), fusidic acid, metronidazole, nitrofurantoin, linezolid, daptomycin, rifampicin, rifabutin, metronidazole), birth weight, £ adjusted for: educational attainment, delivery mode, gender, birth weight, narrow spectrum antibiotics (vancomycin (oral), benzylpenicillin, phenoxymethylpenicillin, dicloxacillin, flucloxacillin, cephalaxine, aztreonam, trimethoprim, sulfamethizole, erythromycin, roxithromycin, clarithromycin, azithromycin, clindamycin, vancomycin, teicoplanin, fusidic acid, metronidazole (intravenous), fusidic acid, metronidazole, nitrofurantoin, linezolid, daptomycin, rifampicin, rifabutin, metronidazole).

**Supplementary table S8. Sensitivity analysis eczema after age 3 in NTR**

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

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 S9. 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)
<b>STR</b>	<b>Unmatched</b>	256/419 (61.1%)	3,184/7,287 (43.7%)	2.39 (1.20-4.74)*, £
	<b>MZ and same sex DZ</b>	133/236 (56.4%)	122/236 (51.7%)	1.33 (0.83-2.14) <sup>§</sup>
	<b>Same sex DZ</b>	75/139 (54.0%)	69/139 (49.6%)	1.26 (0.69-2.31) <sup>§</sup>
	<b>MZ</b>	58/97 (59.8%)	53/97 (54.6%)	1.49 (0.69-3.26) <sup>§</sup>

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 S7). 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 S10. Post-hoc power calculations of different sensitivity analyses in our study

<i>Analysis</i>	<i>Power in current study</i>
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)