Adverse Paediatric Outcomes of Macrolide Antibiotics Treatment in Pregnancy

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Declaration

I, Heng Fan confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Date: 19 January 2020 Signature:

Abstract

Background: Over the last 20 years, concerns have been raised about rare but serious adverse outcomes associated with macrolide use during pregnancy. Currently there was no consensus about whether macrolides are considered safe in pregnancy or not. This PhD study aims to examine the association between maternal exposure of macrolide antibiotics during pregnancy and adverse paediatric outcomes where short-term fetal hypoxia could be aetiologically involved.

Methods: I first conducted a systematic review and meta-analysis of both random controlled trials and observational studies to investigate the association. I prioritized comparisons of macrolides with alternative antibiotics (mainly penicillins) for comparability of indication and effect. I then performed a large cohort study using a mother-baby linkage derived from the Clinical Practice Research Datalink (CPRD), a UK-representative primary care database. The cohort study assessed the association between macrolide (versus penicillin) prescribing during pregnancy and major malformations, cerebral palsy, epilepsy, attention-deficit/hyperactivity disorder (ADHD), and autism spectrum disorder (ASD) in children.

Results: The systematic review and meta-analysis found consistent evidence for an association between macrolide antibiotics use during early pregnancy and an increased risk of miscarriage, inconsistent evidence for cerebral palsy and epilepsy, and insufficient evidence for malformations, stillbirth and neonatal death. The cohort study demonstrated that prescribing macrolides compared with penicillins during the first trimester of pregnancy (4 to 13 Gestational Week) was associated with increased risks of any major malformation and specifically cardiovascular malformations. Macrolide prescribing in any trimester was associated with an increased risk of genital malformations (mostly hypospadias). Erythromycin in the first trimester was found to be associated with an increased risk of any major malformation. Indication bias, unmeasured confounding, live-birth bias and outcome misclassification were unlikely to explain the findings.

Conclusions: Considering the widespread use of macrolides during pregnancy, international collaboration is in urgent need to bring together existing datasets for large-scale analyses of high quality trial and observational cohorts that have accurate measurements of macrolides treatment and specific child outcomes. Analyses should pre-specify treatment exposure periods based on the critical period of specific outcomes. The findings of this study warrant cautious use of macrolides in pregnancy and recommendation of alternative antibiotics where feasible.

Impact Statement

Macrolide antibiotics are one of the most commonly used class of antibiotics worldwide. Over the last 20 years, concerns have been raised about rare but serious adverse outcomes associated with macrolide use during pregnancy. However, there was no consensus about whether macrolides are considered safe in pregnancy or not and policy advice about use of macrolides in pregnancy varies.

The increased risks of miscarriage found in the systematic review and the increased risks of major malformations found in this cohort study provide evidence that macrolide prescribing during pregnancy could be associated with serious pregnancy and child outcomes. If the associations are causal, an additional 5 to 100 miscarriages would occur for every 1000 pregnancies exposed to macrolides instead of penicillins at 6 gestational weeks (with a baseline risk of of 20%) and at 20 gestational weeks (with a baseline risk of 1%), respectively; 4.1 (95% CI: 0.4-9.4) children with cardiovascular malformations and 1.7 (95% CI: 0.4-3.5) children with genital malformations would occur for every 1000 children exposed to macrolides instead of penicillins in the first trimester or in any trimester, respectively.

Given the moderate to high absolute risks of potential adverse outcomes of macrolides and the widespread use of macrolides during pregnancy, as well as the cardiotoxicity of macrolides use in adults, a review of the evidence on macrolide safety in pregnancy by the regulatory agency is warranted. The regulatory agency should examine the use of macrolides in pregnant women to ensure the best outcome for mother and child. Cost-effective alternatives for macrolides for use in women who are allergic to penicillin also need to be identified. Finally, prescription guidelines and patient information leaflets should report the uncertainty about the safety of macrolides, including erythromycin, and evidence of associations with miscarriage and malformations.

International collaboration is in urgent need to bring together existing datasets for large-scale analyses of high quality trial and observational cohorts that have accurate measurement of macrolides treatment and specific child outcomes. Analyses should pre-specify treatment exposure periods based on the critical period of specific outcomes.

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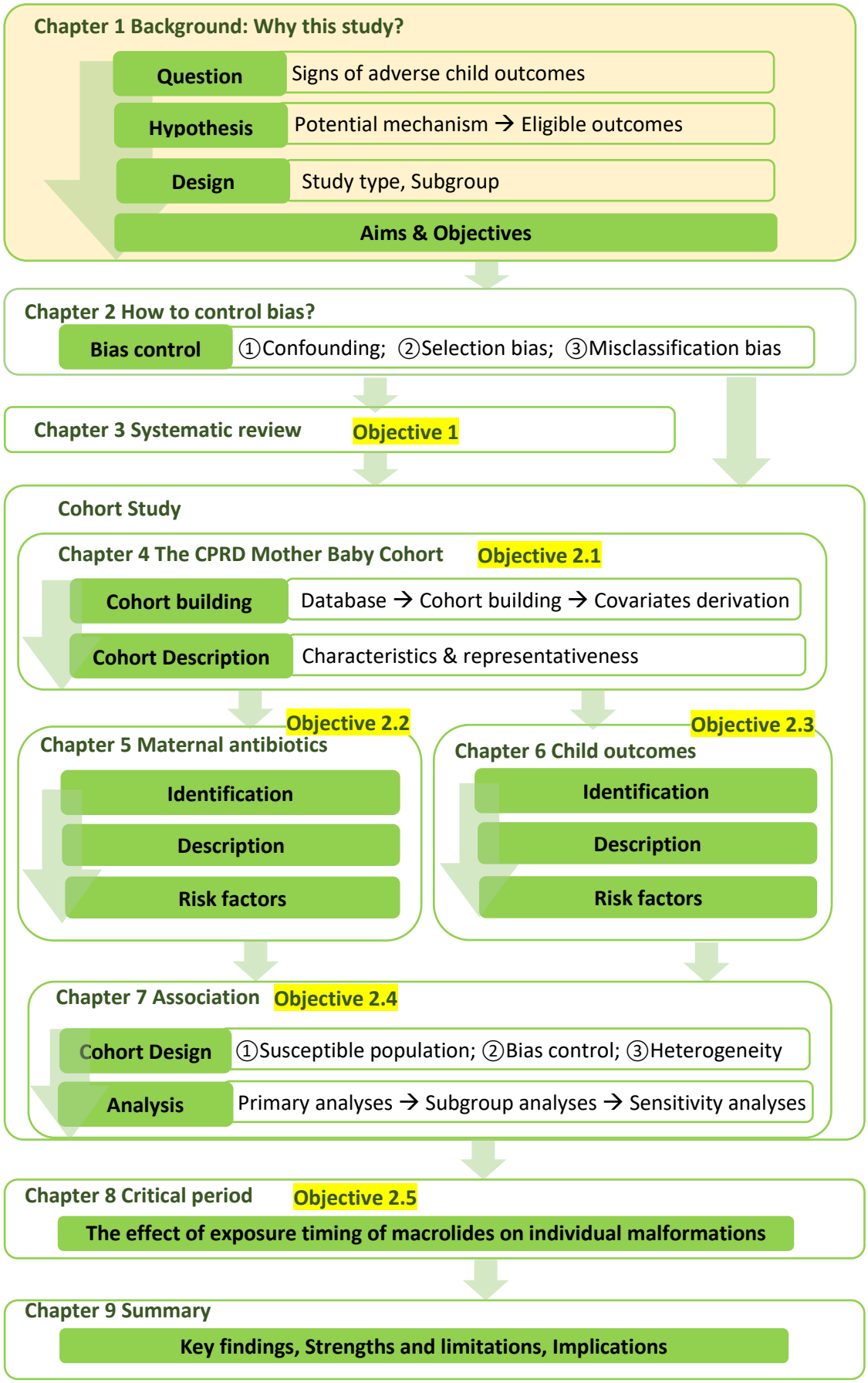
Abbreviations

ADHD	Attention deficit hyperactivity disorder
AIC	Akaike information criterion
AED	Anti-epileptic drug
ASD	Autistic Spectrum Disorder
BMI	Body mass index
BNF	British National Formulary
CC	Case control study
CI	Confidence interval
CNS	Central nervous system
CP	Cerebral palsy
CPRD	Clinical Practice Research Datalink
EEG	Electroencephalography
EUROCAT	European Concerted Action of Congenital Anomalies and Twins
FC	Prof. Finbar O'Callaghan
GBS	Group B streptococcus/streptococcal
GD	Gestational day
GP	General practitioner
GPRD	General Practice Research Database (now CPRD)
GUTIs	Genitourinary tract infections
GW	Gestational week
HELLP	Haemolysis, Elevated Liver enzymes, and Low Platelet count (syndrome)
HIV	Human immunodeficiency virus
HR	Hazard ratio
ICD	International Classification of Diseases
IVF	In vitro fertilisation
kg	Kilogram
LMP	Last menstrual period (date of)
MeSH	Medical Subject Heading
MHRA	The Medicines and Healthcare products Regulatory Agency
mmol	Millimole
MRI	Magnetic resonance imaging
NCAS	National Congenital Anomaly System
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NNH	Number needed to harm
OCS	ORACLE Childhood Study
ONS	UK Office for National Statistics
OR	Odds ratio
PDA	Patent ductus arteriosus
pPROM	Preterm pre-labour (or premature) rupture of membranes
PPV	Positive predictive value
PTL	Preterm labour
RCT	Randomised controlled trial

RG	Prof Ruth Gilbert, UCL
RR	Relative risk / risk ratio
RTI	Respiratory tract infection
SD	Standard deviation
SES	Socio-economic status
SPL	Spontaneous preterm labour
SSRIs	Selective serotonin reuptake inhibitors
Std.diff	Standardized difference
THIN	The Health Improvement Network
TORCH	Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus (CMV), and Herpes infections
UCL	University College London
UK	United Kingdom
US(A)	United States (of America)
UTI	Urinary tract infection
STI	Sexually transmitted infection
VSD	Ventricular septal defect

Thesis Structure



Chapter 1 Thesis background and rationale

1.1 Chapter Overview

The aim of this chapter is to describe the rationale for investigating whether the prescribing of macrolides during pregnancy is associated with adverse child outcomes. Although use of macrolides during pregnancy is generally considered safe, concerns have been raised over the last 20 years about some rare but serious adverse child outcomes.

I first explain why macrolides are used during pregnancy (Section 1.2), and then present an overview of current evidence evaluating the potential adverse fetal and child outcomes of macrolides (Section 1.3). I discuss the hypothesised mechanism of the potential harmful effects of macrolides, and thus the definition of outcomes for the study (Section 1.4). Heterogeneity within the effect is discussed in Section 1.5. I set out the study types, overall aim and specific objectives of this thesis (Section 1.6 and 1.7). Finally, in Section 1.8, I describe the structure of the thesis.

1.2 Macrolides and its use during pregnancy

Macrolides are a class of antibiotics characterised by their large lactone ring structures and by their growth-inhibiting effects on bacteria.[1] Macrolides were first discovered in the 1950s, when scientists isolated erythromycin from the soil bacterium Streptomyces erythraeus. The synthetic derivatives of erythromycin, including clarithromycin and azithromycin, were developed in the 1970s and 1980s. The new agents have fewer gastrointestinal side effects, better tissue penetration and display longer half-lives than erythromycin.[2] Currently available macrolides in the UK include erythromycin and the newer agents clarithromycin, azithromycin, and spiramycin.[3]

Macrolides have an antibacterial spectrum that is similar but not identical to that of penicillin. They are active mainly against Gram-positive bacteria and have limited activity against Gram-negative bacteria. They are active against many-penicillin-resistant staphylococci, but some strains are now also resistant to macrolides.[3] The most frequent indication for their use in pregnancy is therefore as a replacement for suspected penicillin allergy, commonly for upper and lower respiratory tract, and soft tissue infections. Azithromycin is a macrolide with slightly less activity than erythromycin against Gram-positive bacteria, but enhanced activity against some Gram-negative organisms. Clarithromycin is a derivative of erythromycin with slightly

greater activity than the parent compound. Clarithromycin is also one of the most useful antimicrobials against H. pylori, and often prescribed to H. pylori-infected patients who are allergic to penicillin. Spiramycin is used in pregnancy specifically to reduce mother to child transmission of the protozoan parasite *Toxoplasma gondii*.[4]

Specific indications for prescribing macrolides are less common and involved specific organisms. For example, azithromycin and erythromycin are recommended as treatment for uncomplicated chlamydia,[5] and azithromycin appears to be the most effective for chlamydia with a long tissue half-life and is the only single dose treatment available.[6] Macrolides are the most effective antibiotics against Campylobacter infections, where azithromycin (erythromycin in pregnant women) is the primary choice.[7] Also, macrolides are the first-line treatment for Mycoplasma pneumonia and Legionella pneumonia while these pathogens are innately resistant to penicillins.[3,8]

During pregnancy, macrolides are the third most frequently used antibiotics (4% of all pregnant women), after broad-spectrum penicillins (28%) and cephalosporins (11%), according to a research using The Health Improvement Network (THIN) UK primary care database in 2010.[9] In the US, macrolides rank as the second most frequently used antibiotics prescribed during pregnancy.[10] The most common indications of macrolides in pregnancy include respiratory infection, skin and soft tissue infection, probably as replacement for penicillins.[9]

1.3 Review of adverse effects of macrolides reported in clinical and animal studies

1.3.1 Clinical studies

1.3.1.1 In fetuses and children

This section briefly summarises the previous evidence of potential adverse effects of macrolides, with detailed examination of clinical studies in pregnancy reported in the systematic review in Chapter 3.

The first report of adverse children outcomes following macrolide prescribing in pregnancy was an unexpected increased risk of cardiovascular malformations for erythromycin use in early pregnancy in a case-control study using Swedish Medical Birth Register in 2003.[11] A detailed cohort study was then performed by the same author in 2005 using the unselected population-based registry data extended for one further year.[12] In this study, Kallen et al. found an increased adjusted risk for cardiovascular malformation after erythromycin therapy during the

first trimester (34 in 1844 infants, odds ratio [OR] 1.92, 95% confidence interval [CI]: 1.37–2.68), but not after penicillin V therapy (86 in 9110 infants, OR 0.99, 95% CI 0.80-1.23). In response to this study, the Swedish and Norwegian Regulatory Medicines Agencies issued warnings against the use of macrolides during the first trimester.[13,14] Eight other cohort studies investigating cardiovascular malformations reported no statistically significant association with macrolides prescribing during pregnancy, although 4 of the 8 studies reported fewer than 10 malformations and were therefore underpowered to detect an effect. [12,15-21]

The evidence is more consistent of an increased risk of miscarriage, defined as pregnancy loss before 22 to 24 weeks depending on the country. Three cohort studies reported an association between macrolides exposure during early pregnancy and increased risks of miscarriage.[15,22,23] Two of them were large studies using Canadian and Danish birth registries and compared with alternative antibiotics (e.g. phenoxymethylpenicillin). However, multiple trials reported no increased risk for stillbirths or neonatal deaths in mothers randomised to erythromycin, although the trials were restricted to mothers of high risk of bacterial infection.

Further evidence of an adverse effect of macrolides comes from a large randomised controlled trial (RCT, ORACLE Child Study II) of women with spontaneous preterm labour (SPL) who were randomised erythromycin, co-amoxiclav, both or placebo. The trial reported an increased risk of cerebral palsy in children whose mothers received erythromycin (3.3%) compared with no erythromycin (1.7%, OR 1.93, 95% CI 1.21-3.09)[24]. A large population-based study using The Health Improvement Network, a UK primary care database, also reported a higher risk of cerebral palsy and/or epilepsy amongst children of mothers treated with macrolides during pregnancy compared to those of mothers receiving penicillin (28 cases versus 156 cases; HR 1.78, 95%CI 1.18–2.69).[25]

1.3.2.2 In adults

Seven systematic reviews, 19 RCTs and more than 30 observational studies have evaluated the association between macrolides and cardiovascular events in adults. Overall, there is evidence of a rare adverse effect on cardiac outcomes, mainly within high cardiovascular risk populations (Table 1-1). Reported adverse cardiac outcomes with macrolides include QT interval prolongation, torsade de pointes (TdP), ventricular tachycardia, sudden cardiac death and cardiovascular death. Though the findings were discordant, the Food and Drug Administration (FDA) issued a safety communication that azithromycin “can cause abnormal changes in the electrical activity of the heart that may lead to a potentially fatal irregular heart rhythm” in patients with high risk of arrhythmias.[26] The warning was based on a large cohort study that

compared the risks of cardiovascular death in patients treated with the azithromycin, amoxicillin, ciprofloxacin (Cipro), and levofloxacin (Levaquin), or no antibacterial drug. The study reported an increase in cardiovascular deaths, and in the risk of death from any cause, in persons treated with a 5-day course of azithromycin (Zithromax) compared to persons treated with amoxicillin, ciprofloxacin, or no drug during treatment.[27] In addition to the immediate adverse reactions, FDA communication recently raised concerns regarding both short (1 year) and long term (10 year) risk for heart problems or death associated with clarithromycin use in patients with coronary heart disease.[28,29] The CLARICOR study, which triggered the warning, randomised 4373 patients diagnosed with myocardial infarction or angina pectoris to two-weeks of treatment with clarithromycin or placebo. The study found patients in the clarithromycin arm died more often, with a statistically significant increased relative risk for cardiovascular death at follow up 1 year and 10 years after randomisation (Table 1-1).

Table 1-1. Previous studies on adverse effect of macrolides in adults

Year (journal, lead author)	Outcome(s)	Description
Studies reporting adverse effects of macrolides		
2006, CLARICOR Trial[30]	Cardiovascular mortality	A **placebo controlled study** of patients in Denmark with stable coronary heart disease, reporting increased mortality in patients treated with **clarithromycin (14 days, n=2172)** compared with patients who received a placebo (hazard ratio [HR] 1.45; 95% CI, 1.09 to 1.92). The observed difference in mortality became apparent after patients had been followed for one year or longer after the study drug was given.
2012, Ray, NEJM, triggered the FDA warning[31]	Cardiac mortality	**US retrospective cohort study** using Tennessee Medicaid data. Compared with amoxicillin, **azithromycin** (n=347,795 prescriptions) was associated with an increased risk of cardiovascular death (HR, 2.49; 95% CI, 1.38 to 4.50). The risk of cardiovascular death was significantly greater with azithromycin than with ciprofloxacin (quinolones) but did not differ significantly from that with levofloxacin (quinolones).
2013, Schembri, BMJ[32]	Cardiovascular events	Analysis of **two prospectively collected cohort datasets** including the UK chronic obstructive pulmonary disease dataset and the Edinburgh pneumonia cohort. After multivariable adjustment, **clarithromycin** use (n=1261) in acute exacerbations of chronic obstructive pulmonary disease was associated with an increased risk of cardiovascular events and acute coronary syndrome (HR, 1.50; 95% CI 1.13 to 1.97 and HR, 1.67; 95% CI 1.04 to 2.68). After multivariable adjustment, clarithromycin use in community-acquired pneumonia was associated with increased risk of cardiovascular events (1.68, 1.18 to 2.38) but not acute coronary syndrome (1.65, 0.97 to 2.80). Use of beta lactam antibiotics or doxycycline was not associated with increased cardiovascular events in patients with acute exacerbations of chronic obstructive pulmonary disease.
2014,Svanstrom , BMJ[33]	Cardiac mortality	**Danish retrospective cohort study** using national health records, which reported a small increased risk of cardiac death (adjusted Risk Ratio (RR): 1.76, 1.08 to 2.85) for patients over 40 years old treated with **clarithromycin** (n = 160,297) instead of penicillins.
2014 Rao, Ann Fam Med [34]	Cardiac mortality/arrhythmias	**US veteran study** conducted after FDA issued a warning for **azithromycin**. Patients received azithromycin (1-5 days, n = 594,792), had significantly increased risk of death (HR = 1.48; 95% CI, 1.05-2.09) and serious arrhythmia (HR = 1.77; 95% CI, 1.20-2.62). No increased risks were observed on azithromycin treatment days 6 to 10.
2015, Winkel, Int J Cardiol[35]	Mortality and cardiovascular events	**10-year follow-up** via Danish public registers of the **CLARICOR trial**. **Clarithromycin** increased all-cause mortality (HR: 1.10, 95% CI 1.00 to 1.21) and cerebrovascular disease during 10 years (1.19, 1.02 to 1.38).
2015, Chou, Clin Infect Dis[36]	Cardiac mortality and cardiac arrhythmia	**Population based cohort study** using Taiwan National Health Insurance database including 10,684,100 patients who were prescribed oral **azithromycin** (n=66,745)**, clarithromycin** (n=393,243), quinolones (moxifloxacin, levofloxacin, ciprofloxacin), or amoxicillin-clavulanate at outpatient visits. Compared with amoxicillin-clavulanate treatment, the use of azithromycin and moxifloxacin was associated with significant increases in the risks of ventricular arrhythmia and cardiovascular death. The adjusted Odds Ratios (OR) for ventricular arrhythmia were 4.32 95% CI (2.95 to 6.33) for **azithromycin**, 3.30 (2.07-5.25) for moxifloxacin, and 1.41 (0.91 to 2.18) for levofloxacin. For cardiovascular death, the adjusted ORs for azithromycin, moxifloxacin, and levofloxacin were 2.62 (1.69 to 4.06),

		2.31 (1.39 to 3.84), and 1.77 (1.22 to 2.59), respectively. No association was noted between clarithromycin or ciprofloxacin and adverse cardiac outcomes.
2015, Cheng, J American College of Cardiology[37]	SCD, VTA, and Cardiac mortality	Meta-analysis >20 million participants (11 cohorts and 19 randomized controlled trials [RCTs]). The RRs associated with sudden cardiac death (SCD) or ventricular tachyarrhythmias (VTA) were 3.40 (1.68-6.90) for **azithromycin**, 2.16 (1.70-2.74) for **clarithromycin**, and 3.61 (1.09-11.99) for **erythromycin**, respectively. No association was noted between roxithromycin and adverse cardiac outcomes. Treatment with macrolides was associated with an absolute risk increase of 118.1 additional SCDs or VTA, and 38.2 additional cardiovascular deaths per 1 million treatment courses.
2010, Guo[38]	QT prolongation and TdP	Systematic review (18 clinical studies and 30 case reports) shows that **macrolides** are cardiotoxic in a range of patient groups, including preterm babies. Macrolides prolong the QT interval, which can cause acute cardiac events. Erythromycin was more cardiotoxic than clarithromycin, which in turn was more cardiotoxic than azithromycin.
2015, Abdulhak, Am J Ther[39]	Cardiac mortality	Systematic review and meta-analysis of 5 cohort studies of **azithromycin** use and cardiovascular death. No association between azithromycin use and all-cause of cardiovascular death overall. However, subgroup analyses found that current use of azithromycin was associated with a higher risk of death among older patients (HR: 1.64, 1.23 to 2.19).
2016, Li, Eur J Intern Med[40]	Cardiac death in patients >48 y	Systematic review and meta-analysis showed increased risk of cardiac death in patients >48 y (pooled OR 1.99, 95% CI 1.53 to 2.59, based on 3 observational studies of 6 million participants) in **macrolides** group as compared with other antibiotics. No association found between use of macrolides and all-cause mortality or within middle-aged adults.
2017, Wong, Drug Safety[41]	Cardiovascular mortality, myocardial infarction, and arrhythmia	Systematic review and meta-analysis of 19 RCTs and 30 observational studies of **macrolides** use and cardiovascular events. Observational studies were found to have a short-term risk of cardiovascular mortality, myocardial infarction, and arrhythmia associated with macrolides but no risk was found in RCTs. However, no association for long-term risk (ranging from [30 days to [3 years) was observed in observational studies or RCTs.
2018, Gorelik, Antimicrobial Agents and Chemotherapy[42]	Myocardial infarction (MI)	Systematic review and meta-analysis of 33 studies (13 RCTs, 15 cohort studies, and 5 case-control studies) and 22 million subjects. **Macrolide** use was not associated with the risk of arrhythmia or cardiovascular mortality (all age groups). In the primary analysis, macrolide use was associated with a small but statistically significant 15% increase in risk for MI (OR = 1.15 [1.01 to 1.30]). In indirect network meta-analysis, erythromycin and clarithromycin were ranked considerably more likely to be associated with a higher risk for MI and significantly associated with an increased risk of MI compared with azithromycin (OR = 1.58 [1.18 to 2.11] and OR = 1.41 [1.11 to 1.81], respectively).
Studies reporting no evidence of adverse effects		
2014, Khosropour CM, NEJM[43]	Large database study (Oregon Public Health Division) conducted following FDA warning about cardiotoxic effects of azithromycin. Database involved young people treated for sexually transmitted diseases (STDs) – azithromycin (n=162,385). They identified no deaths from cardiovascular causes among patients treated with azithromycin or another drug.	

2016,Trac, CMAJ[44]	Ontario prescribing dataset for older adults. No association were found between macrolides use (n=616,359) and hospital encounter with ventricular arrhthymias (0.03% v. 0.03%; RR 1.06, 0.83–1.36) and were associated with a lower risk of all-cause mortality (0.62% v. 0.76%; RR 0.82, 0.78–0.86), as compared with non-macrolide anitbiotics.
2018,Polgreen, JAHA[45]	Cohort study using Medicare. For azithromycin (n=40,119) and clarithromycin (n=3465), the odds ratio for any cardiac event or death in the following week was statistically significant compared with non-users, but after controlling for a wide range of covariates, the odds ratio decreased to null. The increased risk of atrial fibrillation or atrial flutter kept significant (1.24 [1.11-1.38] for azithromycin and 1.70 [1.23-2.33] for clarithromycin).
2014 Almalki ZS, Am Health Drug Benefits[46]	Meta-analysis of 12 RCTs of 15,588 patients randomising adult patients to azithromycin vs placebo. No evidence of increased risks of death, hospitalisation or interventions for cardiovascular events associated with azithromycin.

1.3.2 Animal studies

Experimental studies of adverse effects of macrolides during pregnancy are largely limited to toxicology studies in animals to determine maximum drug levels to prevent adverse fetal effects. Manufacturers' animal reproduction studies of clarithromycin has demonstrated cardiovascular malformation, cleft palate and/or fetal growth retardation in monkeys at plasma levels 2 to 17 times maximum recommended human concentrations during the period of major organogenesis. Similar animal studies using erythromycin and azithromycin revealed no impairment of fertility or harm to the fetus.[47,48] However, potential teratogenic effect of erythromycin and azithromycin cannot be ruled out, because traditional animal reproduction studies are not always predictive of human response.[49] In fact, one *in vitro* study in 2008 exposed rat embryo to clarithromycin, azithromycin and spiramycin testing on sensitive days of fetal development and found a significant dose-dependent relationship between macrolide dose and decreased growth and developmental parameters .[50]

1.3.3 Summary of evidence, current policies and research question

The FDA has published repeated warnings of the increased risks of cardiovascular event or deaths associated with the used of macrolide antibiotics in high-risk adults.[26,28,29] However, current evidence is less consistent for an association between the use of macrolides during pregnancy and adverse child outcomes. Increased risks of some serious child outcomes were reported in trial and observational studies, including miscarriage, cardiovascular malformation, cerebral palsy and epilepsy. Animal data is limited on macrolides' teratogenicity or embryo toxicity, and harmful fetal effects cannot be excluded.

Currently, there is no consensus about how to respond to this evidence. Swedish national policy advised against the use of erythromycin during early pregnancy in 2005.[51] In April 2015, the UK Medicines and Healthcare products Regulatory Agency (MHRA) reviewed the evidence reported by Meeraus et al. (which identified an association between macrolides and cerebral palsy and/or epilepsy) and decided there was insufficient evidence to warn against the use of macrolides in pregnancy.[25,52] Recent Patient information leaflets (PILs) and Summaries of product characteristics (SPCs) (issued by the UK MHRA) comments erythromycin as "There are no adequate and well-controlled studies in pregnant women. However, observational studies in humans have reported cardiovascular malformations after exposure to medicinal products containing erythromycin during early pregnancy." [53] However, the British National Formulary states that erythromycin is "not known to be harmful", while recommending to avoid clarithromycin and azithromycin during pregnancy unless potential benefit outweighs risk or if adequate alternatives were not available.[3]

Prompt and adequate treatment for infection during pregnancy is vital given the well-established association between maternal infection during pregnancy and adverse birth outcomes, including miscarriage and stillbirth.[54] More evidence is needed on whether there is an association between maternal treatment with macrolide antibiotic during pregnancy and adverse outcomes in children. This PhD project aims to generate further evidence on associations between maternal prescribing of macrolides during pregnancy and adverse child outcomes.

1.4 Potential mechanism for adverse effects of macrolides on child outcomes

1.4.1 Potential mechanisms

The potential mechanisms for the association between adverse child outcomes and prenatal macrolide treatment were discussed following findings reported in the ORACLE II trial. First, the investigators of the ORACLE trial suggested that the antibiotics may suppress but not eradicate intrauterine infection, leading to a prolonged pregnancy and a continuing inflammatory environment which could have led to fetal brain injury and adverse neurological outcomes.[24,55] An alternative explanation is that macrolide treatment prolonged pregnancy, thereby increasing survival of fetuses damaged by infection, who then presented with adverse neurological outcomes or died postnatally.[56] However, the ORACLE trial observed no difference of gestational length at delivery between the erythromycin group and placebo group.[24] Also, a previous systematic review of RCTs found no clear evidence of a significant effect of antibiotic treatment in pregnancy on shifting prenatal death to postnatal death (compared with no antibiotics treatment or placebo), though confidence intervals were wide due to relatively few stillbirths and postnatal deaths (46 prenatal deaths and 133 neonatal deaths).[56]

A second suggested mechanism was that the excess of cerebral palsy in OCSII may have been induced by the immunomodulatory effects of macrolides.[57] The macrolide treatment could stimulate an enhanced immune response and thereby, a surge in cytokines, which has been implicated with the aetiology of cerebral palsy. [58]

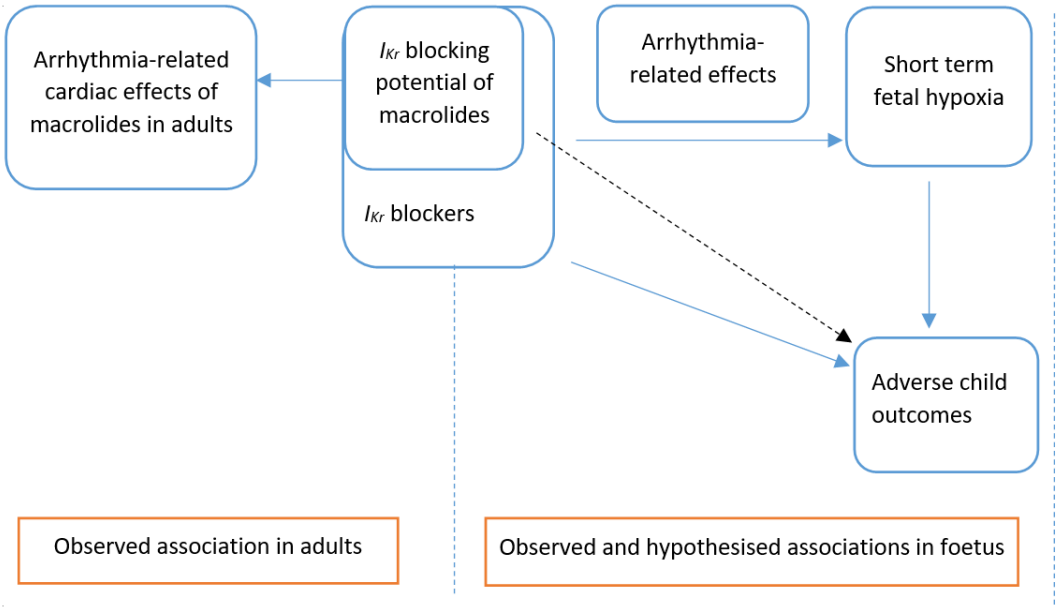
Fetal hypoxia induced by the arrhythmic effect of macrolides

A third possible mechanism relates to the arrhythmic effect of macrolides. The QT-interval prolongation potential of macrolides may lead to short-term fetal hypoxia, and the fetal hypoxia may in turn cause ischaemic damage and adverse outcomes in the fetus or child (Figure 1-1).

The QT-interval prolongation effect of macrolide antibiotics has been well established in adults. As is mentioned in Section 1.3.1, the increased risk of cardiovascular events, including cardiac death in adults is thought to be attributable to the QT-interval prolongation potential of macrolides.[26,28] The FDA comments in its warnings that "Pharmacologic and epidemiologic data point to lethal arrhythmias as a potential consequence of QT-interval prolongation with use of azithromycin".

Macrolides prolong QT interval and inducing arrhythmic cardiac events because they can block the delayed rectifier channel (I_{Kr}).[59,60] This I_{Kr} channel plays a major role in human cardiac rhythm regulation in both adult and fetal cardiomyocytes, which first presents in human embryonic cardiomyocytes when the heart starts to beat during 4-5 weeks of gestation.[59,61] *In vitro* studies have demonstrated that macrolides can block the I_{Kr} channel both in human and animal cardiomyocytes.[59] The proarrhythmic properties of macrolides may be exacerbated by their interaction with other CYP450 (Cytochromes P450, a family of enzymes that plays a key role in the metabolism of drugs) metabolised QT-prolonging drugs, because macrolides can inhibit CYP450 enzymes at the same time.[60,62] (Figure 1-1)

Figure 1-1. Hypothesised pathway from macrolide exposure during pregnancy to adverse child outcomes: through an induced short-term fetal hypoxia.



*Solid arrow: observed association; Dashed arrow: hypothesised association. I_{Kr}: rapidly activating component of delayed rectifier K current, which conducts potassium (K+) ions out of cardiac myocytes.

For other I_{Kr} -blockers (e.g. dofetilide, almokalant, and sotalol), teratology studies in rats and rabbits have demonstrated that embryonic or fetal exposure to these drugs can lead to embryonic death and a wide range of malformations, including digital defects, orofacial clefts and cardiovascular defects, especially ventricular-septal defects.[63] Observational studies of mothers prescribed other I_{Kr} -blocking drugs, e.g. antidepressants citalopram, clomipramine, fluoxetine and paroxetine, reported an increased risk of fetal adverse effects including cardiac-septal defects and miscarriage.[64,65]

Evidence from animal studies and observational studies suggested that the adverse fetal effects of selected I_{Kr} blockers could be through hypoxic pathways. A dose-response relationship was observed between I_{Kr} blockers (astemizole and dofetilide) and bradycardia and/or arrhythmia of the embryonic heart. The cardiotoxicity in turn induce an interrupted or decreased oxygen supply, hypoxia-reoxygenation damage and malformations at critical times during fetal development that were also dose-dependent.[49,66-69] There is also indirect evidence from animal studies to suggest that similar malformations to those associated with I_{Kr} -blockers can be induced by interrupted or decreased oxygen supply during the embryonic period (Table 1-2).[63,70,71]

Although studies have observed on macrolides the effect of QT-prolonging, developmental toxicity and teratogenicity, direct evidence of underlying fetal hypoxia in the process has not known to be studied before. However, based on these findings in animals, and clinical evidence from studies in adults and pregnant women, I hypothesise that fetal exposure to macrolides, as IKr blockers, can also induce short-term periodic hypoxia and result in hypoxic adverse effects in human fetuses.

1.4.2 Study outcome

This thesis aim to investigate whether there is an association between macrolides prescription during pregnancy and adverse child outcomes. By assuming that macrolides affect the fetus through short-term fetal hypoxia, I specify the outcomes of interest in this study as those that could potentially result from short-term fetal hypoxia. The specification avoids possible fishing expedition among wide range of outcomes. Potential effects of fetal hypoxia include fetal death, malformation and neurodevelopmental disorders (Table 1-3). While some specific adverse childhood outcomes of prenatal macrolides exposure have been studied, other potential adverse outcomes may have not been studied in previous studies.

The spectrum of hypoxia-related outcomes

In the human fetus, potential effects of fetal hypoxia include congenital malformations, brain injuries and fetal loss.[72-75] Animal studies have demonstrated that fetal hypoxia is teratogenic. Observed malformations that could be induced by fetal hypoxia include limb defects, facial defects including cleft palate/lip, cardiovascular malformations etc. (Table 1-2).[76,77] The evidence of hypoxia causing malformation in the human comes from studies of fetuses lacking hemoglobin (Hb) F where at least 17% of newborns have one congenital anomaly.[78] Also, maternal asthma exacerbation during the first trimester of pregnancy was reported to increase the risk for congenital malformations including the risk of cardiovascular malformations.[79] Fetal hypoxic and/or ischaemic injury is also thought to be at least one of the contributory factors to neurodevelopmental disorders including cerebral palsy, epilepsy, ADHD and ASD among other pathways (e.g. inflammatory/infective insults and preterm birth), as shown by both preclinical and clinical evidence.[80] Fetal hypoxia can lead to neuronal damage of white matter and grey matter including the cerebral cortex, hippocampus, cerebellum, and ensuing neurodevelopmental disorders.[81] Previous studies (the ORCALE trial in SPL women and a population-based cohort study) also suggested an association between macrolides use in pregnancy and neurodevelopmental disorders (cerebral palsy and epilepsy).[24,25] Lastly, serious fetal hypoxia could lead to fetal loss (miscarriage or stillbirth) (Table 1-3).[75]

The timing, severity and duration of fetal hypoxia are critical for determining the pattern and extent of fetal damage. Acute and serious fetal hypoxia, according to a study of eight fetal sheep made hypoxemic for 12 hours with an O_2 saturation of 50%-60%, can result in neuronal death, white matter damage and reduced growth of neural processes.[82] Based on animal studies, chronic mild placental insufficiency leads to different pattern of damage, including deficits in neural connectivity and myelination, fetal growth restriction, asymmetric fetal growth, cerebral vasodilatation and low birth weight.[83,84] Fetal hypoxia induced by macrolides might be short-term. This assertion is based on evidence from the pooled results in a systematic review of 14 observational studies in adults.[41] Most studies presented only short-term cardiac effects in adults who received macrolide (vs no macrolide) antibiotics,[37,41,85] although a recent study reported an increased risk of all-cause mortality (HR: 1.10, 95% CI 1.00 to 1.21) and cerebrovascular disease in 10-year follow-up via Danish public registers of the CLARICOR trial.[35]

Consistent with the hypothesised mechanism, outcomes that can also be induced by postnatal insults (e.g. abnormal cerebral ultrasonography) or commonly induced by chronic hypoxia (e.g. low birthweight, intrauterine growth restriction and asymmetric fetal growth) are excluded. (Table 1-3)

Table 1-2. Animal studies of teratogenicity of hypoxia on fetal organ development (the mark "+" indicates that the specific malformation was identified or significantly associated with fetal hypoxia in literature).

Age (GD)	Species	Sample Size	Hypoxia proxy	Duration	Adverse Outcomes										Refe-rence
					CNS	Skeleton	Heart	Limb	Lip/Palate	Ears	Eyes	Urogenital	Visceral	Mortality	
1-6.5	mouse	90	high altitude	5 h	+	+	+		+					10%	[86]
3-6	rat	70	clamp artery	0.5-3 h										60%	[87]
6- 12	mouse	177	vaso drugs*	1 dose	+	+		+		+	+			0-100%	[88]
7-14	rat	70	clamp artery	0.5-3 h	+						+			6%-26%	[87]
7.5-17.5	mouse	180	high altitude	5 h	+	+	+		+			+		37%	[86]
10-13	rat	88	brad drugs**	1 dose			+	+	+		+	+		Resorbed	[89]
10-14	rat	300	brad drugs	1 dose	+	+	+	+	+			+	+	-	[90]
14	rat	120	clamp artery	0.5-1.5 h		+		+	+					10%-50%	[71]
14-19	rat	52	vaso drugs	1-2 dose	+			+				+	+	Resorbed	[91]
16	rat	10	clamp artery	2- 30 min	+						+			-	[92]
5- 20	rat	7	10% O_2 gas	16 d	+										[93]
7-18	rabbit	80	brad drugs	12 d	+			+	+		+		+	Resorbed	[94]
1-12	rat	20	vaso drugs	12 d	+			+			+			60%	[95]

*Vaso drug: vasoconstriction drugs, e.g. epinephrine and vasopressin.

**Brad drugs: drugs inducing bradycardia or arrhythmia, e.g. phenytoin and almokalant.

Table 1-3. Eligible adverse child outcomes in this PhD thesis.

Fetal and neonatal death	1) Miscarriage 2) Stillbirth 3) Neonatal death
Congenital malformations	4) Major malformation overall 5) System-specific malformations
Neurological outcomes	6) Cerebral palsy 7) Epilepsy 8) Attention deficit hyperactivity disorder (ADHD) 9) Autism spectrum disorder (ASD)

1.4.3 Definitions and aetiologies of outcomes of interest

The outcomes in Table 1-3 differ in their aetiology. The consensus is that these outcomes are multifactorial and no single risk factor is sufficiently responsible. While fetal hypoxia could be an important pathway between macrolide and outcomes, the relative contribution of fetal hypoxia differs between outcomes, and there could be other aetiological pathways for a specific outcome. For example, more than 70% of cerebral palsy cases have non-genetic antenatal causes, while only 25% of epilepsy cases are believed to be associated with antecedent central nervous system (CNS) damage including fetal hypoxia.[96,97] The contribution of fetal hypoxia for an outcome can differ between subtypes of macrolides and populations, e.g. clarithromycin seems to be more risky of cardiovascular deaths in adults with existing cardiovascular disease, compared with other macrolides, suggesting a multifactorial aetiology underlying the potential risk.

1.4.3.1 Fetal and neonatal death

Miscarriage, also known as spontaneous abortion or pregnancy loss, is defined as "the spontaneous loss of a pregnancy before 24 weeks of gestation", according to the National Institute for Health and Care Excellence (NICE) guideline.[98] Among women who know they are pregnant, the miscarriage rate is roughly 10% to 20%, while rates among all conceptions is around 30% to 50%.[99] Chromosomal abnormalities are the most common cause of first trimester miscarriage (detected in 50–85% of pregnancy tissue specimens after spontaneous miscarriage).[100] No cause of recurrent miscarriage can be determined in about 50% of couples, while identifiable causes include thrombophilic abnormalities, immunological abnormalities, infective causes, endocrinological causes, maternal age and number of previous miscarriages.[99] Maternal exposure to high doses of toxic agents may also be a risk factor. Immunogenic, hypoxic,

and vascular causes may lead to a final common pathway of severe villous or placental dysfunction resulting in embryonic or fetal demise.[101]

Stillbirth, defined as "a baby delivered with no signs of life and known to have died after 24 completed weeks of pregnancy", occurs in around 1 in 200 fetuses. The cause of stillbirth is often unknown, but around 90% occur before the onset of labour.[102] Genetic causes and maternal/fetal infection may be responsible for 6 – 12% and 10–25% of all stillbirths, respectively.[103] Common fetally damaging infectious organisms include Parvovirus B-19, Enteroviruses and Cytomegalovirus (CMV). Maternal characteristics or conditions including pre-pregnancy obesity, older maternal age, stress and diabetes are associated with increased stillbirth rates.[103] Pathologically, acute or chronic intrauterine asphyxia is the final common pathway for most causes of fetal death (miscarriage and stillbirth). In a study on 765 stillbirths, hypoxia accounted for 43% of deaths; the remainder were due to antepartum haemorrhage, congenital anomalies, diabetes mellitus, trauma, or unclassified/miscellaneous.[104] The subgroups and frequencies of hypoxia-related fetal death were fetal growth restriction (26%), cord accidents (18%), maternal hypertension (17%), placental insufficiency (17%), postmaturity (13%), and other (13%).

Neonatal death is defined as the death of a live-born baby before 28 days of age. The rate of neonatal mortality in the UK is about 2 per 1000 live births.[105] According to the Centre for Maternal and Child Enquiries (CMACE) report of 2009, the latest report into causes of perinatal death in England, the most common causes/associated factors of neonatal deaths were associated obstetric factors (27%), major congenital anomalies (24%) and infection (10%).[106]

1.4.3.2 Major malformations

Major malformation: According to the definition of World Health Organisation (WHO), malformation is "structural or functional anomalies (for example, metabolic disorders) that occur during intrauterine life and can be identified prenatally, at birth, or sometimes may only be detected later in infancy, such as hearing defects". Major malformations are abnormalities that have medical, surgical, or cosmetic significance that occur in approximately 2 to 4 percent of livebirths. Some suspected causes are summarised by Robert L. Brent (Table 1-4).[107] While the definition of malformation also includes functional anomalies, I emphasis the structural anomalies where malformation is mentioned in this thesis. I regard neurodevelopmental disorders as functional anomalies.

Table 1-4 Cause of Human Congenital Malformations (by Robert L. Brent).[107]

Suspected Cause	% of Total
Unknown	65–75
Polygenic	
Multifactorial (gene-environment interactions)	
Spontaneous errors of development	
Synergistic interactions of teratogens	
Genetic	15–25
Autosomal and sex-linked inherited genetic disease	
Cytogenetic (chromosomal abnormalities)	
New mutations	
Environmental	10
Maternal conditions: alcoholism, diabetes, endocrinopathies, phenylketonuria, smoking and nicotine, starvation, nutritional deficits	4
Infectious agents: rubella, toxoplasmosis, syphilis, herpes simplex, cytomegalovirus, varicella zoster, Venezuelan equine encephalitis, parvovirus B19	3
Mechanical problems (deformations): amniotic band contrictions, umbilical cord constraint, disparity in uterine size and uterine contents	1–2
Chemicals, prescription drugs, high-dose ionizing radiation, hyperthermia	<1

1.4.3.3 Neurodevelopmental disorders

Cerebral palsy

A unifying definition for cerebral palsy is that of Rosenbaum and Bax from 2006:

> "*Cerebral palsy describes a group of permanent disorders of the development of movement and posture, causing activity limitation that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, behaviour, by epilepsy, and by secondary musculoskeletal problems".*[108] The prevalence of cerebral palsy ranges from 1.5 to 3 per 1,000 live births or children in western countries.[108]

Current understanding about the pathophysiology is that most cases of cerebral palsy result from an interference in brain development in utero. The risk of cerebral palsy increases steadily with declining gestational age at birth among preterm births. Two mechanistic pathways have been proposed: 1) the hypoxia and ischaemia pathway (e.g. deep CNS hypoxia, cerebral cortex hypoxia and transient or irreversible ischaemia); and, 2) the infection-inflammation (or fetal inflammatory syndrome) pathway.[109,110] 30% of cerebral palsy cases have been attributed to genetic variance.[97] Specific maternal infections including rubella, chorioamnionitis and cytomegalovirus also increase the risk of cerebral palsy.

Epilepsy is defined as a disease of the brain characterised by repeated unprovoked seizures (e.g. at least two unprovoked seizures occurring more than 24 hours apart), with a prevalence of

about 7 per 1,000 people under the age of 16 years.[111] About two thirds of people with epilepsy in the UK do not have an anatomically identifiable cause (i.e. "idiopathic epilepsy", reserved for syndromes of presumed genetic origin), especially among younger people. Around 25% of epilepsy is associated with an antecedent central nervous system (CNS) injury (e.g., head trauma, stroke, or brain infection) and accordingly is classified as "symptomatic".

NICE defines **Attention deficit hyperactivity disorder (ADHD)** as "a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development".[112] Increasing recognition of ADHD has resulted in a rising prevalence of the condition from 0.5 per 1,000 children diagnosed in the UK 30 years ago, to more than 3 per 1,000 receiving medication for ADHD in the late 1990s. The prevalence ratio between male and female is estimated at 3 to 1 in UK. Genetic variance contributes substantially to ADHD, with a mean heritability of 76% demonstrated in twin studies. Environmental factors known to be most strongly associated with ADHD are low birth weight and maternal smoking during pregnancy. Other risk factors include preterm delivery, epilepsy, acquired brain injury, lead exposure, iron deficiency, alcohol exposure during pregnancy, psychosocial adversity, and adverse maternal mental health. In utero exposure to Ischemic-hypoxic conditions, especially birth asphyxia, respiratory distress syndrome, and preeclampsia, are also reported to be associated with ADHD.[113]

Autism spectrum disorder (ASD) is a complex developmental condition characterized by 1) developmental impairments in social interaction and social communication; 2) restricted, repetitive patterns of behaviours, interests, or activities.[114] The estimated prevalence in children is at least 1%, with more boys affected compared with girls (approximately 3:1). Similar to ADHD, the precise cause of ASD is unknown and suspected to be an interplay of genetic and environmental factors. Factors associated with an increased prevalence of ASD include having a sibling with ASD, preterm birth before 35 weeks of gestation, parental schizophrenia-like psychosis or affective disorder, maternal use of sodium valproate during pregnancy, gastrointestinal abnormalities and immune imbalance, and birth defects associated with central nervous system malformation and/or dysfunction including cerebral palsy.[115] Fetal hypoxia have also been implicated in the pathogenesis of ASD based on evidence from epidemiological and animal studies. Kolevzon et al hypothesised that neonatal or fetal hypoxia are implicated in ASD, by summarising epidemiological studies of obstetric variables as surrogates of fetal hypoxia, including low Apgar score, fetal distress, cesarean delivery, threatened abortion, and bleeding during pregnancy.[116] A study in juvenile mice following also demonstrated that prenatal hypoxic

insults could induce development of maladaptive stress responses associated with many neurodevelopmental disorders such as ASD, and males seem to be more susceptible.[117]

1.5 Heterogeneity within the potential effect

1.5.1 Exposure timing during pregnancy

The timing of fetal hypoxia crucially influences the types and extent of adverse child outcomes. The first two weeks from the last menstrual period (i.e. gestational week [GW]) occur before conception, while the third and fourth weeks comprise the pre- and implantation periods of zygotes and blastocysts, including omnipotent stem cells. Thus congenital malformation is less susceptible to teratogen in the first month of gestation.[118] The critical period for most major malformation is during the second and third gestational months (e.g. 5-9 GW is the most vulnerable period for heart malformations), but some malformation such as cleft palate and hypospadias may also be triggered after the third gestational month (Figure 1-2). Minor anomalies and functional defects usually take place from the second to third trimester during continued growth and differentiation.[72] An acute fetal hypoxia during later gestation could also be less hazardous in general, because as approaching term fetal cardiovascular system can adopt strategies to spare fetal brain.[81] Although the neurological system keeps developing and remains at risk throughout the whole of fetal development, the susceptible region differs according to the timing of insult. (Figure 1-2) [82] For example, the periventricular region of the brain, the most commonly affected structure in cerebral palsy, is particularly vulnerable to perfusion fluctuation during GW 26-36 when the periventricular vasculature develops.[119,120] Therefore, I may expect a higher risk of major malformations overall for macrolides exposure during early pregnancy compared with macrolides exposure later in pregnancy. While for cerebral palsy, the etiological relevant window may extend to later pregnancy.

1.5.2 Exposure subtypes

Macrolides subtypes (e.g. erythromycin, clarithromycin and azithromycin) differ in their bioavailability, transplacental transfer, I_{Kr} -blocking potential and therefore potential harm to fetus. Fewer gastrointestinal side-effects and better pharmacokinetic profiles (e.g. better oral bioavailability, tissue penetration and longer half-life of elimination) of clarithromycin and azithromycin may increase their maternal and fetal bioavailability compared with erythromycin.[121] An ex vivo experiment with term human placentas demonstrated that azithromycin, clarithromycin and erythromycin show transplacental transfer of 2.6%, 3% and 6%, respectively.[122] The percentages were calculated as the ratio between the steady-state level

in fetal venous and maternal arterial sides. Studies in both animals and non-pregnant adults suggest that compared with other macrolides, clarithromycin may have higher QT prolongation potential to the fetus and a stronger arrhythmic effect.[26,28,123,124] If the underlying mechanism is fetal-hypoxia related, a higher risk of adverse effect after clarithromycin exposure might be expected compared with erythromycin or azithromycin.

1.5.3 Exposure duration

The duration of macrolide exposure is also likely to be correlated with the risk of adverse outcomes. A short treatment period may be less associated with harm to the fetus, as compared with longer exposure and more severe fetal hypoxia, especially during late gestation when the fetal cardiovascular system could adopt strategies to spare the fetal brain. In other words, it is reasonable to expect a dose-response relationship between macrolides exposure and the risk of adverse child outcomes.

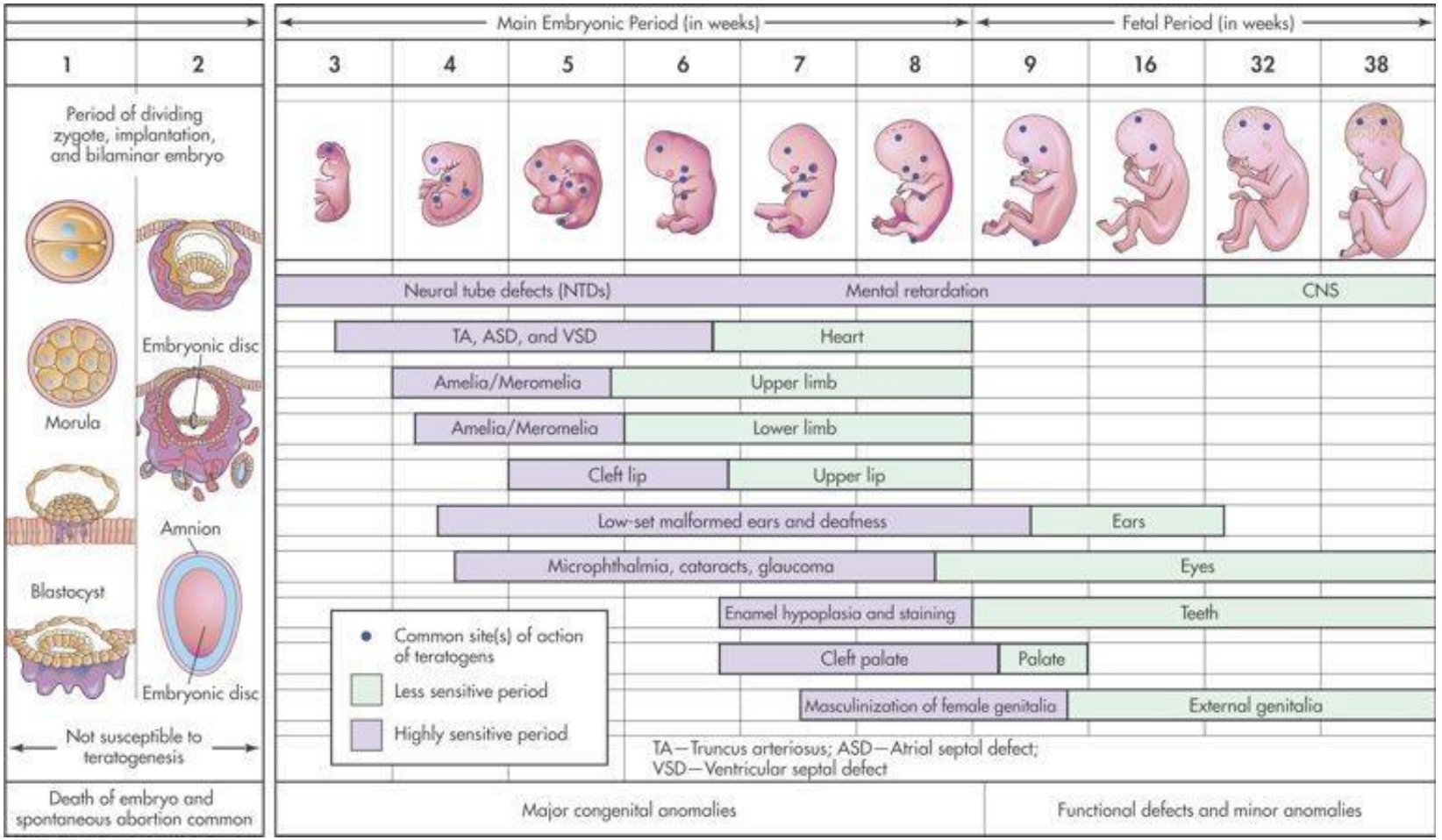
To conclude, if macrolides exposure during pregnancy has a direct harmful effect on the fetus, I would expect the strength of association between macrolides and risk of adverse child outcomes vary by: 1) timing of macrolides exposure during pregnancy; 2) macrolides subtypes; and 3) the duration of macrolide treatment.

1.5.4 Baby gender

Baby sex is believed to be an important factor involved in the multifactorial, known or not known mechanisms of adverse pregnancy and child outcomes. The male fetus is at greater risk of death or damage from almost all the obstetric complications.[125] Perinatal brain damage, cerebral palsy, congenital malformations of the genitalia and limbs, premature birth, and stillbirth are more frequent in boys.[126] These increased risks could be related to sex-dependent physiologic and pathologic changes in the placenta and the fetus,[127] although the specific impact of sex in the mechanisms of the outcomes remains unclear. I thus performed subgroup analyses by baby sex where possible.

Figure 1-2. Critical periods in human development.

(Source: *The Developing Human: Clinically Oriented Embryology,* Moore and Persaud, 1998).[128]



1.6 Study type

1. Systematic review and meta-analysis

In this study, I first conducted a systematic review to synthesise existing evidence on associations between macrolides exposure during pregnancy and a range of outcomes that can be at least partially induced by a short-term fetal hypoxia based on evidence from human or animal studies (Table 1-3). I included both RCTs and observational studies in this systematic review and meta-analysis to evaluate whether the current evidence was sufficient and consistent regarding each interested outcome. Besides the ORACLE II trial, a number of RCTs investigated outcomes in offspring whose mother had infections (e.g. Chlamydia trachomatis and preterm premature rupture of membranes (pPROM)) and macrolides in pregnancy, as part of the safety evaluation of macrolides.

2. Retrospective cohort study based on CPRD

I used a retrospective cohort derived from the CPRD to examine the association between prenatal macrolide prescription and adverse outcomes in the offspring. Given the observed adverse effect of macrolides in OCS II and the rarity of the outcomes,[24] further RCTs on the effect of macrolide antibiotics use during pregnancy would be unethical, and prospective studies requiring a large sample size with long follow-up would be expensive. A large retrospective cohort study is thus required. Using the large UK primary care database (CPRD), I compared prenatal macrolide antibiotic prescriptions with other type(s) of antibiotics prescribed for a similar indication, to determine evidence of any association with multiple adverse outcomes in childhood. Such databases are sufficiently large for studying rare events. CPRD captures real-life events, has been validated for the study of teratogenic and other effects of prescribed medications in pregnancy, and substantially used for pharmacoepidemiology and drug safety studies.[129,130]

1.7 PhD aims and objectives

As I outlined above, there is a clear need for robust evidence on the association between maternal exposure of macrolide antibiotics during pregnancy and a later risk of adverse outcomes in the fetus and during childhood. The overall aim of this PhD is to examine the association between maternal exposure of macrolides antibiotics during pregnancy and child adverse outcomes where short-term fetal hypoxia could be aetiologically involved; I also evaluate the effect of timing, subtypes and duration of macrolides exposure on this association.

The specific objectives are to:

1. Conduct a systematic review and meta-analysis to synthesise existing evidence and identify adverse outcomes of short-term fetal hypoxia where there is a consistent evidence for a significantly increased risk after prenatal exposure to macrolide antibiotics, and outcomes where further exploration is needed.
2. Determine the association between maternal exposure of macrolide antibiotics during pregnancy and adverse child outcomes by using UK primary care data, which involves:
   2.1 To develop a mother-baby cohort using an UK administrative general practice database.
   2.2 To investigate the patterns of prenatal macrolide and other antibiotics prescribing and determine their association with maternal characteristics including infection during pregnancy.
   2.3 To develop and validate the indicators of each eligible outcome in children.
   2.4 To conduct a retrospective cohort study to determine whether there was an association between macrolide (versus penicillin) prescribing in pregnancy and adverse child outcomes relating to short-term fetal hypoxia, where the effect of exposure timing, macrolides subtypes, treatment duration and baby gender will be evaluated.
   2.5 Explore the effect of exposure timing on the association between macrolides and specific outcomes, e.g. VSDs.

1.8 Thesis structure

In **Chapter 2**, because this PhD study relies on historically summarised data (systematic review) or observed data (cohort study), bias control is crucial to estimate the effect of macrolides. I systematically describe three major source of potential bias (confounding bias, selection bias and misclassification bias) and corresponding solutions as analyses strategies.

In **Chapter 3**, I conduct a systematic review and meta-analysis to address Objective 1. The systematic review covers all outcomes interested, and evaluates whether the current evidence was sufficient and consistent regarding each adverse outcome.

In **Chapter 4**, I present work towards objective 2. I describe the development of the CPRD Mother Baby Cohort given the CPRD mother-baby linkage. I present methods for building the CPRD mother-baby linkage (briefly) and the development of the CPRD Mother Baby Cohort, derive mothers' demographic and pregnancy-related characteristics (including gestational age),

describe the CPRD Mother Baby Cohort and discuss the representativeness of the cohort for the pregnancies in the UK (Objective 2.1).

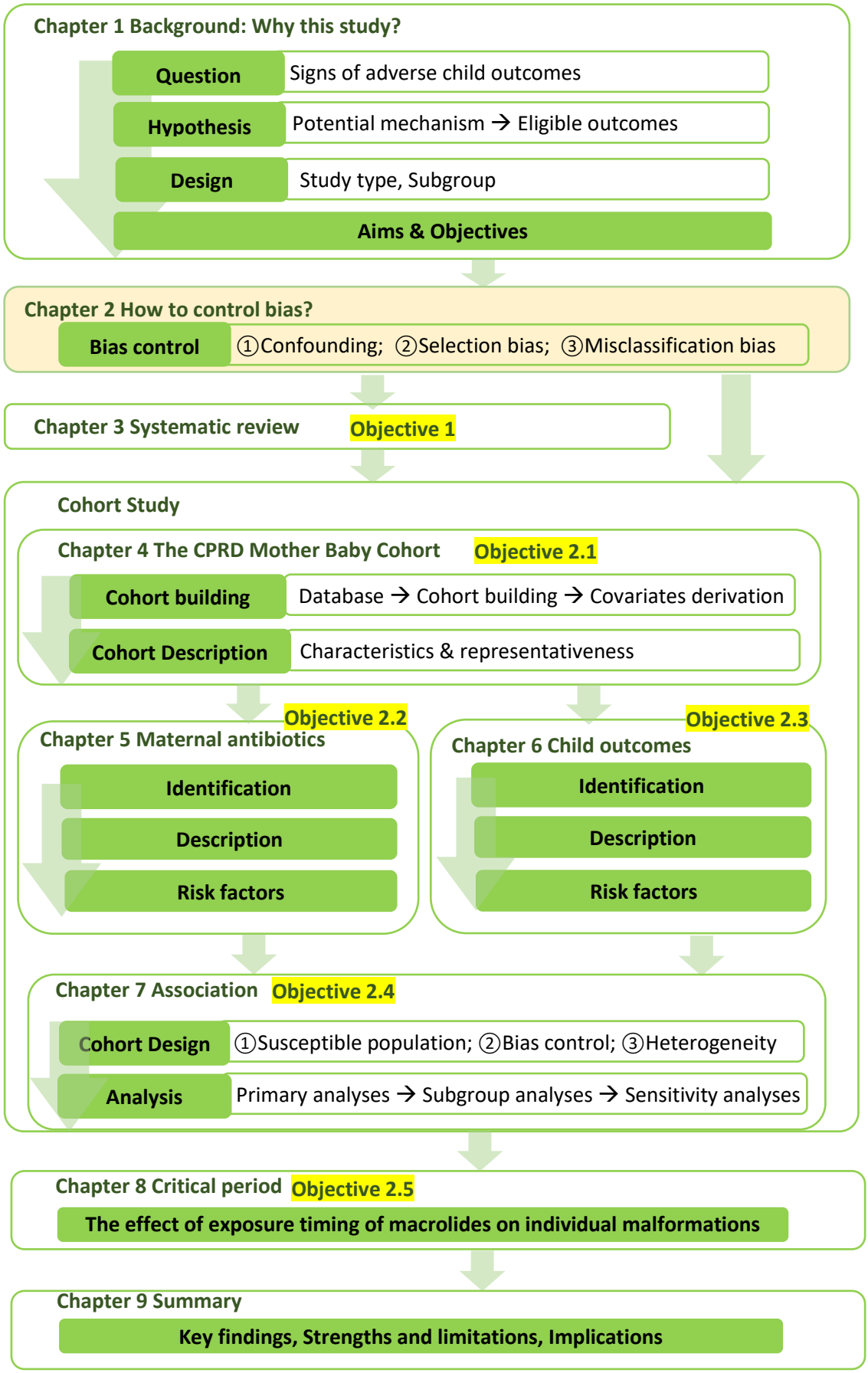
Further work towards objective 2 is described in **Chapter 5 and Chapter 6,** where antibiotics exposure and child adverse outcomes in the CPRD Mother Baby Cohort were defined and described, respectively. In **Chapter 5** I explore whether antibiotic prescribing in pregnancy is adequately captured in the primary care database. I also seek to understand the associations between potential risk factors and infection indications with antibiotic prescribing in pregnancy, which may confound the association between fetal exposure to macrolides and adverse child outcomes (Objective 2.2). In **Chapter 6** I identify and validate each adverse child outcome using the CPRD Mother Baby Cohort, with potential risk factors for the outcomes evaluated and discussed (Objective 2.3).

In **Chapter 7**, I perform a cohort study using the CPRD Mother Baby Cohort to assess the association between macrolide prescribing during pregnancy and adverse child outcomes, and evaluate the heterogeneity within the association according to the timing of macrolide prescribing, subtypes, duration of treatment and baby gender (Objective 2.4).

In **Chapter 8**, to address the dilution effect of unspecific measurement of outcome and exposure in previous studies, I explore the exposure time-specific effect of macrolides on a continuous time scale for common specific outcomes (Objective 2.5).

In **Chapter 9,** I discuss the key findings from the thesis, describe the limitations of using administrative data to study child outcomes of pregnancy antibiotics exposure, and discuss the need for large-scale analyses of high quality trial and observational cohorts that have accurate measurement of treatment and specific child outcomes.

Thesis Structure

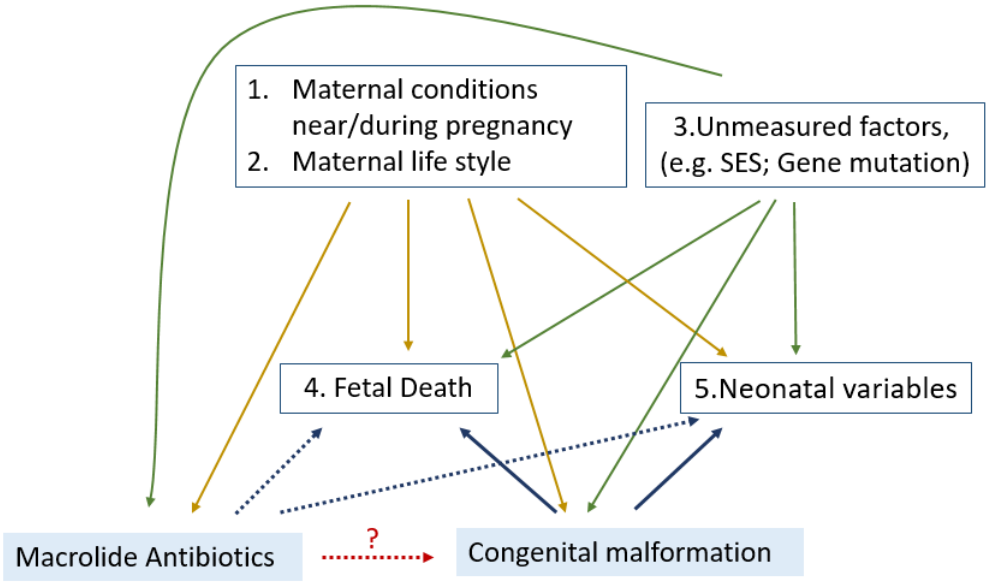


Chapter 2 Methodological considerations: bias control

This study aims to provide evidence on the causal relationship between macrolides exposure during pregnancy and adverse outcomes, based on a systematic review and a retrospective cohort design. Because this study relies on historically summarised data (systematic review) or observed data (cohort study), bias control is crucial to estimate the effect of macrolides. Multiple sources of bias exist, including confounding bias, selection bias and misclassification bias.

In the following, I discuss sources of bias in the context of observational studies, which also applies to the systematic review. Bias specific to RCTs is discussed when specified. I use a Directed Acyclic Graph (DAG) to present the relationships between macrolides exposure, child outcomes (e.g. congenital malformations), and associated factors categories (Figure 2-1). Associated factors in each categories are listed in Table 2-1.

Figure 2-1. A Directed Acyclic Graph (DAG) for the association between macrolides and malformations in children.



*Dashed lines represent causal relationships that may exist. SES: social-economic status.

Table 2-1. Potential risk factors.

1. Maternal conditions near/during pregnancy
Maternal infection, pregnancy year, maternal age, hypertension, diabetes, epilepsy, depression, anxiety, chronic medical treatments, parity, and multiple birth.
2. Maternal life style
Alcohol misuse, illicit drug use, smoking/tobacco use, and obesity.

3. Unmeasured factors
Social-economic status (SES); genetic variants.
4. Fetal Death
5. Neonatal variables
Gestational age, birth weight, and Apgar score

2.1 Confounding bias (Factor category 1-3)

a. Indication bias (Factor category 1)

Confounding could be the source of bias which observation studies are probably most susceptible to. For observational studies on the effect of antibiotics, the most common confounding may be indication bias due to infection. This bias occurs because some infection during pregnancy can be a cause of adverse outcomes in the offspring (as discussed in section 1.4.3), while infection (of suspected bacterial aetiology) is also the indication for prescribing antibiotics. Compare mothers exposed to macrolides with mothers not exposed to macrolides, mothers not exposed to antibiotics or the general population would all bias the estimated adverse effect in children towards upwards (overestimating risk).

Solution 1: Head to head comparison. Head to head comparisons of antibiotics for the same underlying infection provides particularly useful information on their adverse effect in pharmacoepidemiology studies. In the context of this study, penicillin is a preferable comparator for macrolides for two reasons. First, as mentioned in the first chapter, the most frequent indication for macrolides' use in pregnancy is as a replacement for suspected penicillin allergy, e.g. for upper and lower respiratory tract and soft tissue infections. According to the antibiotics prescribing guidelines issued by the National Institute for Health and Care Excellence, common indications of erythromycin and clarithromycin include sore throat, sinusitis (acute), cough (acute) and otitis media (acute), which are the most common indications for antibiotic prescriptions during pregnancy. Table 2-2 includes a full list of BNF indications for common macrolides and penicillins (e.g. Benzylpenicillin sodium).[131] Secondly, penicillin itself has long-established safety records during pregnancy.[132] The potential adverse effect of macrolides could therefore be measured with a controlled confounding due to indication, treatment effect and adverse effect. A detailed real-world comparison of the indications between macrolides and penicillins is reported in Chapter 4, based on a retrospective cohort study using a primary care database of mothers linked to children.

Differences in seriousness or comorbidity of infection between two comparison groups could also induce bias. Seriousness is hard to measure, yet the risk could be reduced by restricting the number of macrolides and penicillin prescriptions to e.g. monotherapies.

For RCTs, although indication bias is not a concern due to the random assignment, head-to-head design is still preferable to placebo-controlled design for this study. The reason is that the placebo-controlled design would mask potential adverse effects of macrolides by reducing the adverse fetal effects of infection through effective antibiotic treatment of the macrolides arm. The overall effect would be to bias results towards the null or protective effect.

Solution 2: Restrict analyses to Respiratory tract infections (RTIs). An obvious solution is to compare treated population to untreated population with the same indication. Urinary tract infections (UTIs), though common during pregnancy, is not a suitable indication to control. Pregnant women with UTI are very unlikely to remain untreated since UTI is one of the potentially neurologically-damaging infections and untreated UTI could lead to adverse pregnancy outcomes (e.g. preterm delivery) and severe consequences (e.g. sepsis and pneumonia) in the offspring.[133] The treated and rare untreated women with UTI could therefore significantly differ in their risk profiles, which will induce residual confounding in the estimation. Respiratory tract infection (RTI) is the most common infection during pregnancy: one in five pregnant women have an RTI recorded based on a study using The Health Improvement Network (THIN) UK, a similar primary care database to CPRD in 2010.[9] Because RTIs diagnosed in primary care are usually self-limiting with viral aetiology, any benefits (in terms of a reduction in the risk of adverse child outcome) due to antibiotic treatment of bacterial RTI would be small or negligible, thereby increasing the chance of detecting potential adverse effects of antibiotic treatment.

In summary, the head-to-head comparison (macrolides versus alternative antibiotics) is preferable for both observational studies and RCTs because it can reduce the risk of indication bias due to maternal infection and avoid masking due to the effect of antibiotic treatment on infection. Ideally, only monotherapy macrolides and penicillins would be compared. I will also perform the analyses restricted to RTIs where possible, to further reduce the risk of indication bias.

Table 2-2. British National Formulary (BNF) indications for common macrolides and penicillin (as ordered).

Erythromycin	Clarithromycin	Azithromycin	Benzylpenicillin sodium
Susceptible infections in patients with penicillin hypersensitivity (e.g. respiratory-tract infections (including Legionella infection), skin and oral infections, and campylobacter enteritis)	Respiratory-tract infections, Mild to moderate skin and soft-tissue infections	Prevention of secondary case of invasive group A streptococcal infection in patients who are allergic to penicillin	Mild to moderate susceptible infections, Throat infections, Otitis media, Cellulitis, Pneumonia
Acute otitis media	Acute exacerbation of chronic obstructive pulmonary disease	Respiratory-tract infections, otitis media, skin and soft-tissue infections	Endocarditis (in combination with other antibacterial if necessary)
Early syphilis	Acute exacerbation of bronchiectasis	Uncomplicated genital chlamydial infections, Non-gonococcal urethritis	Anthrax (in combination with other antibacterials)
Uncomplicated genital chlamydia, Non-gonococcal urethritis	Acute otitis media	Uncomplicated gonorrhoea	Intrapartum prophylaxis against group B streptococcal infection
Chronic prostatitis	Prevention of pertussis	Lyme disease [erythema migrans and/or non-focal symptoms]	Meningitis, Meningococcal disease
Prevention and treatment of pertussis	Helicobacter pylori eradication in combination with a proton pump inhibitor and amoxicillin	Mild to moderate typhoid due to multiple-antibacterial resistant organisms	Suspected meningococcal disease (meningitis with non-blanching rash or meningococcal septicaemia) prior to urgent transfer to hospital
Prevention of secondary case of diphtheria in non-immune patient	Helicobacter pylori eradication in combination with a proton pump inhibitor and metronidazole	Community-acquired pneumonia, low to moderate severity/ high severity	Suspected bacterial meningitis without non-blanching rash where patient cannot be transferred to hospital urgently
Prevention of secondary case of invasive group A streptococcal infection in penicillin allergic patients	Acute sinusitis	Antibacterial prophylaxis for insertion of intra-uterine device	
Prevention of pneumococcal infection in asplenia or in patients with sickle-cell disease (if penicillin-allergic)		Trachomatous conjunctivitis caused by Chlamydia trachomatis, Purulent bacterial conjunctivitis	
Prevention of recurrence of rheumatic fever			
Rosacea, Acne			
Gastro-intestinal stasis			

b. Other measured risk factors (Factor category 1 & 2)

While the head-to-head comparison minimises confounding due to infection, it might additionally make other potential confounders more comparable between the groups, assuming infection was associated with maternal characteristics. For example, factors found to be associated with antibiotic prescribing described in the population-based research include age (younger women receive more prescriptions for antibiotics than older women) and social-economic status (welfare recipients and women with high Townsend score are more likely to be prescribed antibiotics).[9] However the comparability of these and other potential confounders cannot be ensured, and a strict control is still needed. As described in Section 1.4.3, some factors are observed to be associated with the risk of these outcomes (though the causal pathways have not been established), such as maternal age, life style, chronic disease, multiple births and some others.

Solution: In the cohort study in this thesis, I will adjust the measured factors potentially precede the outcome (or, risk factors) as covariates in the comparison between macrolides and penicillins, with details methods discussed in Chapter 6.

c. Unmeasured risk factors (Factor category 3)

Not all of the potential risk factors I discussed were measured in the CPRD database, e.g. maternal educational level, social-economic status, or genetic variance. The direction of bias due to the unmeasured factors cannot be determined.

Solution: I use two negative control cohorts to evaluate the effect of the unmeasured factors indirectly. In the first negative control cohort, I compare children born to mothers whose macrolides or penicillins were prescribed before conception (e.g. from 10 to 50 weeks before conception). In the second negative control cohort, I compare siblings of children in the two exposure groups of the main analyses (i.e. children whose mother were prescribed macrolides versus penicillins during pregnancy). If increased risks were observed in the main cohort but not in these negative control cohorts, it is reasonable to assume that confounding owing to family-related factors is unlikely to explain the observed increased risks. Additionally, an effect specific to exposure during critical period would strengthen the causal argument for the association between macrolides and adverse child outcomes.

2.2 Live-birth bias (Factor category 4)

Except for prenatal death, the eligible outcomes listed in Table 1-3 can only be ascertained within livebirths. When both the antibiotics and the outcomes affect the risk of fetal death (directly or by unmeasured common cause), a live-birth bias would be induced by limiting the study population to live births, which is a "collider", or a common effect.[134] Both the direction and magnitude of the live-birth bias cannot be predicted. This live-birth bias is often overlooked in previous studies on the adverse outcomes of macrolides exposure during pregnancy[24,135]

Solution: The comparison between macrolides and penicillins minimises the risk of live birth bias, since macrolide is considered to have a similar treatment effect on infection (including the effect on fetal loss) as penicillin. Within women with the same treatment indication (infection), the relative risk of fetal loss for treated and untreated women would be difficult to predict because of the combination of an increased risk of fetal loss due to the effect of serious infection and a reduced risk of fetal loss due to the effect of treatment on infection. Meanwhile, the risk of fetal loss would be more comparable between women treated by macrolides and women treated by penicillin due to comparable seriousness and treatment effect. Thus the causal link between treatment and fetal loss is weakened and the risk of live-birth bias is reduced. This applies to both RCTs and observational studies as long as the comparator involves antibiotics that are similarly effective to macrolides (e.g. penicillin and cephalosporin).

Nevertheless, this live birth bias may still exist within head-to-head comparisons and a further simulation (e.g. multiple bias analyses) will be needed to evaluate the direction and magnitude of the bias.

2.3 Potential descendants or mediators (Factor category 5)

Neonatal variables such as gestational age, birth weight and Apgar score should not be adjusted for in the analysis of any outcomes. This is because neonatal variables are strongly associated with the risks of adverse outcomes as either potential descending proxies (e.g. for malformation) or mediators on the causal path from macrolides to adverse child outcome (e.g. cerebral palsy). Adjustment for neonatal variables is thus not only unnecessary but likely harmful.

2.4 Misclassification bias

Outcomes misclassification is possible in this cohort study using records from primary care practice.

Since any misclassification is probably non-differential between the exposure groups, the misclassification may therefore bias the risk (or hazard) ratio towards null. However, as a drug safety study, the quantification of the potential underestimation of risk is still needed.

Potential exposure misclassification can be induced by two source. First, in observational studies, filled prescriptions are often used as proxies for maternal exposure to antibiotics. Such studies do not record whether the antibiotics were actually taken, and this could differ between macrolides and penicillins because the gastrointestinal side effects of erythromycin reduce compliance. However, this is unlikely to lead to an overestimated bias due to more untreated infection in macrolides group, because the antibiotics may predominantly be prescribed for virus infections in primary care settings.[136] Second, because the pregnancy start date is achieved through estimation in CPRD, the non-perfect estimation could also lead to potential misclassification of exposure (e.g. exposure before the pregnancy start date could be classified as exposure during pregnancy if an earlier pregnancy start date was estimated).

Both trials and observational studies are susceptible to another important source of misclassification, i.e. non-specific outcome grouping and non-specific exposure measurement. Individual outcomes are often grouped together because of limited number of cases (e.g. specific malformations into "major malformation" or "system-specific malformation"), even though causes and critical periods for individual malformations differ within these groups. The possible dilution effect in epidemiological studies due to grouping phenotypes with different inherent causes has been highlighted by Jenkins et al.[137] A example of the dilution effect is the study of Romøren et al, where the odds ratio estimates for cardiovascular malformations increased from 1.2 [95% CI 0.8, 1.8] for erythromycin measured during the first trimester to 1.6 [95% CI 0.9–3.0] for erythromycin measured during days 28–56 of gestation (though not statistically significant at the 5% level).[12]

Solution 1: I summarise previous validation studies on the sensitivity and positive predictive value of outcomes recording in CPRD, and perform probabilistic bias analyses on outcome misclassification to quantify the magnitude of the bias.

Solution 2: Exposure timing-specific effect can be explored for most common specific malformations, e.g. ventricular septal defects (VSDs), using suitable statistical method (e.g. generalised additive model).

2.5 Summary

To evaluate the association between macrolides use during pregnancy and adverse child outcomes, the study needs to be designed to control the risk of three major types of bias: 1) confounding (indication bias, measured and unmeasured confounders); 2) misclassification bias; and 3) live-birth bias. Heterogeneity of the association should also be evaluated using subgroup analyses (Section 1.5). The discussion above on potential bias lead to the following considerations on design:

For comparison group:

a. Head-to-head comparison of antibiotics (macrolides versus alternative antibiotics, e.g. penicillins) is preferable in RCTs and observational studies. Such a comparison reduces the risk of indication and live-birth bias, and allows separation of the adverse effect of macrolides from its beneficial effect through reduced fetal infection. Ideally, only monotherapy prescription of macrolide and alternative antibiotics were compared.
b. In RCTs, the comparison of macrolides versus placebo is a secondary choice since there are residual live-birth biases and the adverse effect of macrolides cannot be separated from treatment effects.

Subgroup analyses are planned where the analyses were stratified by (where feasible):

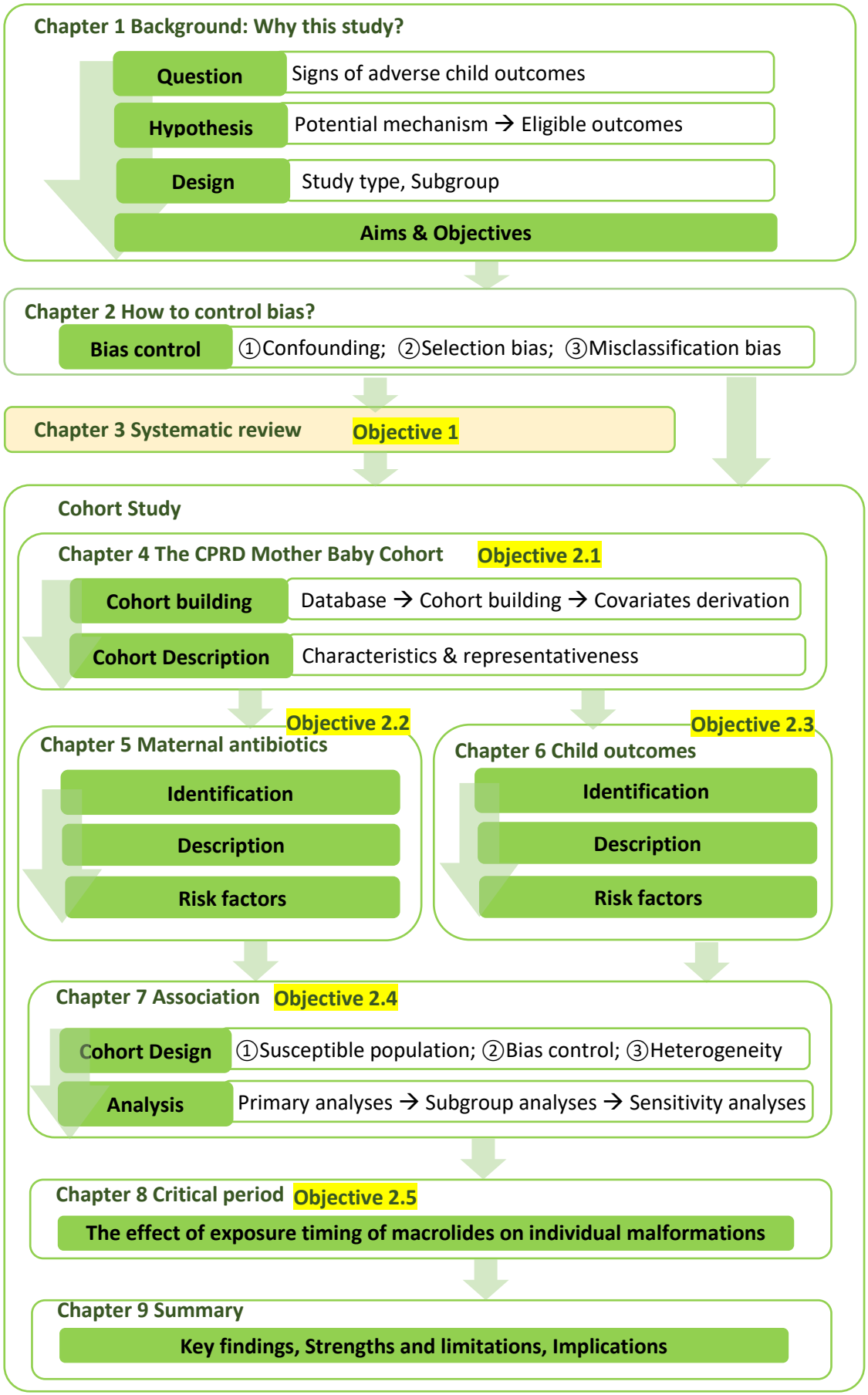
a. Exposure (prescription) timing of macrolides
b. Macrolides subtypes
c. Duration of prescription
d. Baby gender

To control bias, following methods/analyses are planned where feasible:

a. Adjusted for maternal conditions near or during pregnancy and maternal life style factors in observational studies, to reduce the risk of confounding bias.
b. Two negative control cohorts to evaluate the effect of unmeasured confounders:
    I. Within children whose mother were prescribed antibiotics before conception;
    II. Within siblings of children whose mothers were prescribed antibiotics during pregnancy.
c. Sensitivity analyses:
    I. Restricting to children whose mother were prescribed for RTIs.
    II. Quantifying the direction and magnitude of outcome misclassification and live-birth bias using multiple bias analyses.

Exploratory studies will be performed on the exposure-timing-specific effect on common specific outcomes, e.g. ASDs.

Thesis Structure



Chapter 3 Systematic review and meta-analyses

Earlier I described existing sign of macrolides' adverse child effect and an assumed pathway through induced fetal hypoxia. The evidence from current clinical studies is inconsistent for the association between macrolides and various adverse outcomes. In this chapter, I synthesised evidence from previous RCTs and observational studies to evaluate whether the current evidence was sufficient and consistent regarding the association between macrolides treatment in pregnancy and each fetal-hypoxia related adverse child outcome.

3.1 Chapter overview

Studies exists which provide evidence on the risk of various adverse outcomes in fetuses and children after maternal macrolide antibiotic exposure during pregnancy. Besides the ORACLE/OCS studies, some RCTs measured the outcomes in offspring as the safety evaluation of macrolides, mainly within women with prenatal infections (e.g. Chlamydia trachomatis and pPROM). Several observational studies were designed to detect adverse outcomes of macrolides, using population- and cohort-based data.

I conducted a systematic review and meta-analysis to examine the effects of macrolide treatment during pregnancy on paediatric (fetal and child) outcomes. The systematic review prioritized comparisons between macrolides and alternative antibiotics (mainly penicillins or cephalosporins), because I assume their indication and treatment effects were comparable. I regarded RCTs where one of the arms included placebo or drug combinations as secondary comparisons because they are susceptible to incomparable treatment effects. Based on previous evidence from animal and epidemiological studies, I hypothesized that an underlying mechanism of observed adverse effects of macrolides could be short-term fetal hypoxia induced by fetal arrhythmia. The outcomes of interest in this systematic review are therefore outcomes that could potentially result from short-term fetal hypoxia. I presented pooled effects of macrolides use in pregnancy on each outcome by study types and explored heterogeneity of the effect according to specific types of macrolides.

A complete PRISMA harm checklist for this review is available in Appendix 3-1. A protocol for this review has not been previously published.

3.2 Methods

3.2.1 Eligibility criteria

3.2.1.1 Study types

I sought comparative studies which examined macrolide treatment during pregnancy and adverse fetal and/or childhood outcomes that have been reported to be associated with fetal hypoxia. I included randomised controlled trials and observational (cohort or case-control) studies and set different eligibility criteria for the comparator group according to study type in order to address the risk of bias as explained below.

3.2.1.2 Participants, interventions and comparisons

The exposed populations were the fetuses or children whose mothers were prescribed macrolide antibiotics during pregnancy. I included studies that included all pregnancies or those that reported outcomes only in live-births.

The primary analysis included studies comparing macrolide antibiotics with alternative antibiotics e.g. penicillin or cephalosporin. Macrolides are recommended as the alternative for women with suspected allergy to penicillin or cephalosporin, thereby minimising the risk of indication bias due to infection [3,132]. Penicillin and cephalosporin are also comparable with macrolides in treatment effect with long-established safety records during pregnancy [132]. This head-to-head comparison thus also allows the separation of possible harm of macrolides from their benefits of treatment on infection.

The secondary analysis includes RCTs with the following two comparisons: macrolide versus placebo and macrolides plus alternative antibiotics versus the same alternatives. Though RCTs avoid indication bias by design, true adverse effects of macrolides may be masked by the benefits of macrolides in reducing infection, thereby underestimating adverse effects.

3.2.1.3 Outcomes

I reviewed cohort studies of pregnant women and their children and experimental studies in animals to identify outcomes that could potentially result from short term fetal hypoxia, including fetal and neonatal death, congenital malformations, and conditions resulting from central nervous system damage (i.e. epilepsy, cerebral palsy, ADHD and autism). I excluded outcomes commonly related to chronic hypoxia (e.g. low birthweight, intrauterine growth restriction) or outcomes that might result from postnatal events (e.g. abnormal cerebral ultrasonography).

3.2.2 Search Strategy

Systematic literature searches were conducted in PubMed, Embase, Cochrane Library, Conference Proceeding Citation Index-Science and ClinicalTrials.gov from their respective inception until Februry 15, 2018 (Appendix 3-2). I included conference abstracts if sufficient data were provided. Further relevant studies were retrieved by hand searching the reference lists of eligible papers and by using 'Similar articles' function within databases. No language restrictions were applied.

3.2.3 Study selection

After removal of duplicates, the titles and abstracts of all identified records (full-text articles and abstracts) were evaluated by reviewer HF, using a "decision tree" (Appendix 3-3). Ten percent of the records were also reviewed by another reviewer LW, and an inter-rater agreement was calculated. A total of 967 studies were double reviewed with an inter-rater agreement of 81%. All studies potentially meeting the inclusion criteria were reviewed in full by reviewer HF, and 10% of the potentially eligible studies were reviewed by a third reviewer (LL). I resolved any discrepancies through discussion. Reviewers were not blinded to authors, journals or institutions.

3.2.4 Data extraction

I developed and piloted a data extraction sheet on 15 relevant studies. Reviewer HF extracted relevant data from included studies. I contacted authors of potentially eligible manuscripts by email for relevant data if this could not be extracted from the publication. Where the same cohort was reported more than once, I extracted data from the study with the largest sample size.

For studies reporting multiple comparisons of each specific type of macrolides (vs same comparator), I divided the comparator group equally for each comparison. For studies presenting multiple estimates of exposure on both whole pregnancy and the first trimester, the estimate of exposure on the first trimester was included in the meta-analysis to avoid a potential dilution effect.

3.2.5 Risk of bias assessment

I used risk of bias assessment tools for RCTs (Cochrane Collaboration's tool for assessing risk of bias in randomised trials) and observational studies (Risk of Bias In Non-Randomised Studies – of Interventions (ROBIN-I)). The tool for RCTs was modified by including sections on masked adverse effect (susceptible when one of the arms was placebo or drug combinations, as mentioned in the eligibility criteria). I considered observational comparisons of macrolides versus alternative antibiotics to be at low or moderate risk of indication bias, provided there is no evidence of macrolides indication apart from that of alternative antibiotics (Appendix 3-4, Appendix 3-5) [138,139].

3.2.6 Data synthesis and analysis

I estimated the pooled ORs for each adverse outcome using a random-effects meta-analysis, considering the heterogeneity among studies that was measured by I^2 statistics. RCTs and observational studies were analysed separately. In the primary analysis, I compared macrolides with alternative antibiotics in RCTs and observational studies. In the secondary analyses, I included RCTs that compared (1) macrolides with alternative antibiotics, (2) macrolides with placebo and (3) macrolides plus alternative antibiotics with the same alternatives. I summarized the results according to specific types of macrolide in subgroup analysis. Sensitivity analysis was performed according to risks of bias.

Studies with no observed events were excluded from the meta-analysis. For studies with no events in one of the two groups, I applied a correction proportional to the reciprocal of the size of the contrasting study arm [140]. For observational studies, I used the adjusted OR if reported, otherwise, I re-calculated the crude OR using the data reported. Funnel plot asymmetry was not assessed because of an insufficient number of studies. Analyses were performed using R 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria).

3.3 Results

I identified 11,186 citations, and selected 99 abstracts for detailed assessment (Figure 3-1). The primary analysis included 14 articles based on 12 studies (2 RCTs and 10 observational studies, 190,368 pregnancies or live births) which met the eligibility criteria. Secondary analysis included 9 RCTs published in 11 articles of 15,405 pregnancies. I summarised the characteristics of the included studies in Appendix 3-6 [12,15,19,20,22-25,141-153].

Overall, 12/14 (85.7%) articles in the primary analysis were judged to have low or medium overall risk of bias (Figure 3-2). Adjusted ORs were available for four of ten observational studies [15,23,25,147].

Figure 3-1 Study selection.

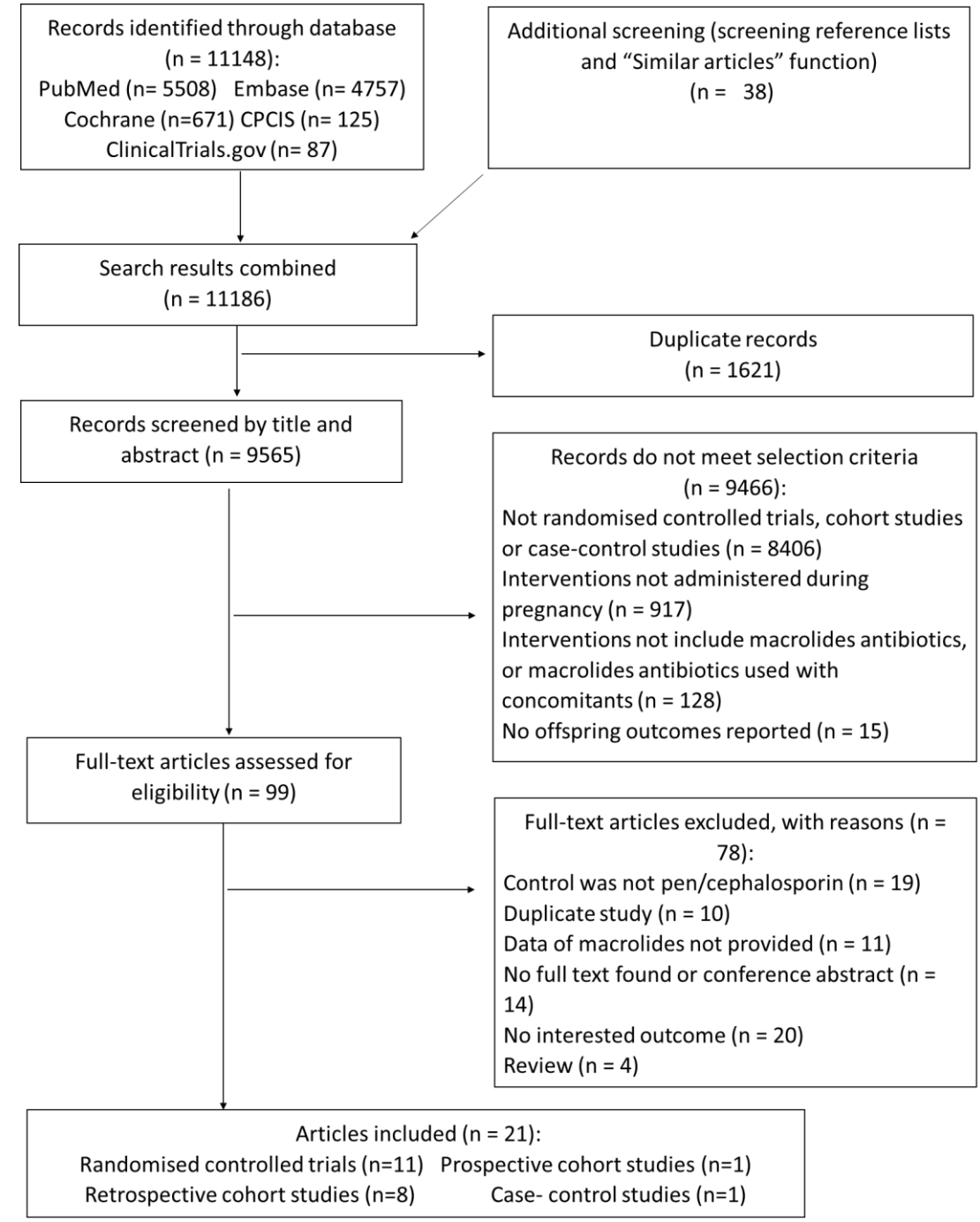


Figure 3-2 Assessment of bias.

RCTs	Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Imcomparable treatment effect	Overall risk of bias	Note
1	Kenyon, et al (2001, ORACLE I)- Neonatal death	○	○	○	○	○	○	○	○	a
2	Kenyon, et al (2001, ORACLE II)- Neonatal death	○	○	○	○	○	○	○	○	a
3	Kenyon, et al (2008, OCS I)- Stillbirth	○	○	○	○	○	○	○	○	
3	Kenyon, et al (2008, OCS I)- Other outcomes	○	○	○	○	○	○	○	○	a
4	Kenyon, et al (2008, OCS II)- Stillbirth	○	○	○	○	○	○	○	○	
4	Kenyon, et al (2008, OCS II)- Other outcomes	○	○	○	○	○	○	○	○	a
5	Eschenbach (1991)	○	●	●	●	◐	○	◐	●	b
6	Kwak (2013)	○	●	●	○	◐	○	◐	●	b
7	Martin (1997)	○	●	●	●	◐	○	◐	●	b
8	McGregor (1991)- Stillbirth	○	●	●	●	○	○	◐	●	b
8	McGregor (1991)- Neonatal death	○	●	●	●	◐	○	◐	●	b
9	Mercer (1992)	○	●	○	○	◐	○	◐	●	b
10	Tita (2016)	○	○	○	○	◐	○	◐	◐	b
11	Ye, Y (2001)	○	●	●	●	◐	○	◐	●	b

Observational studies	Study	Bias due to confounding	Bias in selection of participants	Bias in classification of interventions	Bias due to deviation from intended interventions	Bias due to missing	Bias in measurement of outcomes	Bias in selection of the reported results	Overall risk of bias	Note
12	Andersen (2013)	○	○	○	○	○	○	○	○	c
13	Cooper (2008)	◐	○	○	○	○	○	○	◐	
14	Einarson (1998)	◐	●	○	○	●	●	○	●	
15	Kallen (2005)	◐	○	○	○	○	○	○	◐	
16	Le Nguyen (2017)	○	●	○	○	●	●	○	●	d
17	Lund (2014)	◐	○	○	○	○	○	○	◐	
18	Meeraus (2015)	○	○	○	○	○	○	○	○	e
19	Muanda (2017)- Malformation	◐	○	○	○	○	○	○	◐	
20	Muanda (2017)- Miscarriage	○	○	○	○	○	○	○	○	f
21	Romoren (2012)	◐	○	○	○	○	○	○	◐	

● High risk ◐ Moderate risk ○ Low risk

a: Evaluated as modere risk in secondary analysis (low risk in primary analysis), caused by imcomparable treatment effect.

b: Studies only eligible for secondary analysis.

c: In the study of Andersens (2013), OR was adjusted by maternal age, number of previous miscarriages, income and education.

d: In the study of Le guyen, OR was adjusted by maternal age, long-term illnesses, parity and multiple pregnancy.

e: In the study of Meeraus (2015), HR was adjusted by maternal age, Townsend quintile, year of delivery, smoking/tobacco use, alcohol problems, obesity, illicit drug use, treatment of chronic medical conditions and potentially neurologically-damaging infection during pregnancy.

f: In the study of Muanda (2017), cases and controls were matched by gestational age and year of pregnancy; in the analysis of specific macrolides, OR were adjusted by 11 covariates, e.g. maternal age, education level, chronic comorbidities, maternal infections (urinary tract infection, respiratory tract infection, bacterial vaginosis and sexually transmitted infections) and prior exposure to antibiotics.

In the primary analysis, consistent increased odds of miscarriage for mothers prescribed macrolide antibiotics compared with those prescribed alternative antibiotics were observed in all three observational studies reporting the risk of miscarriage, with a pooled OR of 1.82 (95% CI 1.57-2.11) (Figure 3-3 and Appendix 3-7) [15,22,23]. No RCTs included evaluated the risk of miscarriage. Considering 1) the lowest risk ratio estimate in the three studies was 1.51 and 2) the baseline risks of miscarriage was 20% and 1% from early to mid-gestation, the number needed to harm (NNH) for miscarriage ranges from 10 at 6 weeks' gestation to 196 at 20 weeks.[15,154] In the analysis of macrolides subtype, the increased odds of miscarriage were observed in azithromycin and clarithromycin, but not in erythromycin, compared with alternative antibiotics (Appendix 3-7, Appendix 3-10).

The association with stillbirth, neonatal death or "stillbirth and neonatal mortality" was reported by two RCTs (4 articles) and 2 observational studies[12,22,24,143-145]. No significant pooled association was found between macrolides and alternative antibiotics prescribing. (Figure 3-3, Appendix 3-8).

Two RCTs, one of women with SPL and the other of women with pPROM reported the risks for cerebral palsy or epilepsy, but no increase of risk was found.[24,143] However, the odds for epilepsy and a composite outcome "cerebral palsy and/or epilepsy" increased significantly in mothers prescribed to macrolides compared with penicillins from general population in the large cohort study of Meeraus et al (pooled OR: 1.78 (95% CI 1.18-2.69)). The NNH for the two outcomes were 245 and 214, based on the baseline risks of 0.4% and 0.6%, respectively (Figure 3-3, Appendix 3-8).[25,155]

Seven observational studies but no RCTs evaluated the risk of malformations. No difference in risk was identified for "all malformations", major malformations, cardiovascular malformations and pyloric stenosis. For other organ-specific malformations, the results were dominated by the study of Muanda et al [20]. Evidence were scarce for the effect of specific types of macrolides on malformations. Only azithromycin was significantly associated with major malformations. (Figure 3-3, Appendix 3-10).

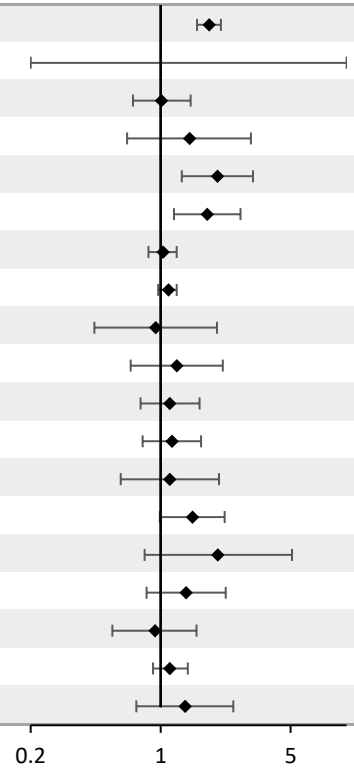
Results from the secondary analyses included RCTs with one arm as drug combinations or placebo were similar to those from the primary analysis [141,146,148-152]. (Figure 3-3, Appendix 3-9). The conclusions of primary analysis did not alter in sensitivity analysis restricted comparisons

with low or moderate risk of bias (applicable to miscarriage, “all malformation” and major malformation) (Appendix 3-12).

Figure 3-3. Primary and secondary analysis for the association between prenatal use of macrolides and adverse child outcomes.

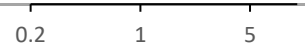
Primary analysis (Observational studies): Macrolides versus alternative antibiotics

	Outcomes	Studies	Events/ Pregnancies (n)	Crude OR or HR (95% CI)	I^2 (%)
Fetal and neonatal death	Miscarriage	3	1348/41029	1.82 [1.57, 2.11]	0
	Stillbirth	1	1/323	168.07 [0, >100]	.
	Stillbirth and neonatal mortality	1	159/22424	1.01 [0.71, 1.45]	.
Neurological outcomes	Cerebral palsy	1	55/30326	1.43 [0.66, 3.06]	.
	Epilepsy	1	142/30326	2.02 [1.30, 3.14]	.
	Cerebral palsy and epilepsy	1	184/30326	1.78 [1.18, 2.69]	.
Congenital malformations	All	4	897/28105	1.03 [0.86, 1.22]	0
	Major	3	1372/19231	1.10 [0.97, 1.25]	0
	Nervous System	2	93/19557	0.94 [0.44, 2.01]	14
	Orafacial	2	82/19557	1.22 [0.69, 2.16]	0
	Cardiovascular	4	523/37981	1.12 [0.78, 1.62]	61
	Ventricular/atrial septal defect	3	308/29862	1.15 [0.80, 1.65]	40
	Respiratory	1	59/11438	1.12 [0.61, 2.06]	.
	Gastrointestinal	2	149/19557	1.48 [0.99, 2.21]	0
	Cleft palate	1	20/11438	2.03 [0.82, 5.09]	.
	Pyloric stenosis	3	169/71686	1.37 [0.84, 2.24]	29
	Genital	1	93/11438	0.93 [0.55, 1.56]	0
	Musculo-skeletal	2	495/19557	1.12 [0.91, 1.40]	0
	Craniosynostosis	1	56/11438	1.35 [0.74, 2.46]	.



Primary analysis (RCTs): Macrolides versus alternative

		Studies	Events/	Prevention	Risk	Crude OR or HR	I^2(%)
Fetal and neonatal death	Stillbirth	2	59/4684			0.89 [0.53, 1.49]	0
	Neonatal Death	2	230/5529			0.96 [0.73, 1.25]	0
Neurological outcomes	Cerebral palsy	2	82/3264			1.31 [0.84, 2.04]	0
	Epilepsy	2	239/3264			1.03 [0.79, 1.35]	0



Secondary analysis (RCTs): Macrolides versus no macrolides

		Studies	Events/	Prevention	Risk	Crude OR or HR	I^2(%)
Fetal and neonatal death	Stillbirth	7	153/11688			0.87 [0.63, 1.21]	0
	Neonatal Death	6	486/13186			0.99 [0.83, 1.19]	0
Neurological outcomes	Cerebral palsy	2	167/6434			1.50 [0.93, 2.42]	0
	Epilepsy	2	473/6434			1.13 [0.89, 1.44]	0

*OR: odds ratio; HR: hazard ratio

3.4 Discussion

I conducted a systematic review and meta-analysis of associations between maternal macrolide antibiotics exposure and adverse child outcomes. I found consistent evidence for an association between macrolide antibiotics use during pregnancy and increased risk of miscarriage, inconsistent evidence for cerebral palsy and epilepsy, and insufficient evidence for malformations, stillbirth and neonatal death.

To our knowledge, the adverse fetal and child outcomes of macrolides use during pregnancy have not been systematically assessed before. The increased risk of miscarriage and inconsistent evidence for cerebral palsy and epilepsy suggests that macrolides may have the potential to cause adverse effects when used in pregnancy. Evidence from experimental and epidemiological studies in adults also indicates biological plausibility of embryotoxicity and cardiotoxity of exposure to azithromycin and clarithromycin. As the aetiology of these fetal adverse outcomes is multifactorial, the increase of absolute risk can be small for some conditions. There could be dependencies between comparisons where the comparator groups of a study were split or more than one outcome from one study was evaluated. Also, the scarcity of evidence for some outcomes could result in imprecision in the heterogeneity estimates.

To address potential bias due to treatment indication, in the primary analysis I included only head-to-head comparisons, most of which (42 out of 47 comparisons, 89%) were between macrolides and penicillins. Macrolides are often used as replacement therapy for patients with penicillin allergy [3]. There are unique indications of macrolides (e.g. azithromycin for gonorrhoea, chlamydia and mycoplasma infection) which could be linked to increased risk of miscarriage [156]. However, the indications of clarithromycin do not include genitourinary tract infections or sexually transmitted infections according to guidelines in both Europe and North America [157-160]. Yet all three studies in this review reported consistent increased risks of miscarriage associated with clarithromycin [15,22,23]. Furthermore, it is estimated that over 20% penicillins prescribed at pregnancy are for genitourinary tract infection [9]. The increased risk of miscarriage observed in this review is thus unlikely to be over-estimated. Among the three studies on miscarriage, two are large studies using Canadian and Danish population-based administrative databases. The Canadian study was a nested case-control study matched by gestational age, with adjustment for types of maternal infections (including sexually transmitted infections) and proxies for infection severity[23]. The Danish study also analysed indication by comparing clarithromycin with erythromycin, phenoxymethylpenicillin, amoxicillin and proton pump inhibitors adjusted by

maternal characteristics, with consistent evidence of increased risks of miscarriage in the clarithromycin group (Appendix 3-6) [15].

Survivor bias is another source of bias in studies on outcomes that often show after birth (e.g. neurodevelopmental disorders and malformations). Both macrolides exposure in pregnancy and adverse child outcomes may lead to increased risk of fetal death, therefore study conditioning on live birth status can distort the association estimates. The observed risk ratios for cerebral palsy, epilepsy and some serious malformations in this review may therefore be underestimated due to survivor bias. The potential underestimation is illustrated by a post-hoc simulation on the association between macrolides (versus penicillins) and major malformation in Appendix 3-13.

Heterogeneity exists among studies due to study design (RCT or observational), population of pregnant women studied, specific types of macrolides, and gestational ages for administering macrolides. Our analyses of RCTs were dominated by studies of mothers at high risk of fetal infection, e.g. mothers with pPROM or urinary tract infections. Compared with mothers randomised to placebo, mothers randomised to macrolides stand to benefit from the antibiotic treatment effect on fetal infection, which could therefore mask evidence of potential harms of macrolides. However, the masked harm due to treatment benefit would reduce in mothers with SPL, who had a relatively low risk of fetal infection. This reduced risk may explain the finding of the significantly increased risk of cerebral palsy in mothers with SPL, but not in mothers with pPROM in secondary analysis (Appendix 3-9).

Evidence is limited for the association with adverse outcomes according to macrolides subtypes. The increased risk of miscarriage was found for azithromycin and clarithromycin prescription during pregnancy, but not for erythromycin (1895 cases). Reasons are not clear for this heterogeneity. Fewer gastrointestinal side effects and better pharmacokinetic profiles (e.g. better oral bioavailability, tissue penetration and longer half-life of elimination) of clarithromycin and azithromycin may increase their maternal and fetal bioavailability compared with erythromycin.[121] Meanwhile, an *ex vivo* experiment using term human placentas found that the transplacental transfer is higher for clarithromycin as compared with erythromycin and azithromycin (percentage transfer at 6%, 2.6% and 3%, respectively).[122]

Timing of exposure according to critical periods in fetal development may also cause heterogeneity. Fetuses are most vulnerable to teratogenic effects during certain time windows (e.g., day 28-56 of gestation is the critical period for heart formation) [61,76] Romoren et al. reported that when the window of macrolides exposure was reduced from the first trimester to

28-56 days of gestation, the estimated OR of cardiovascular malformation increased from 0.96 to 1.36, although they were nonsignificant due to limited power [12]. This increased OR was similar to those reported by Kallen et al in 2005 and 2014[51,142] These findings highlight the possibility of underestimating the adverse effect of macrolides, by measuring the macrolides exposure outside of critical period for fetal development. [10,16,19,20]

Clinical relevance

The British National Formulary (BNF) comments erythromycin usage during pregnancy as "Not known to be harmful", and advises use azithromycin and clarithromycin only if adequate alternatives not available during pregnancy.[3] Given the consistent evidence for an increased risk of miscarriage in mothers prescribed macrolides, with a number needed to harm of 10 in first 6 weeks to 196 at 20 weeks of gestation, guidelines should be updated to avoid macrolides during the first trimester of pregnancy. The uncertain increased risks of cerebral palsy and epilepsy following macrolide treatment at any point during pregnancy should be reported in drug safety leaflets and alternative antibiotics recommended where appropriate.

3.5 Conclusions

Evidence is consistent with an increased risk of miscarriage in mothers prescribed macrolides during pregnancy compared with alternative antibiotics. The risk of cerebral palsy and epilepsy is uncertain, and there is insufficient evidence for adverse effects on congenital malformations. These findings warrant caution about the use of macrolides in pregnancy and use of alternatives where appropriate. As macrolides are the third most frequently used class of antibiotics, it is important to confirm these results with larger, high quality studies to investigate associations between specific types of macrolides and rare events, such as cerebral palsy, epilepsy and organ-specific malformations. Heterogeneity due to baseline maternal risk of infection and exposure timing should also be evaluated.

3.6 Chapter Appendix

Appendix 3-1. PRISMA Checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5-7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	A6
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Figure 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figure 3, A9 -11
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Figure 3, A9-11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	A12-A14
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	12-14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
FUNDING			

Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	9, cover letter

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Appendix 3-2. Search terms.

Database	Search strategy
PubMed	#1 Search (Pregnancy[MeSH Terms]) OR Infant[MeSH Terms] #2 Search (pregnan*[Title/Abstract] OR fetal[Title/Abstract] OR fetus[Title/Abstract] OR maternal[Title/Abstract] OR prenatal[Title/Abstract] OR gestation*[Title/Abstract] OR in utero'[Title/Abstract] OR neona*[Title/Abstract] OR infant*[Title/Abstract] OR infanc*[Title/Abstract]) #3 Search (#1) OR #2 #4 Search macrolide[MeSH Terms] #5 Search (macrolide*[Title/Abstract] OR erythromycin[Title/Abstract] OR clarithromycin[Title/Abstract] OR azithromycin[Title/Abstract]) #6 Search (#4) OR #5 #7 Search (#3) AND #6 #8 Search (("Clinical Trial"[Publication Type] OR groups[Title/Abstract] OR placebo*[Title/Abstract] OR trial*[Title/Abstract] OR random*[Title/Abstract] OR "drug therapy"[Subheading]) NOT ((animals[MeSH Terms]) NOT ((animals[MeSH Terms]) AND (humans[MeSH Terms])))) #9 Search (#7) AND #8 #10 Search (penicillins[MeSH Terms]) OR cephalosporins[MeSH Terms] #11 Search (Penicillin[Text Word] OR Benzylpenicillin[Text Word] OR Phenoxymethylpenicillin[Text Word] OR Crystapen[Text Word] OR Aminopenicillins[Text Word] OR Ampicillin[Text Word] OR Amoxicillin[Text Word] OR Co-amoxiclav[Text Word] OR Amoxil[Text Word] OR Penbritin[Text Word] OR Augmentin[Text Word] OR Flucloxacillin[Text Word] OR CO-FLUAMPICIL[Text Word] OR Magnapen[Text Word] OR Ticarcillin[Text Word] OR Timentin[Text Word] OR PIVMECILLINAM HYDROCHLORIDE[Text Word] OR Selexid[Text Word] OR cephalosporins[Text Word] OR Cefradine[Text Word] OR cefotaxime[Text Word] OR ceftazidime[Text Word] OR cefuroxime[Text Word] OR cefalexin[Text Word] OR ceftriaxone[Text Word] OR CEFACLOR[Text Word] OR Distaclor [Text Word] OR CEFADROXIL[Text Word] OR Baxan[Text Word] OR CEFALEXIN[Text Word] OR Ceporex[Text Word] OR Keflex[Text Word] OR CEFIXIME[Text Word] OR Suprax[Text Word] OR CEFOTAXIME[Text Word] OR CEFPODOXIME[Text Word] OR Orelox [Text Word] OR Velosef[Text Word] OR Fortum[Text Word] OR Kefadim[Text Word] OR Rocephin[Text Word] OR Zinacef[Text Word] OR Zinnat[Text Word]) #12 Search (#10) OR #11 #13 Search (#7) AND #12 #14 Search (#9) OR #13
Cochrane Library	Searched for trials using Cochrane Search Manager: ID Search Hits #1 MeSH descriptor: [Pregnancy] explode all trees #2 MeSH descriptor: [Infant] explode all trees #3 MeSH descriptor: [Macrolides] explode all trees #4 #1 or #2 #5 #4 and #3 #6 macrolide or macrolides or erythromycin or clarithromycin or azithromycin :ti,ab,kw and pregnan* or fetal or fetus or maternal or prenatal or gestation* or 'in utero' or neona* or infant* or infanc*:ti,ab,kw in Trials (Word variations have been searched)

	#7 #6 or #5
Embase	1. exp pregnancy/ 2. exp infant/ 3. 1 or 2 4. exp macrolide/ 5. 3 and 4 6. exp penicillin derivative/ 7. exp cephalosporin derivative/ 8. (Penicillin or Benzylpenicillin or Phenoxymethylpenicillin or Crystapen or Aminopenicillins or Ampicillin or Amoxicillin or Co-amoxiclav or Amoxil or Penbritin or Augmentin or Flucloxacillin or CO-FLUAMPICIL or Magnapen or Ticarcillin or Timentin or PIVMECILLINAM HYDROCHLORIDE or Selexid).tw. 9. (cephalosporins or Cefradine or cefotaxime or ceftazidime or cefuroxime or cefalexin or ceftriaxone or CEFACLOR or Distaclor or CEFADROXIL or Baxan or CEFALEXIN or Ceporex or Keflex or CEFIXIME or Suprax or CEFOTAXIME or CEFPODOXIME or Orelox or Velosef or Fortum or Kefadim or Rocephin or Zinacef or Zinnat).tw. 10. 6 or 7 or 8 or 9 11. 5 and 10 12. random*.ab,ti. or placebo*.de,ab,ti. or (double adj1 blind*).ab,ti. 13. 5 and 12 14. 11 or 13 15. limit 14 to human
Conference Proceeding Citation Index-Science through Web of Science	TOPIC:(macrolide or macrolides or erythromycin or clarithromycin or azithromycin) AND TOPIC: (pregnant or pregnancy or pregnancies or fetal or maternal or prenatal or gestation or gestational or in utero or neona* or infant* or infanc*) Timespan: All years. Indexes: CPCI-S.
ClinicalTrials.gov	"macrolides" OR "macrolide" OR "erythromycin" OR "clarithromycin" OR "azithromycin"| Completed Studies | Studies With Results

Appendix 3-3. Decision tree for including studies.

Decision tree for including studies				
	Title and Abstract Review	**Yes**	**No**	**Maybe**
1	Does the paper present a trial, a cohort study or a case-control study?	2	E	2
2	Does the paper focus on interventions administered during (human) pregnancy?	3	E	3
3	Does one of the interventions include macrolide antibiotic (erythromycin, clarithromycin, azithromycin or macrolide antibiotics as a whole)?	4	E	4
4	Does the paper present data on offspring outcomes?	Full text	E	Full text
	Full text review	**Yes**	**No**	
5	Does the study present a trial?	6	7	
6	In this trial, does any of the comparison pair belong to any of the following types: 1) macrolide antibiotics VS placebo; 2) macrolide antibiotics VS (penicillins or cefalosporins); 3) (macrolide antibiotics + (penicillins or cefalosporins)) VS (penicillins or cefalosporins)	10	E	
7	In this observational study, does the comparison belong to penicillins or cefalosporins, or does the data allow a comparison between macrolide antibiotic and penicillins or cefalosporins?	8	E	
8	Does the study present a case-control study?	9	10	
9	In this case-control study, were the cases and controls selected from a similarly defined population and of which prescription information was collected prospectively?	10	E	
10	Does the study report the risk (or relevant data to compute the risk) of fetus or child conditions that are direct and specific short-term hypoxia-related effects?	Included	E	

Appendix 3-4. Risk of bias assessment for randomised controlled trials.

Bias Domain	Source of bias	Judgment	Support for judgement
Selection Bias	Random sequence generation	Low risk	The investigators describe a random component in the sequence generation process for pregnant women e.g. random number table, computer random number generator or shuffling cards or envelopes etc.
		High risk	The investigators describe a non-random component in the sequence generation process for pregnant women, e.g. date of birth, day of visit, ID, choice of clinician or participant, test results, availability; or there was insufficient information about the sequence generation process
	Allocation concealment	Low risk	Participants and investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation: • Central allocation (including telephone, web-based and pharmacy-controlled randomization); • Sequentially numbered drug containers of identical appearance; • Sequentially numbered, opaque, sealed envelopes.
		High risk	Participants or investigators enrolling participants could possibly foresee assignments and thus introduce selection bias, such as allocation based on: • Using an open random allocation schedule (e.g. a list of random numbers); • Assignment envelopes were used without appropriate safeguards (e.g. if envelopes were unsealed or nonopaque or not sequentially numbered); • Alternation or rotation; • Date of birth; • Case record number; • Any other explicitly unconcealed procedure. Or there was insufficient information to permit judgement
Performance Bias	Blinding of participants and personnel	Low risk	Any one of the following: • Blinding of participants and key study personnel ensured, and unlikely that the blinding could have been broken; • No blinding or incomplete blinding, but the review authors judge that the outcome is not likely to be influenced by lack of blinding.
		High risk	Any one of the following: • No blinding or incomplete blinding, and the outcome is likely to be influenced by lack of blinding; • Blinding of key study participants and personnel attempted, but likely that the blinding could have been broken, and the outcome is likely to be influenced by lack of blinding. Or there was insufficient information to permit judgement.
Detection Bias	Blinding of outcome assessment	Low risk	Any one of the following: • Blinding of outcome assessment ensured, and unlikely that the blinding could have been broken; • No blinding of outcome assessment, but the review authors judge that the outcome measurement is not likely to be influenced by lack of blinding.

		High risk	Any one of the following: • No blinding of outcome assessment, and the outcome measurement is likely to be influenced by lack of blinding; • Blinding of outcome assessment, but likely that the blinding could have been broken, and the outcome measurement is likely to be influenced by lack of blinding. Or there was insufficient information to permit judgement
Attrition Bias	Incomplete outcome data	Low risk	The follow-up length was long enough to detect specific outcomes. (e.g. at least 1 year to detect birth defects; at least 5 year to detect neurological adverse outcomes), and any one of the following: •No missing outcome data; •Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; •The proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; •Missing data have been imputed using appropriate methods.
		High risk	Any one of the following: •The follow-up length was not long enough to detect specific outcomes, which would result in a bias towards null; •The proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; •'As-treated' analysis done with substantial departure of the intervention received from that assigned at randomization; •Potentially inappropriate application of simple imputation. Or there was insufficient information to permit judgement
Reporting Bias	Selective reporting	Low risk	Any of the following: •The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way; •The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon).
		High risk	Any one of the following: •Not all of the study's pre-specified primary outcomes have been reported; •One or more primary outcomes is reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified; •One or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); •One or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis; •The study report fails to include results for a key outcome that would be expected to have been reported for such a study. Or there was insufficient information to permit judgement.
Incomparable treatment effect		Low risk	•Macrolides were compared with alternative antibiotics.
		High risk	RCTs with the following two comparisons: • macrolides versus placebo; • macrolides plus alternative antibiotics versus the alternatives.

Appendix 3-5. Risk of bias assessment for observational studies.

Bias Domain	Source of bias	Judgment	Support for judgement
Pre-intervention	Bias due to confounding	Low risk	• Multivariate analysis, propensity score analyses or matching performed with additional confounders (e.g. gestational age, maternal age, and social economic status).
		Moderate risk	• The comparison between macrolides and alternative antibiotics was not adjust for additional confounders.
		High risk	The risk of bias due to confounding is moderate at worst since I only allow alternative antibiotics as comparator for macrolides, and this comparison itself has controlled for several confounders including infection and social economic status, to a certain degree.
At intervention	Bias in selection of participants into the study	Low risk	• In the selection process, there is no other systematic difference introduced between study groups.
		High risk	• In cohort studies, the investigator's selection of exposed and reference groups introduces a systematic difference, other than the exposure, between the groups, and this systematic difference is associated with the outcome. In case-control studies, cases and controls are recruited in a way that they are not representative of the target population.
	Bias in classification of interventions	Low risk	Any one of the following: • The intervention was measured based on prescription records, healthcare registry etc.; • The intervention was measured before outcome presented.
		High risk	Any one of the following: • The intervention was measured after outcome presented; • The intervention was measured by self-report, which was subject to recall bias. Or there was insufficient information to permit judgement
Post-intervention	Bias due to deviations from intended interventions	Low risk	Observational studies can rarely measure which subjects actually had their prescriptions dispensed and which subjects took antibiotics as dispensed. However, women prescribed macrolides may be less likely to adhere to the treatment than women prescribed penicillin or cephalosporin because of minor side effects of macrolides including nausea, vomiting and diarrhoea, which will result in a bias towards null.
	Bias due to missing	Low risk	• The cohort included methods to document whether the patients were under the follow-up account for censoring, to enable the differentiation between no interested covariates presenting and no information for covariates; or, used person-year to measure the time at risk. And, • For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk is not enough to have an important impact on the intervention effect estimate.

		High risk	Any one of the following: • The cohort did not include methods to document whether the patients were under the follow-up account for censoring, which cannot differentiate between no covariates presenting and no information for covariates; • For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk is large enough to have an important impact on the intervention effect estimate. Or there was insufficient information to permit judgement.
	Bias in measurement of outcomes	Low risk	•The follow-up length was long enough to detect specific outcomes. (e.g. at least 1 year to detect birth defects; at least 5 year to detect neurological adverse outcomes), and; •The cohort included methods to document whether the patients were under the follow-up account for censoring to enable the differentiation between no outcome presenting and no information for outcomes, or used person-year to measure the time at risk.
		High risk	Any one of the following: •The follow-up length was not long enough to detect specific outcomes, which would result in a bias towards null; •The cohort used proportion instead of person-year measurement, and did not include methods to document whether the patients were under the follow-up account for censoring, which cannot differentiate between no outcome presenting and no information for outcomes; • The outcomes was measured based on self-report. Or there was insufficient information to permit judgement.
	Bias in selection of the reported results	Low risk	Outcome and analysis reporting were consistent with designing/ pre-specified.
		High risk	Selective reporting of a specific outcome (e.g., selected follow-up intervals), incomplete reporting of a specific outcome (e.g., incomplete reporting of nonsignificant p values, such as p>0.05), selective reporting of data on subgroups, presentation of adjusted rather than unadjusted analyses. Or there was insufficient information to permit judgement.

Appendix 3-6. Characteristics of included studies.

Outcome	Author (Year) (study number). Comparison	Study Design	Maternal risk group	Macrolide type	Timing, length of prescription and follow up	Comparison type	Macrolides n/N	Comparison n/N	Odds Ratio	95% LCL	95% UCL
Miscarriage	Einarson (1998).1	Prospec cohort	Unselected. All pregnancies of women who inquired about the use of clarithromycin in five centres	Clarithromycin	4-14 w, NS, NS	Nonteratogenic Antibiotics	22/157	11/166	2.30	1.07	4.91
Miscarriage	Anderson (2013)	Retro cohort	Unselected. All pregnancies from national fertility registry and Hospital registry	Clarithromycin	1st tri, NS, NS	Phenoxymethylpenicillin	40/401	-/33469	1.51	1.09	2.12
Miscarriage	Muanda (2017)(1).1	Nested CC	Unselected. All pregnancies of women who were included in the province's drug insurance	Macrolides	Before spontaneous abortion (20w), >=1 prescription, -	Penicilins	264/1789	500/6073	1.88	1.59	2.22
Miscarriage	Muanda (2017)(1).2	Nested CC	Same as above	Azithromycin	Same as above	Penicilins	110/763	500/6073	1.91	1.53	2.39
Miscarriage	Muanda (2017)(1).3	Nested CC	Same as above	Clarithromycin	Same as above	Penicilins	111/547	500/6073	2.73	2.16	3.44
Miscarriage	Muanda (2017)(1).4	Nested CC	Same as above	Erythromycin	Same as above	Penicilins	29/428	500/6073	0.82	0.56	1.19
Stillbirth	Einarson (1998).2	Prospec cohort	Unselected. All pregnancies of women who inquired about the use of clarithromycin in five centres	Clarithromycin	4-14 w, NS, NS	Nonteratogenic Antibiotics	1/157	0/166	168.07	0.00	>100
Stillbirth	Kenyon (2008)(1).1	RCT	Pprom. All pregnancies of pPROM women (Median gestational age: 32 w)	Erythromycin	32 w (at randomisation, 61% delivered within 7 days), 250 mg *4 times a day for 10 days or until delivery, 7 years	Co-amoxiclav	19/1167	22/1180	0.87	0.47	1.62
Stillbirth	Kenyon (2008)(2).1	RCT	SPL. All pregnancies of SPL women (Median gestational age: 31 w)	Erythromycin	31 w (at randomisation, 15.8% delivered within 7 days), 250 mg *4 times a day for 10 days or until delivery, 7 years	Co-amoxiclav	9/1204	9/1133	0.94	0.37	2.38
Stillbirth	Eschenbach (1991)	RCT	U. realyticum. All pregnancies of women with U. realyticum (26-30 w)	Erythromycin	26-35 w, 333mg 3 times daily, until delivery	Placebo	3/590	3/561	0.95	0.19	4.73
Stillbirth	McGregor (1991).1	RCT	pPROM. All pregnancies of pPROM women (30 w)	Erythromycin	31 w (at randomisation), 333 mg *3 time/day until delivery or 7 days, until	Placebo	1/28	0/27	29.04	0.00	>100
Stillbirth	Mercer (1992)	RCT	pPROM. All pregnancies of pPROM women (20-34 w)	Erythromycin	20-34 w (at randomisation), 333 mg*3 time/day until delivery, until delivery	Placebo	2/109	5/114	0.41	0.08	2.15

Stillbirth	Martin (1997)	RCT	Chlamydia trachomatis. All pregnancies of women with Chlamydia trachomatis (29.4 w)	Erythromycin	23-29 w (at randomisation), 333 mg*3 time/day until 35 w, until delivery	Placebo	2/202	1/203	2.02	0.18	22.46
Stillbirth	Ye, Y (2001)	RCT	Mycoplasma. All pregnancies of mycoplasma-positive pregnant women (>=28 w)	Erythromycin	>28 w (at randomisation), 125 mg twice daily for 6 w, until delivery	Placebo	1/241	5/247	0.20	0.02	1.74
Stillbirth	Kenyon (2008)(1).2	RCT	pPROM, same as Kenyon (2008)(1).1	Erythromycin plus co-amoxiclav or	32 w, same as Kenyon (2008)(1).1	Co-amoxiclav or placebo	42/2323	44/2389	0.98	0.64	1.50
Stillbirth	Kenyon (2008)(2).2	RCT	SPL, same as Kenyon (2008)(2).1	Erythromycin plus co-amoxiclav or erythromycin only	31 w, same as Kenyon (2008)(2).1	Co-amoxiclav or placebo	20/2375	24/2279	0.80	0.44	1.45
Neonatal death	Kenyon (2001)(1).1	RCT	pPROM. All pregnancies of pPROM women (Median gestational age: 32 w)	Erythromycin	32 w (at randomisation, 61% delivered within 7 days), 250 mg *4 times a day for 10 days or until delivery	Co-amoxiclav	70/1190	79/1205	0.89	0.64	1.24
Neonatal death	Kenyon (2001)(2).1	RCT	SPL. All pregnancies of SPL women (Median gestational age: 31 w)	Erythromycin	31 w (at randomisation, 15.8% delivered within 7 days), 250 mg *4 times a day for 10 days or until delivery, until delivery	Co-amoxiclav	43/1600	38/1534	1.09	0.70	1.69
Neonatal death	McGregor (1991).2	RCT	pPROM. All pregnancies of pPROM women (30 w)	Erythromycin	31 w (at randomisation), 333 mg *3 time/day until delivery or 7 days, until	Placebo	5/28	0/27	173.03	0.01	>100
Neonatal death	Kenyon (2001)(1).2	RCT	pPROM, same as Kenyon (2001)(1).1	Erythromycin plus co-amoxiclav or erythromycin only	32 w, same as Kenyon (2001)(1).1	Co-amoxiclav or placebo	147/2397	161/2430	0.92	0.73	1.16
Neonatal death	Kenyon (2001)(2).2	RCT	SPL, same as Kenyon (2001)(2).1	Erythromycin plus co-amoxiclav or erythromycin only	31 w, same as Kenyon (2001)(2).1	Co-amoxiclav or placebo	90/3151	77/3090	1.15	0.85	1.57
Neonatal death	Kwak (2013).1	RCT	All pregnancies of pPROM women (22-23 w)	Clarithromycin + cefazolin	22-23 w (at randomisation), 250 mg*4 time/day until 35 w, 1 year	Cefazolin	1/35	1/17	0.47	0.03	8.01

Neonatal death	Kwak (2013).2	RCT	All pregnancies of pPROM women (22-23 w)	Erythromycin+ cefazolin	22-23 w (at randomisation), 250 mg*4 time/day until 35 w, 1 year	Cefazolin	1/31	1/17	0.53	0.03	9.11
Neonatal death	Tita (2016)	RCT	Caesarean delivery. Singletons born to women who had non-elective caesarean delivery during labor or after amniotic membrane rupture (39w)	Azithromycin	29 w (at randomisation), 500 mg, 3 months	Placebo	1/1019	1/944	0.93	0.06	14.83
Stillbirth and neonatal mortality	Romoren (2012).1	Retro cohort	Unselected. All singleton pregnancies.	Macrolides	1st tri, 90% received 1 course of AB, at least until delivery	Penicilins	41/5729	118/16695	1.01	0.71	1.45
Cerebral palsy	Kenyon (2008)(1).3	RCT	pPROM, same as Kenyon (2008)(1).1	Erythromycin	32 w, same as Kenyon (2008)(1).1	Co-amoxiclav	28/807	21/849	1.42	0.80	2.52
Cerebral palsy	Kenyon (2008)(2).3	RCT	SPL, same as Kenyon (2008)(2).1	Erythromycin	31 w, same as Kenyon (2008)(2).1	Co-amoxiclav	18/816	15/792	1.17	0.58	2.33
Cerebral palsy	Meeraus (2015).1	Retro cohort	Unselected. Term singleton live-births	Macrolides	1-3 tri, single prescription, until 7 years old (median: 3.6 years)	Penicilins	-/2749	<55/27577	1.43	0.66[g]	3.06
Cerebral palsy	Kenyon (2008)(1).4	RCT	pPROM, same as Kenyon (2008)(1).1	Erythromycin plus co-	32 w, same as Kenyon (2008)(1).1	Co-amoxiclav or placebo	46/1590	41/1671	1.18	0.77	1.81
Cerebral palsy	Kenyon (2008)(2).4	RCT	SPL, same as Kenyon (2008)(2).1	Erythromycin plus co-amoxiclav or erythromycin only	31 w, same as Kenyon (2008)(2).1	Co-amoxiclav or placebo	53/1611	27/1562	1.93	1.21	3.09
Epilepsy	Kenyon (2008)(1).5	RCT	pPROM, same as Kenyon (2008)(1).1	Erythromycin	32 w, same as Kenyon (2008)(1).1	Co-amoxiclav	53/807	55/849	1.01	0.69	1.50
Epilepsy	Kenyon (2008)(2).5	RCT	SPL, same as Kenyon (2008)(2).1	Erythromycin	31 w, same as Kenyon (2008)(2).1	Co-amoxiclav	68/816	63/792	1.05	0.74	1.50
Epilepsy	Meeraus (2015).2	Retro cohort	Unselected. Term singleton live-births	Macrolides	1-3 tri, single prescription, until 7 years old (median: 3.6 years)	Penicilins	-/2749	<142/27577	2.02	1.30[g]	3.14
Epilepsy	Kenyon (2008)(1).6	RCT	pPROM, same as Kenyon (2008)(1).1	Erythromycin plus co-amoxiclav or erythromycin only	32 w, same as Kenyon (2008)(1).1	Co-amoxiclav or placebo	101/1590	107/1671	0.99	0.75	1.31
Epilepsy	Kenyon (2008)(2).6	RCT	SPL, same as Kenyon (2008)(2).1	Erythromycin plus co-	31 w, same as Kenyon (2008)(2).1	Co-amoxiclav or placebo	149/1611	116/1562	1.27	0.99	1.64
Cerebral palsy and/or epilepsy	Meeraus (2015).3	Retro cohort	Unselected. Term singleton live-births	Macrolides	1-3 tri, single prescription, until 7 years old (median: 3.6 years)	Penicilins	28/2749	156/27577	1.78[g]	1.18	2.69

Malformation: All	Einarson (1998).3	Prospec cohort	Unselected. All pregnancies of women who inquired about the use of clarithromycin in five centres	Clarithromycin	4-14 w, NS, NS	Nonteratogenic Antibiotics	10/157	9/166	1.19	0.47	3.00
Malformation: All	Cooper (2008).1	Retro cohort	Unselected. All pregnancies from registry	Erythromycin	1st tri (0-4 months) , NS , 1 year	Amoxicillin	23/903	232/7216	0.79	0.51	1.21
Malformation: All	Romoren (2012).2	Retro cohort	Unselected. All singleton pregnancies.	Macrolides	1st tri, 90% received 1 course of AB, at least until delivery	Penicilins	127/2549	218/4921	1.13	0.90	1.42
Malformation: All	Le Nguyen (2017)	Retro cohort	Unselected. All pregnancies of women in the registry French EFEMERIS	Macrolides	1st tri, NS, NS	Penicilins	47/2473	231/9720	0.93	0.6	1.37
Malformation: Major	Einarson (1998).4	Prospec cohort	Unselected. All pregnancies of women who inquired about the use of clarithromycin in five centres	Clarithromycin	4-14 w, NS, NS	Nonteratogenic Antibiotics	3/157	2/166	1.60	0.26	9.69
Malformation: Major	Romoren (2012).3	Retro cohort	Unselected. All singleton pregnancies.	Macrolides	1st tri, 90% received 1 course of AB, at least until delivery	Penicilins	69/2549	139/4921	0.96	0.71	1.28
Malformation: Major	Muanda (2017)(2).1	Retro cohort	Unselected. Live-born singletons of women in the national birth registry Quebec pregnancy cohort	Macrolides	1st tri, NS, 1 year	Penicilins	265/2332	894/9106	1.13	0.98	1.31
Malformation: Major	Muanda (2017)(2).10	Retro cohort	Unselected, see Muanda (2017)(2).1	Azithromycin	1st tri, NS, 1 year	Amoxicillin	118/883	584/5950	1.25	1.01	1.53
Malformation: Major	Muanda (2017)(2).11	Retro cohort	Unselected, see Muanda (2017)(2).1	Clarithromycin	1st tri, NS, 1 year	Amoxicillin	77/658	584/5950	1.15	0.90	1.48
Malformation: Major	Muanda (2017)(2).12	Retro cohort	Unselected, see Muanda (2017)(2).1	Erythromycin	1st tri, NS, 1 year	Amoxicillin	64/697	584/5950	1.02	0.78	1.34
Malformation: Nervous System	Cooper (2008).2	Retro cohort	Unselected. All pregnancies from registry	Erythromycin	1st tri (0-4 months) , NS , 1 year	Amoxicillin	1/903	23/7216	0.35	0.05	2.57
Malformation: Nervous System	Muanda (2017)(2).2	Retro cohort	Unselected, see Muanda (2017)(2).1	Macrolides	1st tri, NS, 1 year	Penicilins	16/2332	53/9106	1.09	0.62	1.91
Malformation: Nervous System	Muanda (2017)(2).13	Retro cohort	Unselected, see Muanda (2017)(2).1	Azithromycin	1st tri, NS, 1 year	Amoxicillin	8/883	33/5950	1.35	0.63	2.91
Malformation: Nervous System	Muanda (2017)(2).14	Retro cohort	Unselected, see Muanda (2017)(2).1	Clarithromycin	1st tri, NS, 1 year	Amoxicillin	4/658	33/5950	0.95	0.34	2.59
Malformation: Nervous System	Muanda (2017)(2).15	Retro cohort	Unselected, see Muanda (2017)(2).1	Erythromycin	1st tri, NS, 1 year	Amoxicillin	2/697	33/5950	0.5	0.12	2.12
Malformation: Orofacial	Cooper (2008).3	Retro cohort	Unselected. All pregnancies from registry	Erythromycin	1st tri (0-4 months) , NS , 1 year	Amoxicillin	0/903	15/7216	0.00	0.00	>100
Malformation: Orofacial	Muanda (2017)(2).3	Retro cohort	Unselected, see Muanda (2017)(2).1	Macrolides	1st tri, NS, 1 year	Penicilins	16/2332	51/9106	1.22	0.69	2.15

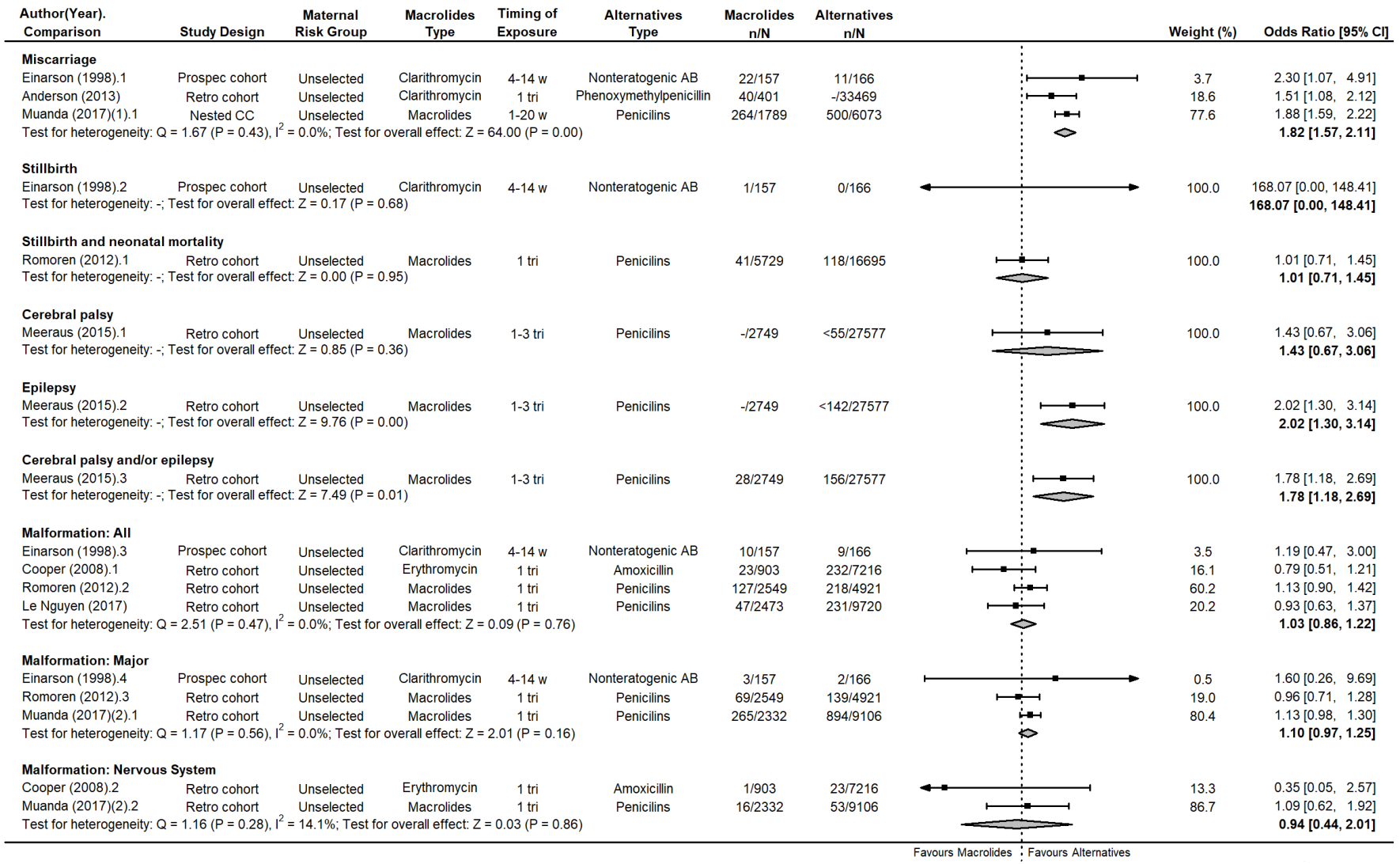
Malformation: Orofacial	Muanda (2017)(2).16	Retro cohort	Unselected, see Muanda (2017)(2).1	Azithromycin	1st tri, NS, 1 year	Amoxicillin	2/883	1479354	1.6	0.75	3.4
Malformation: Orofacial	Muanda (2017)(2).17	Retro cohort	Unselected, see Muanda (2017)(2).1	Clarithromycin	1st tri, NS, 1 year	Amoxicillin	2/658	1479354	1.05	0.38	2.88
Malformation: Orofacial	Muanda (2017)(2).18	Retro cohort	Unselected, see Muanda (2017)(2).1	Erythromycin	1st tri, NS, 1 year	Amoxicillin	3/697	1479354	1.1	0.4	3.02
Malformation: Cardiovascular	Kallen (2005).1	Retro cohort	Unselected. Livebirths of women in the national birth	Erythromycin	1st tri, NS, 1 year	Penicilins	31/1844	84/9110	1.84	1.21	2.78
Malformation: Cardiovascular	Cooper (2008).4	Retro cohort	Unselected. All pregnancies from registry	Erythromycin	1st tri (0-4 months) , NS , 1 year	Amoxicillin	9/903	89/7216	0.81	0.40	1.61
Malformation: Cardiovascular	Romoren (2012).4	Retro cohort	Unselected. All singleton pregnancies.	Macrolides	1st tri, 90% received 1 course of AB, at least until delivery	Penicilins	25/2549	46/4921	1.05	0.64	1.71
Malformation: Cardiovascular	Muanda (2017)(2).4	Retro cohort	Unselected, see Muanda (2017)(2).1	Macrolides	1st tri, NS, 1 year	Penicilins	47/2332	192/9106	0.92	0.66	1.27
Malformation: Cardiovascular	Muanda (2017)(2).19	Retro cohort	Unselected, see Muanda (2017)(2).1	Azithromycin	1st tri, NS, 1 year	Amoxicillin	19/883	117/5950	0.92	0.57	1.49
Malformation: Cardiovascular	Muanda (2017)(2).20	Retro cohort	Unselected, see Muanda (2017)(2).1	Clarithromycin	1st tri, NS, 1 year	Amoxicillin	12/658	117/5950	0.82	0.46	1.48
Malformation: Cardiovascular	Muanda (2017)(2).21	Retro cohort	Unselected, see Muanda (2017)(2).1	Erythromycin	1st tri, NS, 1 year	Amoxicillin	15/697	117/5950	1.09	0.64	1.86
Malformation: VSD/ASD	Kallen (2005).2	Retro cohort	Unselected. Livebirths of women in the national birth	Erythromycin	1st tri, NS, 1 year	Penicilins	18/1844	57/9110	1.57	0.92	2.67
Malformation: VSD/ASD	Romoren (2012).5	Retro cohort	Unselected. All singleton pregnancies.	Macrolides	1st tri, 90% received 1 course of AB, at least until delivery	Penicilins	19/2549	29/4921	1.27	0.71	2.26
Malformation: VSD/ASD	Muanda (2017)(2).5	Retro cohort	Unselected, see Muanda (2017)(2).1	Macrolides	1st tri, NS, 1 year	Penicilins	35/2332	150/9106	0.88	0.61	1.28
Malformation: VSD/ASD	Muanda (2017)(2).22	Retro cohort	Unselected, see Muanda (2017)(2).1	Azithromycin	1st tri, NS, 1 year	Amoxicillin	14/883	93/5950	0.88	0.5	1.54
Malformation: VSD/ASD	Muanda (2017)(2).23	Retro cohort	Unselected, see Muanda (2017)(2).1	Clarithromycin	1st tri, NS, 1 year	Amoxicillin	9/658	93/5950	0.8	0.4	1.56
Malformation: VSD/ASD	Muanda (2017)(2).24	Retro cohort	Unselected, see Muanda (2017)(2).1	Erythromycin	1st tri, NS, 1 year	Amoxicillin	11/697	93/5950	1.02	0.55	1.9
Malformation: Respiratory	Muanda (2017)(2).6	Retro cohort	Unselected, see Muanda (2017)(2).1	Macrolides	1st tri, NS, 1 year	Penicilins	14/2332	45/9106	1.12	0.61	2.05
Malformation: Respiratory	Muanda (2017)(2).25	Retro cohort	Unselected, see Muanda (2017)(2).1	Azithromycin	1st tri, NS, 1 year	Amoxicillin	6/883	30/5950	1.15	0.48	2.77
Malformation: Respiratory	Muanda (2017)(2).26	Retro cohort	Unselected, see Muanda (2017)(2).1	Clarithromycin	1st tri, NS, 1 year	Amoxicillin	4/658	30/5950	1.09	0.38	3.11
Malformation: Respiratory	Muanda (2017)(2).27	Retro cohort	Unselected, see Muanda (2017)(2).1	Erythromycin	1st tri, NS, 1 year	Amoxicillin	4/697	30/5950	1.23	0.44	3.5
Malformation: Gastrointestinal	Cooper (2008).5	Retro cohort	Unselected. All pregnancies from registry	Erythromycin	1st tri (0-4 months) , NS , 1 year	Amoxicillin	0/903	26/7216	0.00	0.00	>100

Malformation: Gastrointestinal	Muanda (2017)(2).7	Retro cohort	Unselected, see Muanda (2017)(2).1	Macrolides	1st tri, NS, 1 year	Penicilins	35/2332	88/9106	1.48	0.99	2.2
Malformation: Gastrointestinal	Muanda (2017)(2).28	Retro cohort	Unselected, see Muanda (2017)(2).1	Azithromycin	1st tri, NS, 1 year	Amoxicillin	15/883	54/5950	1.56	0.89	2.72
Malformation: Gastrointestinal	Muanda (2017)(2).29	Retro cohort	Unselected, see Muanda (2017)(2).1	Clarithromycin	1st tri, NS, 1 year	Amoxicillin	10/658	54/5950	1.46	0.75	2.81
Malformation: Gastrointestinal	Muanda (2017)(2).30	Retro cohort	Unselected, see Muanda (2017)(2).1	Erythromycin	1st tri, NS, 1 year	Amoxicillin	10/697	54/5950	1.58	0.81	3.05
Malformation: Cleft palate/lip	Muanda (2017)(2).8	Retro cohort	Unselected, see Muanda (2017)(2).1	Macrolides	1st tri, NS, 1 year	Penicilins	157968	13/9106	2.03	0.81	5.07
Malformation: Cleft palate/lip	Muanda (2017)(2).31	Retro cohort	Unselected, see Muanda (2017)(2).1	Azithromycin	1st tri, NS, 1 year	Amoxicillin	2/883	5/5950	1.54	0.35	6.84
Malformation: Cleft palate/lip	Muanda (2017)(2).32	Retro cohort	Unselected, see Muanda (2017)(2).1	Clarithromycin	1st tri, NS, 1 year	Amoxicillin	2/658	5/5951	1.92	0.43	8.49
Malformation: Cleft palate/lip	Muanda (2017)(2).33	Retro cohort	Unselected, see Muanda (2017)(2).1	Erythromycin	1st tri, NS, 1 year	Amoxicillin	3/697	5/5952	3.04	0.87	10.65
Malformation: Pyloric stenosis	Kallen (2005).3	Retro cohort	Unselected. Live-births of women in the national birth registry	Erythromycin	1st tri, NS, 1 year	Penicilins	4/1844	6/5953	2.47	0.62	9.90
Malformation: Pyloric stenosis	Lund (2014).1	Retro cohort	Unselected. Live-born singletons from Danish national patient register	Macrolides	1-2 tri, NS, NS	Penicilins	20/7569	89/34222	1.02	0.63	1.65
Malformation: Pyloric stenosis	Lund (2014).3	Retro cohort	Unselected. Live-born singletons from Danish national patient register	Azithromycin	1-2 tri, NS, NS	Penicilins	7/1574	89/34222	1.71	0.79	3.69
Malformation: Pyloric stenosis	Lund (2014).4	Retro cohort	Unselected. Live-born singletons from Danish national patient register	Clarithromycin	1-2 tri, NS, NS	Penicilins	1/223	89/34222	1.72	0.24	12.38
Malformation: Pyloric stenosis	Lund (2014).5	Retro cohort	Unselected. Live-born singletons from Danish national patient register	Erythromycin	1-2 tri, NS, NS	Penicilins	6/4528	89/34222	0.51	0.22	1.16
Malformation: Pyloric stenosis	Lund (2014).2	Retro cohort	Unselected. Live-born singletons from Danish national patient register	Macrolides	1-2 tri, NS, NS	Penicilins	10/2286	40/16655	1.82	0.91	3.64
Malformation: Genital	Muanda (2017)(2).9	Retro cohort	Unselected, see Muanda (2017)(2).1	Macrolides	1st tri, NS, 1 year	Penicilins	41/2332	154/9106	0.93	0.55	1.56
Malformation: Genital	Muanda (2017)(2).34	Retro cohort	Unselected, see Muanda (2017)(2).1	Azithromycin	1st tri, NS, 1 year	Amoxicillin	21/883	102/5950	1.15	0.58	2.32
Malformation: Genital	Muanda (2017)(2).35	Retro cohort	Unselected, see Muanda (2017)(2).1	Clarithromycin	1st tri, NS, 1 year	Amoxicillin	9/658	102/5950	1.27	0.59	2.76
Malformation: Genital	Muanda (2017)(2).36	Retro cohort	Unselected, see Muanda (2017)(2).1	Erythromycin	1st tri, NS, 1 year	Amoxicillin	11/697	102/5950	0.38	0.09	1.59
Malformation: Musculoskeletal	Cooper (2008).7	Retro cohort	Unselected. All pregnancies from registry	Erythromycin	1st tri (0-4 months) , NS , 1 year	Amoxicillin	5/903	52/7216	0.77	0.31	1.93

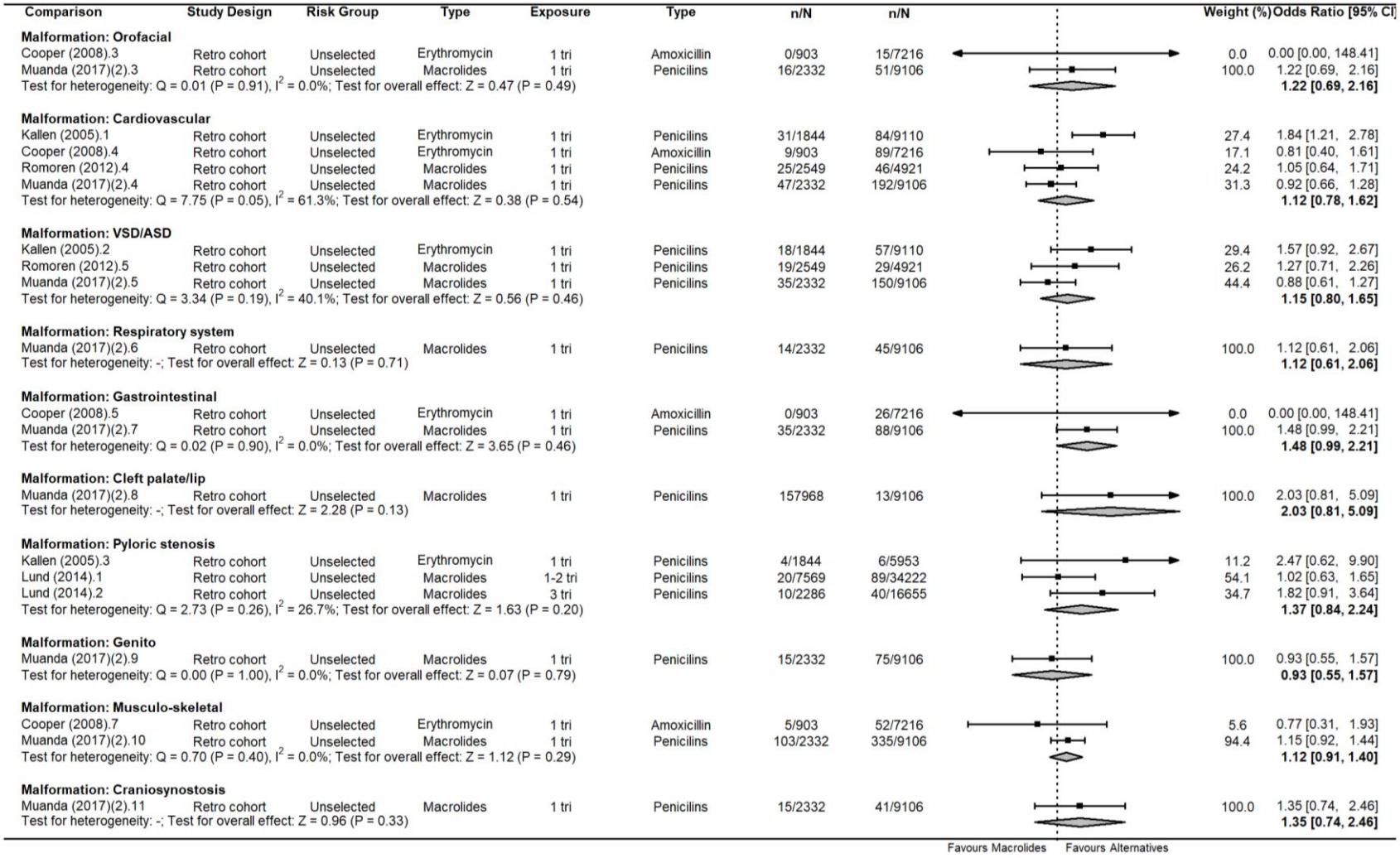
Malformation: Musculoskeletal	Muanda (2017)(2).10	Retro cohort	Unselected, see Muanda (2017)(2).1	Macrolides	1st tri, NS, 1 year	Penicilins	103/2332	335/9106	1.15	0.92	1.44
Malformation: Musculoskeletal	Muanda (2017)(2).37	Retro cohort	Unselected, see Muanda (2017)(2).1	Azithromycin	1st tri, NS, 1 year	Amoxicillin	48/883	230/5950	1.28	0.94	1.75
Malformation: Musculoskeletal	Muanda (2017)(2).38	Retro cohort	Unselected, see Muanda (2017)(2).1	Clarithromycin	1st tri, NS, 1 year	Amoxicillin	30/658	230/5950	1.17	0.85	1.83
Malformation: Musculoskeletal	Muanda (2017)(2).39	Retro cohort	Unselected, see Muanda (2017)(2).1	Erythromycin	1st tri, NS, 1 year	Amoxicillin	25/697	230/5950	1.12	0.74	1.7
Malformation: Craniosynostosis	Muanda (2017)(2).11	Retro cohort	Unselected, see Muanda (2017)(2).1	Macrolides	1st tri, NS, 1 year	Penicilins	15/2332	41/9106	1.35	0.74	2.45
Malformation: Craniosynostosis	Muanda (2017)(2).40	Retro cohort	Unselected, see Muanda (2017)(2).1	Azithromycin	1st tri, NS, 1 year	Amoxicillin	8/883	27/5950	1.63	0.75	3.54
Malformation: Craniosynostosis	Muanda (2017)(2).41	Retro cohort	Unselected, see Muanda (2017)(2).1	Clarithromycin	1st tri, NS, 1 year	Amoxicillin	4/658	27/5950	1.33	0.48	3.72
Malformation: Craniosynostosis	Muanda (2017)(2).42	Retro cohort	Unselected, see Muanda (2017)(2).1	Erythromycin	1st tri, NS, 1 year	Amoxicillin	3/697	27/5950	1.09	0.34	3.55

*1) Priority of timing was given to median gestation age of exposure or randomisation, followed by mean, range and approximate time window of exposure; w: gestational week. 2) Adjusted odds ratio/ hazard ratios were shown if available. 3) In the study of Andersen (2013), OR was adjusted by maternal age, number of previous miscarriages, income and education. Number of miscarriage in the comparison group was not given. 4) In the study of Muanda (2017), cases and controls were matched by gestational age and year of pregnancy; OR was adjusted by 11 covariates, e.g. maternal age, education level, chronic comorbidities, maternal infections (urinary tract infection, respiratory tract infection, bacterial vaginosis and sexually transmitted infections) and prior exposure to antibiotics. 5) In the study of Einarson (1998), the count in clarithromycin arm was adjusted by adding the reciprocal of the size of the opposite treatment arm size (1/166) and non-teratogenic antibiotics arm adjusted by adding 1/157, due to zero event. 6) In the study of Meeraus (2015), there were a total of 55 cerebral palsy cases and 142 epilepsy cases in macrolides group and penicillins group, with specific number in each group not given. The hazard ratio was adjusted by maternal age, Townsend quintile, year of delivery, smoking/tobacco use, alcohol problems, obesity, illicit drug use, treatment of chronic medical conditions and potentially neurologically-damaging infection during pregnancy. 7) In the study of Le guyen, OR was adjusted by maternal age, long-term illnesses, parity and multiple pregnancy. 8) In the study of Cooper (2008), the counts in erythromycin arm were adjusted by adding the reciprocal of the size of the opposite treatment arm size (1/7216) and Amoxicillin arm adjusted by adding 1/903, due to zero event. 9) In the study of Muanda (2017), the odds ratios were adjusted for the following variables: maternal age on the 1DG, maternal marital status (living alone or cohabiting), receipt of social assistance during pregnancy, calendar year of delivery, Infant gender, education level in years (≤12 or >12), and area of residence on the 1DG (urban or rural); maternal chronic co-morbidities assessed using physician-based diagnoses or filled prescriptions of related medications in the year before and during the first trimester of pregnancy (chronic hypertension, depression, diabetes mellitus , asthma, epilepsy, polyarthritis rheumatoid and systemic lupus erythematosus, thyroid disorders) , endometriosis and maternal infections (urinary tract infection, respiratory tract infection, bacterial vaginosis, and sexually transmitted diseases) assessed using physician-based diagnoses in the year before and during the first trimester of pregnancy); Use of healthcare services in the year before pregnancy. Prospec: prospective; Retro: retrospective; CC: case control; AB: antibiotics.

Appendix 3-7. Primary analysis (observational studies) for the association between adverse child outcomes and prenatal use of macrolides versus alternative antibiotics

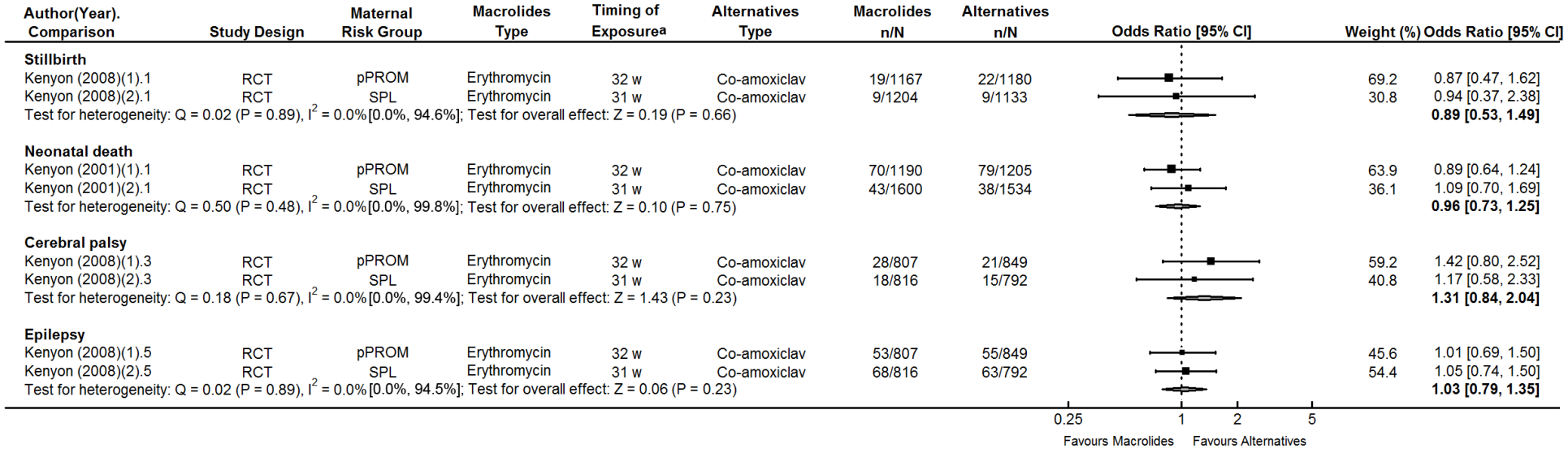


(Appendix 3-7 continued)



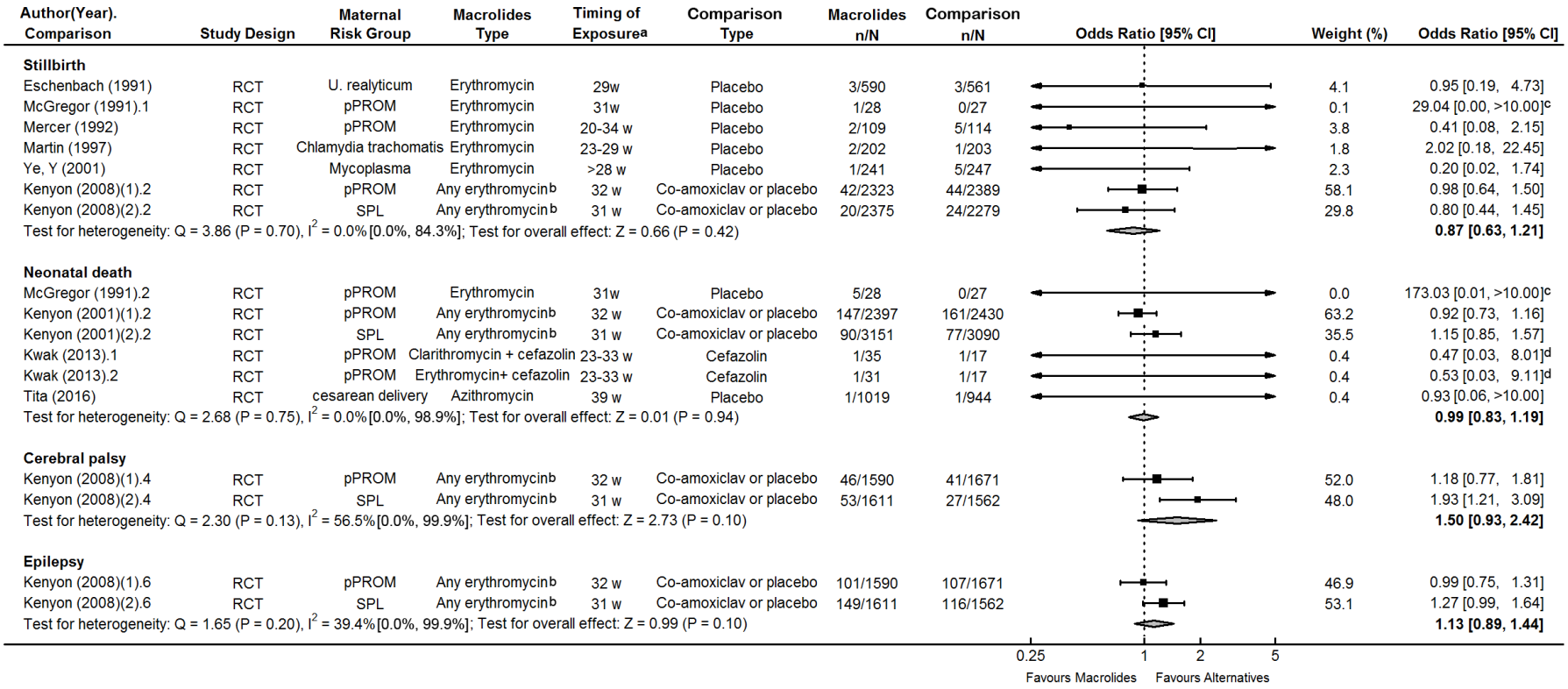
*1) Priority of timing was given to median gestation age of exposure or randomisation, followed by mean, range and approximate time window of exposure; w: gestational week. 2) Adjusted odds ratio/ hazard ratios were shown if available. 3) In the study of Andersen (2013), OR was adjusted by maternal age, number of previous miscarriages, income and education. Number of miscarriage in the comparison group was not given. 4) In the study of Muanda (2017), cases and controls were matched by gestational age and year of pregnancy; OR was adjusted by 11 covariates, e.g. maternal age, education level, chronic comorbidities, maternal infections (urinary tract infection, respiratory tract infection, bacterial vaginosis and sexually transmitted infections) and prior exposure to antibiotics. 5) In the study of Einarson (1998), the count in clarithromycin arm was adjusted by adding the reciprocal of the size of the opposite treatment arm size (1/166) and non-teratogenic antibiotics arm adjusted by adding 1/157, due to zero event. 6) In the study of Meeraus (2015), there were a total of 55 cerebral palsy cases and 142 epilepsy cases in macrolides group and penicillins group, with specific number in each group not given. The hazard ratio was adjusted by maternal age, Townsend quintile, year of delivery, smoking/tobacco use, alcohol problems, obesity, illicit drug use, treatment of chronic medical conditions and potentially neurologically-damaging infection during pregnancy. 7) In the study of Le guyen, OR was adjusted by maternal age, long-term illnesses, parity and multiple pregnancy. 8) In the study of Cooper (2008), the counts in erythromycin arm were adjusted by adding the reciprocal of the size of the opposite treatment arm size (1/7216) and Amoxicillin arm adjusted by adding 1/903, due to zero event. 9) In the study of Muanda (2017), the odds ratios were adjusted for the following variables: maternal age on the 1DG, maternal marital status (living alone or cohabiting), receipt of social assistance during pregnancy, calendar year of delivery, Infant gender, education level in years (≤12 or >12), and area of residence on the 1DG (urban or rural); maternal chronic co-morbidities assessed using physician-based diagnoses or filled prescriptions of related medications in the year before and during the first trimester of pregnancy (chronic hypertension, depression, diabetes mellitus , asthma, epilepsy, polyarthritis rheumatoid and systemic lupus erythematosus, thyroid disorders) , endometriosis and maternal infections (urinary tract infection, respiratory tract infection, bacterial vaginosis, and sexually transmitted diseases) assessed using physician-based diagnoses in the year before and during the first trimester of pregnancy); Use of healthcare services in the year before pregnancy. Prospec: prospective; Retro: retrospective; CC: case control; AB: antibiotics.

Appendix 3-8. Primary analysis (RCTs) for the association between adverse child outcomes and prenatal use of macrolides versus alternative antibiotics.



a. Priority of timing was given to median gestation age of exposure or randomisation, followed by mean, range and approximate time window of exposure; w: gestational week. pPROM: Preterm premature rupture of membranes; SPL: spontaneous preterm labour.

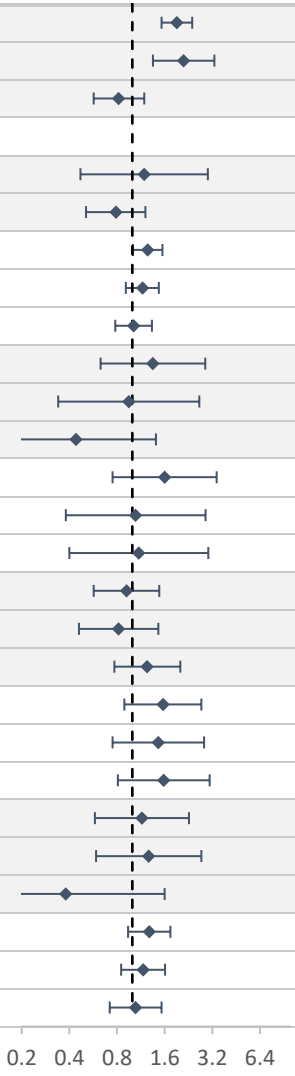
Appendix 3-9. Secondary analysis (RCTs) for the association between adverse child outcomes and prenatal use of macrolides versus no macrolides



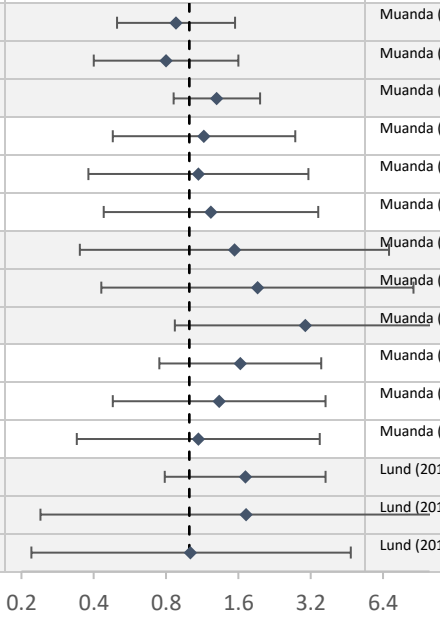
a. Priority of timing was given to median gestation age of exposure or randomisation, followed by mean, range and approximate time window of exposure; w: gestational week. b. Any erythromycin: erythromycin + co-amoxiclav or erythromycin only. c. In the study of McGregor (1991), the counts in erythromycin arm were adjusted by adding the reciprocal of the size of the opposite treatment arm size (1/27) and placebo arm adjusted by adding 1/28, due to zero event. d. In the study of Kwak (2013), participants in the cefazolin group (n = 34, including 2 events) were evenly split into 2 comparison groups to avoid double-counting.

Appendix 3-10. Subgroup analysis: pooled results for specific macrolide types (observational studies).

Outcomes	Subgroup	Number of Studies	Macrolides n/N	Alternatives n/N	I^2	Pooled OR (95% CI)	Pooled OR (95% CI)	Author (year)(paper number), comparison number
Miscarriage	Azithromycin	1	110/763	500/6073	-	1.91 [1.53, 2.39]		Muanda (2017)(1).2
	Clarithromycin	3	133/704	511/6239	75	2.11 [1.35, 3.30]		Muanda (2017)(1).3, Einarson (1998).1, Anderson (2013)
	Erythromycin	1	29/428	500/6073	-	0.82 [0.57, 1.19]		Muanda (2017)(1).4
Stillbirth	Clarithromycin	1	1/157	0/166	-	168.07 [0, >100.00]		Einarson (1998).2
Malformation: All	Clarithromycin	1	10/157	9/166	-	1.19 [0.47, 3.00]		Einarson (1998).3
	Erythromycin	1	23/903	232/7216	-	0.79 [0.51, 1.21]		Cooper (2008).1
Malformation: Major	Azithromycin	1	118/883	894/9106	-	1.25[1.01, 1.53]		Muanda (2017)(2).10
	Clarithromycin	2	80/815	896/9272	0	1.16 [0.91, 1.47]		Muanda (2017)(2).11, Einarson (1998).4
	Erythromycin	1	64/697	894/9106	-	1.02 [0.78, 1.33]		Muanda (2017)(2).12
Malformation: Nervous System	Azithromycin	1	8/883	53/9106	-	1.35 [0.63, 2.89]		Muanda (2017)(2).13
	Clarithromycin	1	4/658	33/5950	-	0.95 [0.34, 2.65]		Muanda (2017)(2).14
	Erythromycin	2	3/1600	56/13166	0	0.44 [0.14, 1.41]		Muanda (2017)(2).15, Cooper (2008).2
Malformation: Orafacial	Azithromycin	1	2/883	51/9106	-	1.6 [0.75, 3.41]		Muanda (2017)(2).16
	Clarithromycin	1	2/658	51/9106	-	1.05 [0.38, 2.9]		Muanda (2017)(2).17
	Erythromycin	2	3/1600	66/16322	0	1.1 [0.4, 3.02]		Muanda (2017)(2).18, Cooper (2008).3
Malformation: Cardiovascular	Azithromycin	1	19/883	192/9106	-	0.92 [0.57, 1.48]		Muanda (2017)(2).19
	Clarithromycin	1	12/658	192/9106	-	0.82 [0.46, 1.46]		Muanda (2017)(2).20
	Erythromycin	3	55/3444	365/25432	59	1.24 [0.77, 2.01]		Muanda (2017)(2).21, Kallen (2005).1, Cooper (2008).4
Malformation: Gastrointestinal	Azithromycin	1	15/883	54/5950	-	1.56 [0.89, 2.73]		Muanda (2017)(2).28
	Clarithromycin	1	10/658	54/5950	-	1.46 [0.75, 2.84]		Muanda (2017)(2).29
	Erythromycin	2	10/1600	80/13166	0	1.58 [0.81, 3.08]		Muanda (2017)(2).30, Cooper (2008).5
Malformation: Genital	Azithromycin	1	9/883	75/9106	-	1.15 [0.58, 2.28]		Muanda (2017)(2).34
	Clarithromycin	1	7/658	75/9106	-	1.27 [0.59, 2.73]		Muanda (2017)(2).35
	Erythromycin	1	2/1697	75/9106	0	0.38 [0.09, 1.6]		Muanda (2017)(2).36
Malformation: Musculoskeletal	Azithromycin	1	48/883	335/9106	-	1.28 [0.94, 1.74]		Muanda (2017)(2).37
	Clarithromycin	1	30/658	335/9106	-	1.17 [0.85, 1.61]		Muanda (2017)(2).38
	Erythromycin	2	30/1600	387/16322	0	1.05 [0.72, 1.53]		Muanda (2017)(2).39, Cooper (2008).7

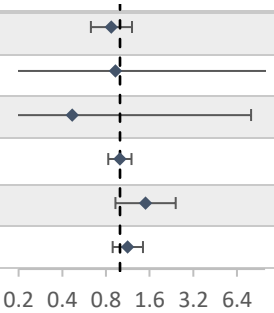


Malformation: VSD/ASD	Azithromycin	1	14/883	150/9106	-	0.88 [0.5, 1.55]		Muanda (2017)(2).22
	Clarithromycin	1	9/658	150/9106	-	0.8 [0.4, 1.6]		Muanda (2017)(2).23
	Erythromycin	2	29/2541	207/18216	6	1.3 [0.86, 1.97]		Muanda (2017)(2).24, Kallen (2005).2
Malformation: Respiratory system	Azithromycin	1	6/883	45/9106	-	1.15 [0.48, 2.76]		Muanda (2017)(2).25
	Clarithromycin	1	4/658	45/9106	-	1.09 [0.38, 3.13]		Muanda (2017)(2).26
	Erythromycin	1	4/697	45/9106	-	1.23 [0.44, 3.44]		Muanda (2017)(2).27
Malformation: Cleft palate/lip	Azithromycin	1	2/883	13/9106	-	1.54 [0.35, 6.78]		Muanda (2017)(2).31
	Clarithromycin	1	2/658	13/9106	-	1.92 [0.43, 8.57]		Muanda (2017)(2).32
	Erythromycin	1	3/697	13/9106	-	3.04 [0.87, 10.62]		Muanda (2017)(2).33
Malformation: Craniosynostosis	Azithromycin	1	8/883	41/9106	-	1.63 [0.75, 3.54]		Muanda (2017)(2).40
	Clarithromycin	1	4/658	41/9106	-	1.33 [0.48, 3.69]		Muanda (2017)(2).41
	Erythromycin	1	3/697	41/9106	-	1.09 [0.34, 3.49]		Muanda (2017)(2).42
Malformation: Pyloric stenosis	Azithromycin	1	7/1574	89/34222	-	1.71 [0.79, 3.69]		Lund (2014).3
	Clarithromycin	1	1/223	89/34222	-	1.72 [0.24, 12.38]		Lund (2014).4
	Erythromycin	2	10/6372	95/43332	73	1.01 [0.22, 4.7]		Lund (2014).5, Kallen (2005).3



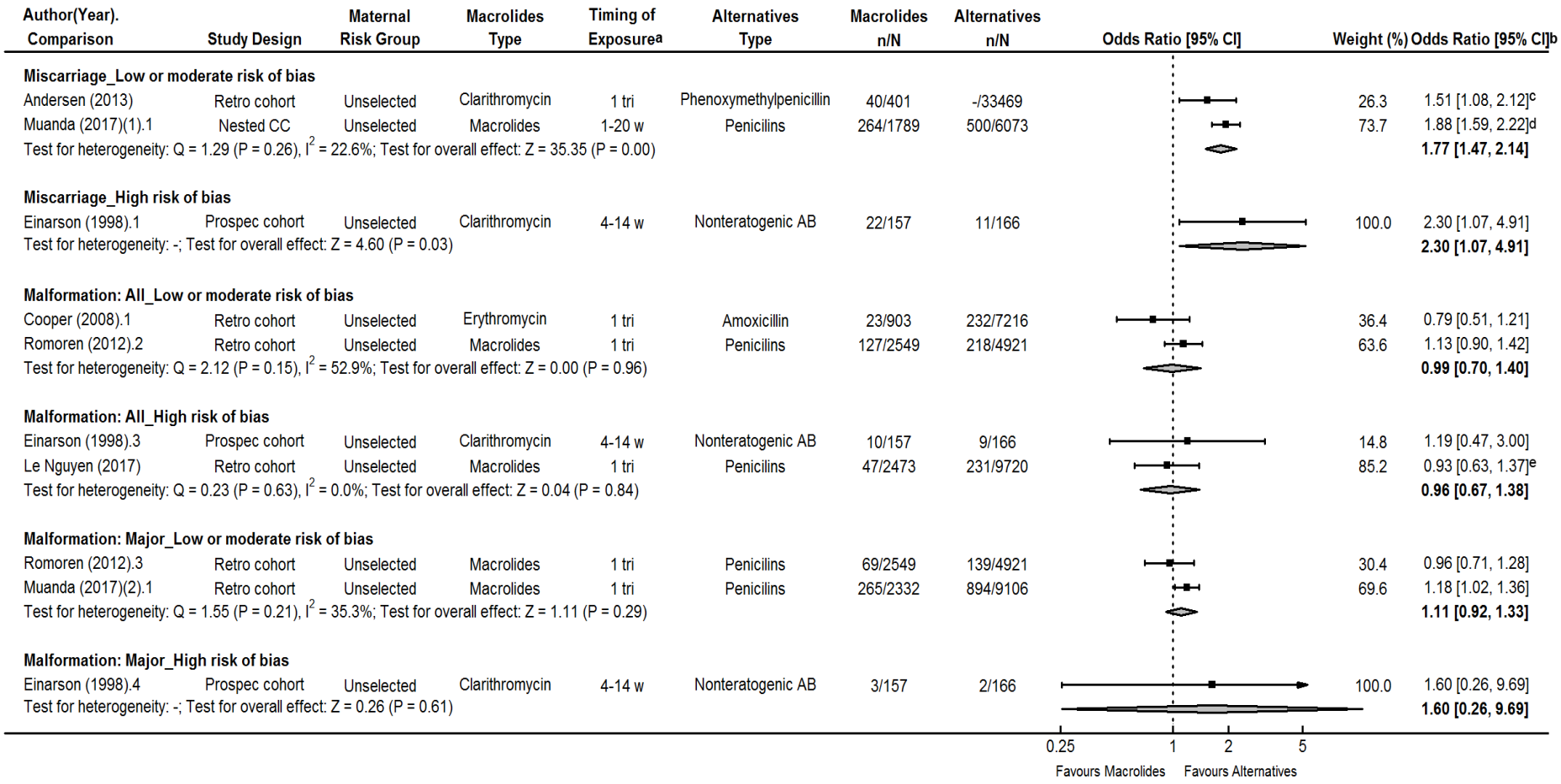
Appendix 3-11. Subgroup analysis: pooled results for specific macrolide types (RCTs, macrolides versus no macrolides).

Outcomes	Subgroup	Number of Studies	Macrolides n/N	No Macrolides n/N	I^2	Pooled OR (95% CI)	Pooled Odds Ratio (95% CI)	Author (year).comparison number
Stillbirth	Erythromycin	7	71/5868	82/5820	0	0.87 [0.63, 1.21]		*
Neonatal death	Azithromycin	1	1/1019	1/944	0	0.93 [0.06, 14.83]		Tita (2016)
	Clarithromycin	1	1/35	1/17	0	0.47 [0.03, 8.01]		Kwak (2013).1
	Erythromycin	4	243/5607	239/5564	0	1.00 [0.83, 1.20]		McGregor (1991).2, Kwak (2013).2, Kenyon (2001)(2).2, Kenyon (2001)(1).2
Cerebral palsy	Erythromycin	2	99/3201	68/3233	56.49	1.50 [0.93, 2.42]		Kenyon (2008)(2).4, Kenyon (2008)(1).4
Epilepsy	Erythromycin	2	250/3201	223/3233	39.37	1.13 [0.89, 1.44]		Kenyon (2008)(2).6, Kenyon (2008)(1).6



*Ye, Y (2001), Mercer (1992), McGregor (1991).1, Martin (1997), Kenyon (2008)(2).2, Kenyon (2008)(1).2, Eschenbach (1991)

Appendix 3-12. Sensitivity analysis according to risk of bias (based on primary analysis).



a. Priority of timing was given to median gestation age of exposure or randomisation, followed by mean, range and approximate time window of exposure; w: gestational week. b. Adjusted odds ratio/ hazard ratio was also shown if available. c. In the study of Andersen (2013), OR was adjusted by maternal age, number of previous miscarriages, income and education. Number of miscarriage in comparison group not given. d. In the study of Muanda (2017), cases and controls were matched by gestational age and year of pregnancy;

OR were adjusted by 11 covariates, e.g. maternal age, education level, chronic comorbidities, maternal infections (urinary tract infection, respiratory tract infection, bacterial vaginosis and sexually transmitted infections) and prior exposure to antibiotics. e. In the study of Le guyen, OR was adjusted by maternal age, long-term illnesses, parity and multiple pregnancy. OR: Odds Ratio; CC: case control; AB: antibiotics

Appendix 3-13. Simulation on the effect of survival bias.

This simulation was performed assuming a study on the association between major malformation and macrolide antibiotics exposure during pregnancy (versus penicillins). Ideally, results of all pregnancies would be observed as shown in table 10.1.

Table 10.1 Numbers of subjects observed in all pregnancies.

Number of subjects	With major malformation	Without major malformation
Macrolides	a	b
Penicillins	c	d

Odds Ratio (in all pregnancies) = ad/bc.

However, both malformation and macrolides were associated with an increased risk of fetal death. Risks of fetal death are estimated as following and in table 10.2:

- The risk of miscarriage in fetuses exposed to penicillin is estimated at 6.7% based on previous observational studies.[15,22]
- Compared to penicillin antibiotics, the risk of fetal death in macrolides group is increased by 50% (about 10.1%, Andersen et al[15]).
- The risk of fetal death in fetuses with major malformation is about 4.5 times of that in unselected population (risk of stillbirth is 0.43% in unselected fetuses and 2% in fetuses with major malformation).[154,161] For simplicity, I estimated that the risk of fetal death would also increase by 4.5 times in fetus with malformation in both exposure groups.

Table 10.2 Risk of fetal death in all pregnancies.

	With major malformation	Without major malformation
Macrolides	45.5%	10.1%
Penicillins	30.2%	6.7%

Thereby the proportions of livebirths in all pregnancies (i.e. those would be selected into future study) are:

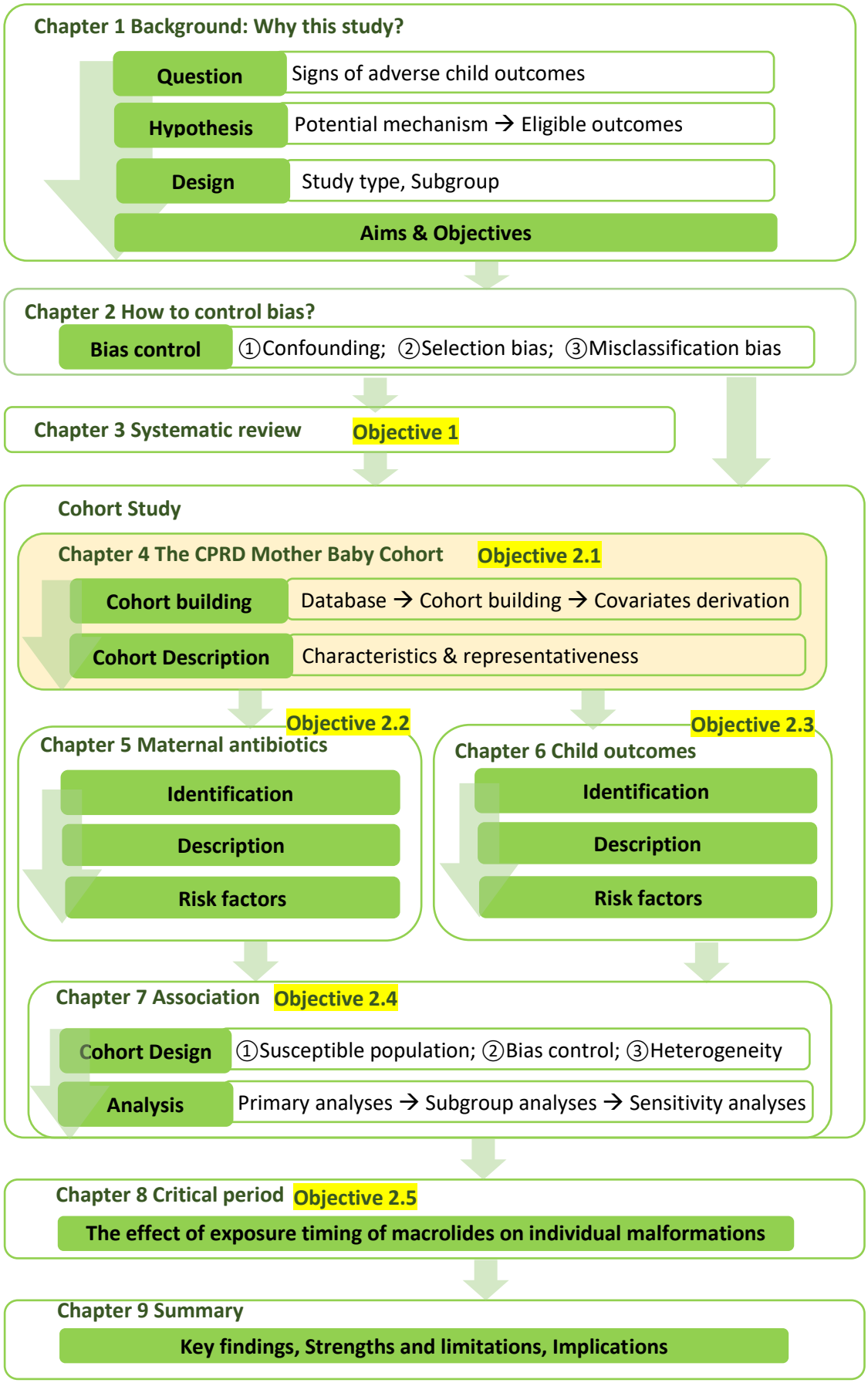
Table 10.3 Proportion of livebirths in all pregnancies.

	With major malformation	Without major malformation
Macrolides	54.5%	89.9%
Penicillins	69.8%	93.3%

Odds Ratio (in livebirths) = (54.5*93.3/(89.9*69.8)) *(ad/bc) =0.81* Odds Ratio (in all pregnancies)

I.e. the OR for the association between macrolides and major malformation measured only in livebirths is underestimated by 19% given the estimation.

Thesis Structure



Chapter 4 The CPRD Mother Baby Cohort

4.1 Chapter overview

In the previous chapter, I synthesised existing evidence from human studies on the association between macrolide prescribing during pregnancy and adverse child outcomes. Combined with evidence from animal studies, there is evidence of some harmful effects of fetal exposure to macrolides. However, consistent evidence on which outcomes are affected and when in pregnancy, is lacking. In this PhD, I performed a cohort study to determine whether there is an association between macrolide antibiotic prescribing in pregnancy and adverse child outcomes, using the CPRD Mother Baby Cohort.

This chapter presents work towards objective 2.1 of this thesis: "To develop a mother-baby cohort using an UK administrative primary care database." I present methods for developing a mother-baby cohort within the CPRD database, and evaluate the validity of the mother-baby cohort. In this chapter, I first introduce CPRD. I then describe methods for deriving a mother-baby linkage from the CPRD database and for deriving the CPRD Mother Baby Cohort using the mother-baby linkage. Next, I derive and describe maternal and birth characteristics in the cohort (including gestational age), and compare them with external evidence to validate the representativeness of the cohort against the population of pregnancies in England. The CPRD Mother Baby Cohort was used as the target population for the main cohort study in the following Chapter 5 to 8.

4.2 The Clinical Practice Research Datalink (CPRD)

4.2.1 Overview

The data source utilised in the cohort study in this PhD was the primary care database, the UK Clinical Practice Research Datalink, which contains anonymised data on patients from over 694 NHS primary care practices from across the UK with approximately 12 million total patients since 1987. Patient can register with the practice at some point during the study period. When a practice agrees to contribute patient data to CPRD, CPRD receives a full historic collection of the coded part of the practice's electronic health records. The data in CPRD therefore includes data on deceased patients and those who have left the practice. General practitioners or other practice staff record the data as part of routine clinical care (e.g. prescribing and specialist referrals). All prescriptions issued in primary care practices contributing to CPRD are captured. In addition to the routinely collected data from primary care consultations, information may also be recorded from some secondary sources that has been provided to primary care clinicians

(such as major diagnoses made in hospital). The CPRD recording guidelines state that the GP should make at least one entry in the medical history for each episode of illness or new occurrence of a symptom.[162] Patient characteristics (e.g. height, weight, smoking status and alcohol use), symptoms, immunizations and tests were also recorded in CPRD. The general practices were required to establish and maintains a register of patients with obesity, and provide continue support for current smokers.[163]

In CPRD, each practice is issued with a set of GPRD recording guidelines, describing how to record all significant morbidity events in each patient's medical history.[4] The raw data provided from each practice undergo extensive quality control and validity checks by a research team based at the MHRA before release. These data are assessed by an 'up to standard' audit, confirming data recording in several key areas. Practices meeting this standard are included in the GPRD data warehouse. Patient-level data are also assessed, with patients considered 'acceptable' for inclusion in the GPRD if recorded details are internally consistent in four areas: age, sex, registration details, and event recording. CPRD also reimburses the GPs for providing quality data.

CPRD has been shown to be broadly representative of the UK population.[164] Epidemiological research has been performed using the database for over 20 years, resulting in over 2,200 peer-reviewed publications investigating drug safety and use of medicines etc. Overall, estimates of diagnoses validity of CPRD were high.[165] CPRD has been extensively used for drug safety research within academic, regulatory, and pharmaceutical organisations to inform policies, due to its high completeness and validity.[130]

While studies using CPRD benefit from real-life data and large sample sizes, primary care data are recorded for clinical rather than research purposes. Not all aspects of a consultation are clinically important enough to record and not all GPs code in a similar way or use the same codes for the same entity. Incidence estimates in CPRD measure rates of recording rather than true rates of occurrence in the population.

4.2.2 Data Format

Each patient has been assigned an encrypted unique identifier in CPRD (patient ID). Patients' data are coded with two coding systems within CPRD. Read codes are based on a detailed coded thesaurus of clinical terms used for recording patient findings and procedures. Prescriptions are coded with Prod codes, which are categorized based on the British National Formula. Additionally, GPs may enter free-text alongside codes as comments in consultations. Free-text

is only available in an anonymized form from MHRA for a fee and not normally included with the full dataset as the fields contain personal information.[166] Indications and prescriptions can be linked using temporal criteria. Searchable dictionaries of read code and prod code used in the database are provided.

Data is saved in a number of separate databases, where each entry contains at least the unique patient identifier, the code of the event, and the date of the event. Table 4-1 provides details of the CPRD data files.

Table 4-1. Description of CPRD data files.

Data file	Data organisation	Description
Patient	One record per patient	Contains demographic data such as gender, year and month of birth, and death date (if applicable).
Clinical	Multiple records per patient	All the medical history data entered on the GP system, including symptoms, signs, life style factors, preventative care provided and diagnoses.
Therapy	Multiple records per patient	All drug prescriptions for the patient, including therapy code, term, dates, strength and formulation.
Test	Multiple records per patient	All test records for the patient, including test type, date and result
Referral	Multiple records per patient	Inbound and outbound patient referrals to or from external care centres, including details of the patient's medical history and diagnosis associated with referral.
Additional	Multiple records per patient	Contains additional information that can be used to derive smoking, BMI and alcohol status

4.3 Develop the CPRD Mother Baby Cohort

This section presents all steps taken to develop a representative mother-baby cohort (referred to as CPRD Mother Baby Cohort) using de-identified match keys to allow linkage of mothers to their baby. Raw data were based on two CPRD data extracts ISAC codes 14_038 and 14_039. The study protocol was approved by the University College London Research Ethics Committee (14569.001) and the Independent Scientific Advisory Committee for MHRA Database Research. The cohort study is registered at www.clinicaltrials.gov NCT01905462.

4.3.1 Develop the CPRD mother-baby linkage

The CPRD Mother Baby Cohort includes eligible mother-baby pairs from the existing CPRD mother-baby linkage. The mother-baby linkage was linked by a designated data scientist, Gonzalez Arturo, in Institute of Health Informatics, University College London using the de-identified match key provided by CPRD. This linkage includes all potential mother-baby pairs identified in CPRD without providing pregnancy start dates. The method of generating and validating the mother-baby linkage was briefly described by CPRD (and explained in section

4.3.1.1 to 4.3.1.3).[167] In brief, the process of creating the mother-baby linkage from the CPRD database involves 1) identifying deliveries in pregnant women, 2) identifying babies born within the appropriate time period, 3) linking mothers with their babies and 4) estimate the pregnancy start date.

4.3.1.1 Identify maternal deliveries

First appended all Read codes available in data files in Table 4-1 into an event list where each row includes the patient ID, the code of the event (including prescription) and the date of event (except the "Additional data file"). Records belong to "Maternity" in "Additional data file" were also extracted. I defined an entire population of women of childbearing age in CPRD and grouped all codes indicating an outcome of pregnancy into four categories: (1) stillbirths; (2) elective terminations; (3) spontaneous abortions or miscarriages; and (4) livebirths: normal full-term births/deliveries, pre-term and post-term births.

Because similar codes can represented the same pregnancy, duplicated records should be removed. For each woman, the date of the earliest code indicating an outcome of a pregnancy was designated as the delivery date. I then discarded the following codes of the same pregnancy category within a specified timeframe afterwards (i.e. 60 days within category 3 - spontaneous abortion; 210 days wihtin category 1, 2 and 4). This timeframe represented the minimum time gap between two different pregnancy outcomes. Within the three pregnancy categories, each subsequent code that was beyond these timeframes was considered a new pregnancy. At this stage, more than one pregnancies categories conflictive to each other could be identified within an adjacent time period for a woman.

Select the final pregnancy outcome

I selected the true pregnancy outcome from a series of adjacent pregnancy categories. Women diagnosed with a stillbirth (category 1) could have a later record indicating delivery (category 4) (e.g. "birth details") or elective termination (category 3, e.g. "termination of pregnancy") depending on the interval between these different pregnancy categories. Here according to practice, the record of "birth details" or "termination of pregnancy" is likely to indicate the result of a stillbirth instead of live-birth and therefore should be ignored. Livebirths were considered correct only when they were not in conflict with codes of other pregnancy categories. The value of time frames is available in the work of Devine et al.[168]

4.3.1.2 Identify live born

All registered patients born after 1986 were extracted. Patients whose year of registration was before their birth year, or whose birth year was after the last collection year were excluded. Since CPRD do not collect full date of birth, birth date was estimated as the 15th day of the given month and year. For patients without a month of birth, birth date was estimated as date of the patient first registered with the practice in CPRD if in the same year. Otherwise, the 30th June of the birth year was assumed (very few).

4.3.1.3 Link Births to Deliveries

A Cartesian join of mothers to babies by practice and family number was undertaken and only those records where the absolute difference between the delivery date and the estimated birth date was within 60 days were retained. Where more than one delivery record per mother baby pair was available, the record with the smallest absolute difference between the delivery date and the estimated birth date was selected. No restriction was applied to the difference between date of children registration with the GP practice and their birth date at this step. Where a delivery was matched to more than one child, the delivery included only if the children had the same birth date and the same registration date, in an effort to keep multiple births (e.g. twins).

4.3.1.4 Estimate the pregnancy start date

I estimated the pregnancy start date for each pregnancy included in CPRD mother-baby linkage using the raw data from CPRD. While most previous studies estimated pregnancy start date simply by imputing full-term for every pregnancy without evidence of premature, a more accurate approach to estimate pregnancy start date needs to develop multiple code list groups. Two previous studies reported similar approaches to estimate more accurate pregnancy start dates using code list groups (Matcho et al.[code list provided][169] and Minassian et al.[code list not available at the time][170]). This study applied a hierarchy of available pregnancy markers, referenced from Matcho et al., to reflect their potential accuracy to estimate start of the pregnancy episode.

Firstly, pregnancy markers that directly provide gestational age such as the date of LMP, or gestational age in weeks on specific dates, whether at birth or at prenatal examinations, or fertility procedures (In vitro fertilisation [IVF]) reflecting conception date, were ranked at the top of the hierarchy. Secondly, I used markers such as ranges of gestational week indicators (e.g. premature 24-26 weeks) and outcome-specific estimates. For example, premature labour was imputed as 36 weeks, because around 60% live preterm births, defined as before 37 weeks, are born at 36 gestational weeks. Thirdly, if no higher ranking information was available, weeks of

gestation were imputed from birthweight, based on the intrauterine growth curves published by Irene E. Olsen et al.[171] For pregnancies with no information available for the above three hierarchies of markers, full term births were assumed and gestational week 40 was used to calculate the pregnancy start date. The distribution of births according to each step in the hierarchy for estimating LMP is reported in Table 4-2.

Table 4-2. Proportion of births according to hierarchical method for estimating LMP in the CPRD mother-baby linkage.

Hierarchy of conception markers	Description	Proportion in all livebirths
Pdate1: Accurate dates indicators (the most accurate)	- Codes indicate specific gestational week, e.g. AN 32 weeks exam/ Premature 36 weeks - Gestational weeks recorded in "Additional data file" maternity section. - "Term infant/pregnancy at term": 40 GW - Fertility procedures (IVF): 2GW - LMP dates recorded in "Additional data file" maternity section: 0 GW	27.8%
Pdate2: week range, inferred dates	- Week range, e.g. premature 24-26 weeks: 25 GW; premature 28-37 weeks: 36 GW - 2. Inferred dates: e.g. Premature labour: 36 GW; Post-mature infancy:42 GW	14.3%
Pdate3: impute by birthweight	Birthweight records in Additional data file-maternity or Read codes.	8.2%
Pdate4: impute as full terms (the least accurate)	For livebirths, if pdate1-3 are missing, then assuming them as full term births, i.e. start date= birthday - 40 GW	49.6%

4.3.1.5 Validate the CPRD mother-baby linkage

The CPRD mother-baby linkage issued to us included 1,126,568 mother-baby pairs (1,111,091 pregnancies and 771,871 mothers). Each pair included patient ID for mother and baby, family number, practice number, baby gender, delivery date. I generated an estimated pregnancy start date for each mother-baby pair, using the method described in the section above.

To evaluate the validity of the CPRD mother-baby linkage and the estimated conception date, I derived the following additional variables: maternal age at conception, baby's calendar year of birth, baby's gestational age, baby's multiple birth status, parity (number of previous delivery), and length of follow up for mothers and babies.

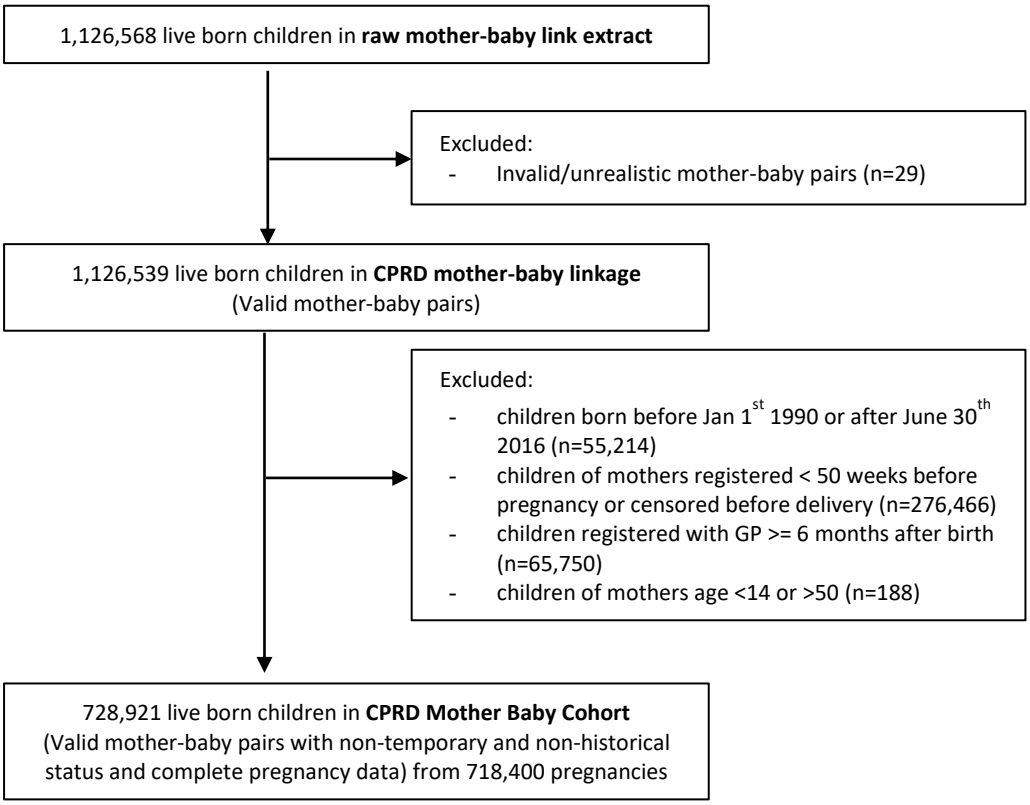
I excluded implausible multiple pregnancies as follows: of the 1,111,091 pregnancies, one pregnancy resulted in sextuplets (6 babies) and another resulted in 19 babies, both of which were unlikely. Since it was not possible to confirm the correct children, these pregnancies (25 babies) were excluded from the linkage. Four pregnancies with missing year of birth for mothers were also excluded. The final CPRD mother-baby linkage included 1,126,539 children (Figure 4-1).

4.3.2 Develop the CPRD Mother Baby Cohort

I applied additional exclusion criteria to the raw data extract (the CPRD mother-baby linkage) to remove mothers and babies who were likely to be "temporarily registered patients" and who might therefore have incomplete records on their life behaviour, chronic disease status, or follow-up (Figure 4-1). I excluded mother-child pairs in which the child:

- was registered with the GP later than 6 months after birth. The 6 months' timeframe allowed for a grace period between birth and registration with a GP, designed for children unable to register immediately following birth because they were kept in hospital, such as those born prematurely; or
- was born before Jan 1st 1990 or after June 30th 2016 (the end of the study); or
- whose mother had been registered with the GP later than 50 weeks before the estimated pregnancy start date or censored before delivery (this 50 weeks' timeframe ensured the capture of baseline characteristics and prescriptions of mothers before and during pregnancy); or
- whose mother was aged < 14 or > 50 years.

Figure 4-1. Flow chart illustrating how the CPRD Mother Baby Cohort was developed from the raw linkage extract. Mothers could have had more than one pregnancy.



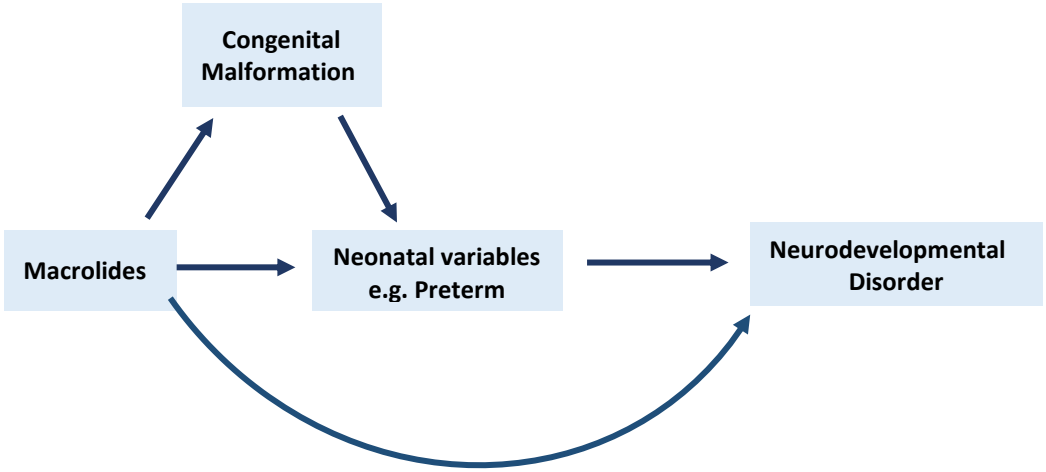
4.4 Derive potential risk factors

In Chapter 1 I explained that, to minimise indication bias due to maternal infection, I chose to compare children whose mother was prescribed macrolides during pregnancy with a comparator group of children whose mother was prescribed penicillins during pregnancy. However, residual confounding can still exist if macrolides were prescribed for specific indications (e.g. chlamydia infection of the reproductive tract), or there were other potential systematic differences in the two antibiotics groups which were also suspected risk factors. To address this problem, I developed indicators to control for risk factors for adverse child outcomes in CPRD Mother Baby Cohort. As was discussed in section 2.1, I considered maternal life style factors and maternal characteristics or conditions during pregnancy as potential risk factors in this study. These risk factors include maternal life style (alcohol misuse, illicit drug use, tobacco use and obesity), maternal characteristics (pregnancy year and maternal age), underlying conditions during pregnancy likely to increase the risk of adverse child outcomes (hypertension, diabetes, anxiety, depression, epilepsy, chronic medical treatments), potential fetal-damaging maternal infections occurring during pregnancy (genitourinary tract infections and sexually transmitted infections [STIs]), and pregnancy indicators (parity and multiple birth).

Respiratory tract infection, skin infection and gastrointestinal infections are common during pregnancy and generally considered benign if uncomplicated, although evidence is limited on associated fetal and child outcomes.[172] However, certain infections can be transmitted from the mother to the fetus before or during birth and damage the fetus. The classic group of teratogenic pathogens are referred to as "TORCH": Toxoplasmosis, Other (syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus (CMV), and Herpes infections.[156] Other STIs and genitourinary tract infections have also been associated with preterm labour (and/or congenital malformation) to varying extent and therefore adjusted as potential confounders.[173,174] Chlamydial infection, the specific indication for azithromycin and less often for erythromycin, was also adjusted as one STI.

I did not adjust for neonatal variables (e.g. gestational age at delivery and Apgar score at 5 minutes after birth) as they are either potential consequences of the adverse outcome (e.g. malformation) or mediating cause of the outcome (e.g. cerebral palsy) on the causal path from macrolides to outcomes (Figure 4-2).

Figure 4-2. Diagram for the effect of neonatal variables.



The detailed definition and derivation algorithms for deriving these variables were included in Table 4-3. Unless otherwise noted, an absence of information regarding a particular condition or behaviour was assumed to indicate an absence of that condition or behaviour.

Table 4-3. Derivation of variables in the CPRD Mother Baby Cohort.

Covariates	Time for measurement	Value	Description
Maternal age at delivery	-	Grouped into categories of 5 calendar years (roughly): 14-19; 20-24; 25-29; 30-34; 35-50.	Defined as year of the date of delivery minus mothers' year of birth.
Calendar year at delivery	-	Grouped into categories of 5 calendar years: 1990-1994; 1995-1999; 2000-2004; 2005-2009; 2010-2016.	-
Parity	-	Categorised as "0", and ">= 1"	Number of previous live births captured in the CPRD Mother Baby Link (i.e. births after registry with GPs) before the current pregnancy.
Reported multiple birth status	-	"Singleton", and "(One of the) Twin, triplets, or quadruplets captured in the database".	
Reported maternal alcohol misuse	Most recent measurement from up to 10 years before pregnancy to the end of pregnancy.	"Yes" and "No"	Alcohol misuse was defined as >=14 units of alcohol per week, moderate or severe drinker. Self-reported alcohol consumption was collected prospectively and coded by general practitioners or practice nurses on the consultation date in CPRD. The most recent alcohol consumption record was used to classify women's drinking behaviour, and "ex-drinker" was categorised as not alcohol misuser. Alcohol misuse was defined using: 1) One of the read codes indicating excessive alcohol consumption; or, 2) A prescription for disulfarim or acamprosate; or, 3) Self-reported average weekly alcohol intakes >= 14 units in the "Additional Clinical Details" I applied the code list of alcohol consumption developed by Bell at al.[175]
Reported illicit drug use	Most recent measurement from up to 10 years before pregnancy to the end of pregnancy.	"Yes" and "No"	Illicit drug use was defined using: 1) read codes recorded for drug use, addiction, or overdose; or 2) prescriptions for methadone treatment I assumed that although a mother may stop using illicit drugs, the underlying behaviour was unlikely to vary significantly over time.
Reported maternal obesity	Most recent measurement from 3 years before pregnancy till the end of the first trimester.	"Yes" and "No"	Mothers who were obese prior to the 2nd trimester of pregnancy were identified from the Read codes for obesity (or a BMI of ≥30 kg/m^2 - either directly entered or calculated from the most recent height measurement and median pre-pregnancy weight after outliers, i.e. height outside the range 1-2m

			and weight outside the range 35-300kg, were removed). It was assumed that once a mother reached clinical obesity, the chance of her returning to a normal BMI was minimal (Section 4.2.1).
Reported recent tobacco use	Most recent measurement from 3 years before pregnancy to the end of pregnancy.	"Yes" and "No"	Tobacco use was defined as daily cigarette consumption of 1-100 cigarettes per day or other tobacco use. The most recent tobacco consumption record was used to classify participants drinking behaviour, and "ex-smoker" was categorised as non-recent smoker if there was evidence of smoking cessation before pregnancy start. Recent tobacco use was defined using: 1) One of the read codes indicating tobacco consumption; or, 2) A prescription for smoking cessation aid; or, 3) Self-reported daily cigarette consumption of 1-100 cigarettes per day in the "Additional Clinical Details"
Reported hypertension	50 weeks prior to pregnancy (LMP)	"Yes" and "No"	Mothers with hypertension during pregnancy were identified based on 1) Systolic and diastolic blood pressure was above 140mmHg and 90mmHg, respectively, or 2) Read codes for hypertension and associated diagnoses (including pre-eclampsia, eclampsia and HELLP syndrome), or 3) Prescriptions for hypertension drugs from sections 2.2 and 2.5 of the BNF. This variable identified mothers with both treated and untreated hypertension in pregnancy.
Reported diabetes	50 weeks prior to pregnancy	"Yes" and "No"	Mothers with diabetes during pregnancy were identified based on: 1) Read codes for type I, type II, or gestational diabetes; or 2) Two or more prescriptions for anti-diabetic medication; or 3) Laboratory tests indicative of diabetes (defined as ≥2 abnormal glucose tests, fasting glucose >7.0 millimoles per litre [mmol/L] or >126 milligrams per decilitre [mg/dL], plasma glucose after glucose tolerance test >11.1 mmol/L or 200mg/dL, glycated haemoglobin >=6.5%, or within diabetes annual review) recorded in the "Additional Clinical Details".
Reported epilepsy	50 weeks prior to pregnancy	"Yes" and "No"	2 prescriptions of antiepileptic drugs (AEDs) within 4 months or >= 1 diagnosis
Reported depression	50 weeks prior to pregnancy	"Yes" and "No"	>= 2 occurrences of diagnostic code, treatment code or symptom
Reported anxiety	50 weeks prior to pregnancy	"Yes" and "No"	>= 2 occurrences of diagnostic code, treatment code or symptom
Reported treatment of chronic medical	During pregnancy	"Yes" and "No"	Existence of chronic medical conditions are defined as conditions that are sufficiently severe to require on-going treatment during pregnancy. Mothers

conditions during pregnancy			were considered to have a chronic medical condition if they were issued two or more prescriptions (on separate days during pregnancy and not more than four months apart) for drugs from the same BNF section or paragraph.[1] Drugs used to treat common conditions in pregnancy such as reflux (BNF section 1.2), nausea and vomiting (BNF section 4.6), and constipation (BNF section 1.3) were not included.
Reported genitourinary tract infection	During pregnancy	"Yes" and "No"	Include urinary tract infection, cystitis, vaginitis and the prescription of Nitrofurantoin.
Reported Sexually Transmitted Infection	During pregnancy	"Yes" and "No"	Include chlamydia infection, trachoma, "TORCH" (Toxoplasmosis, Other agents such as HIV, Rubella, Cytomegalovirus and Herpes simplex) and other sexually transmitted infections (STIs).

*When the key codes indicating a binary condition were not identified in the medical history of a subject, I classified the subject as absence of the condition. There were no missing for multi-categorical covariates in this study ("Age at delivery" and "Calendar year of delivery").

[1] The following BNF sections and paragraphs were included in the algorithm: Chronic bowel disorders 1.5; Stoma care 1.8; Drugs affecting intestinal secretions 1.9; Positive inotropic drugs 2.1; Diuretics 2.2; Anti-arrhythmic drugs 2.3; Beta-adrenoceptor blocking drugs 2.4; Hypertension and heart failure 2.5; Nitrates, calcium-channel blockers, and other antianginal drugs 2.6; Sympathomimetics 2.7; Anticoagulants and protamine 2.8; Antiplatelet drugs 2.9; Stable angina, acute coronary syndromes, and fibrinolysis 2.10; Antifibrinolytic drugs and haemostatics 2.11; Lipid-regulating drugs 2.12; Local sclerosants 2.13; Bronchodilators 3.1; Corticosteroids 3.2; Respiratory stimulants and pulmonary surfactants 3.5; Oxygen 3.6; Mucolytics 3.7; Hypnotics and anxiolytics 4.1; Drugs used in psychoses and related disorders 4.2; Antidepressant drugs 4.3; CNS stimulants and drugs used for attention deficit hyperactivity disorder 4.4; Drugs used in the treatment of obesity 4.5; Drugs used in nausea and vertigo 4.6; Analgesics (not Non-opioid analgesics) 4.7.2, 4.7.3, 4.7.4; Anti-epileptic drugs 4.8; Drugs used in parkinsonism and related disorders 4.9; Drugs for dementia 4.11; Antibacterial drugs (Antituberculosis and Antileprotic drugs only) 5.1.9, 5.1.10; Antiviral drugs (HIV and Viral hepatitis only) 5.3.3; Antiprotozoals (Leishmaniacides and Drugs for pneumocystis pneumonia only) 5.4.5, 5.4.8; Drugs used in diabetes 6.1; Thyroid and antithyroid drugs 06.02; Corticosteroids 6.3; Hypothalamic and pituitary hormones and anti-oestrogens 6.5; Drugs affecting bone metabolism 6.6; Other endocrine drugs 06.07; Cytotoxic drugs 8.1; Drugs affecting the immune response 8.2; Intravenous nutrition 9.3; Metabolic disorders 9.8; Drugs used in rheumatic diseases (not Non-steroidal anti-inflammatory drugs) 10.1.2, 10.1.3, 10.1.4; Drugs used in neuromuscular disorders 10.2; and, Preparations for eczema and psoriasis 13.5.

4.5 Characteristics and representativeness of the CPRD Mother Baby Cohort

After applying the above criteria in Section 4.3.2, 728,921 children born to 514,139 mothers (in 718,400 pregnancies) remained and were included in the CPRD Mother Baby Cohort (Figure 4-1). Patients temporarily registered at GP in mother-baby linkage were excluded from the cohort.

Characteristics of the CPRD mother-baby linkage and CPRD Mother Baby Cohort are shown in Table 4-4. Mothers in the cohort were followed up for a total of nine million person years, with a median of 16 years (inter-quartile range (IQR): 9-25) follow-up per mother from their registration with general practice. Mothers included in the CPRD Mother Baby Cohort have complete follow-up from 50 weeks before LMP until delivery. Children in the cohort were followed up for a total of six million person years, with a median follow-up per child of 6 years (IQR: 2-13). Follow-up for the children began at the date of birth, and ended at the earliest of the day of the last (any) event, the date of de-registration from the primary-care practice, or the date of death, whichever the latest.

The CPRD Mother Baby Cohort included women born between 1943 and 2002, who had deliveries between January 1990 and June 2016. Women in the cohort were older at delivery than the general population of women delivering in England and Wales (32.3% of women were 30-34 years and 23.1% of women were 35-50 years in the cohort, compared to 29.4% and 17.6% respectively according to data of England and Wales Birth Statistics from 1997 to 2007). [176]

There are several potential explanations for this discrepancy. Firstly, more children of the cohort were born in more recent years (Table 4-4). However, comparison by year of birth e.g. 2004 still shows a higher proportion of older mothers than the national statistics in England and Wales that year (26% versus 19% mother aged 35-50), meaning that more recent enrolment cannot explain the older maternal age structure. Instead, a structural difference may exist between the cohort and general population. National statistics include mothers who are not registered with a GP or those registered only temporarily, or registered after 50 weeks before delivery, or those not linked to a child, who were removed from my study cohort. Mothers who register late or not at all are more likely to be disadvantaged, which is strongly associated with young maternal age. Consistent with this assumption, an age structure more comparable to general population can be seen in CPRD mother-baby linkage, before the eligibility criteria is applied (Table 4-4). Furthermore, practices participating in primary care databases (e.g. THIN) are thought to serve more affluent populations, which would be associated with older maternal age.[177] However, the

CPRD Mother Baby Cohort is still preferable due to its completeness of recording in exposure, covariates and outcomes. Give the differences in the distributions of maternal age between this cohort and general population, the generalisability of findings in this study may be affected if there was an interaction between maternal age and macrolides on the association with adverse outcomes. Therefore, I would test the interaction terms between maternal age and macrolide prescribing in the cohort study (Chapter 7), to evaluate whether the older maternal age structure would affect the generalisability of findings from the CPRD Mother Baby Cohort.

Table 4-4. Characteristics of the CPRD mother-baby linkage and Mother Baby Cohort (temporary patients excluded).

	CPRD mother-baby linkage		CPRD Mother Baby Cohort	
	Mothers	**Children**	**Mothers**	**Children**
Number of mother-child pairs				
	771865	1126539	514139	728921
Total years follow up				
	11560083	9267195	9299643	5874426
Median years follow up (IQR)				
	12 (5-23)	6 (2-13)	16 (9-25)	6 (2-13)
Mothers' year of birth (%)				
1940s-1960s	261428 (33.9)		171057 (33.3)	
1970s	280443 (36.3)		195988 (38.1)	
1980s	196990 (25.5)		126183 (24.5)	
1990s	32982 (4.3)		20895 (4.1)	
2000s	22 (0)		16 (0)	
Maternal age at delivery (%)				
13-19		47368 (4.2)		28272 (3.9)
20-24		181470 (16.1)		104365 (14.3)
25-29		314354 (27.9)		192245 (26.4)
30-34		351529 (31.2)		235317 (32.3)
35-50		231818 (20.6)		168722 (23.1)
Babies' year of birth (%)				
1986-1989		36883 (3.3)		
1990-1994		144791 (12.9)		80403 (11)
1995-1999		185721 (16.5)		129860 (17.8)
2000-2004		222953 (19.8)		154684 (21.2)
2005-2009		258255 (22.9)		174720 (24)
2010-2016		277936 (24.7)		189254 (26)
Gestational weeks at birth				
23-27		4730 (0.4)		3724 (0.5)
28-31		7428 (0.7)		5746 (0.8)
32-34		13944 (1.2)		10386 (1.4)
35-36		47793 (4.2)		34595 (4.7)
37-38		125774 (11.2)		92313 (12.7)
>=39		926870 (82.3)		582157 (79.9)
Multiple births captured in the cohort				
Single birth	758589 (98.3)	1095919 (97.3)	505019 (98.2)	708087 (97.1)
Multiple births	13276 (1.7)	30620 (2.7)	9120 (1.8)	20834 (2.9)
Parity				
First child	489992 (63.5)	771865 (68.5)	322304 (62.7)	489696 (67.2)
With sibling(s)	281873 (36.5)	354674 (31.5)	191835 (37.3)	239225 (32.8)

Pregnancy start dates as well as gestational age at birth were estimated for all pregnancies in the CPRD Mother Child Cohort, with a comparable gestational age structure with the population-level statistics. The proportion of preterm birth in this cohort (e.g. births occurring prior to 37 completed weeks gestation) was 7.5%[2], which is comparable to the proportion of preterm reported by the Office for National Statistics (7.1%).[178] Nevertheless, about 6%-7% full-term births with "true" gestational age of 37-38 weeks might have been estimated to be with 39 or longer gestational weeks (12.7% versus 19.3% of pregnancies with 37-38 weeks according to national statistics). The gestational age estimation in this cohort is an improvement over many previous pregnancy cohorts created using routine data where all pregnancies are assumed term unless there is evidence for preterm births, which is prone to misclassify preterm as term babies. A recent paper reported to estimate the pregnancy start dates using algorithms similar to this study (using hierarchical code groups and imputing).[170] In that study, 42% of imputed gestational age were reported, and the pregnancies were validated using other studies of CPRD, showing close agreement with external data. The representative gestational age structure also demonstrated that the six-month window allowed in the linkage algorithm could be enough to capture premature babies who register late with the GP.

The incidence of multiple birth (1.45%, as from 2.9%/2) per pregnancy was also comparable to the national statistics (1.5%). However, there were more firstborn children (67.2%) in our cohort, compared with 41.3% according to England and Wales Birth Statistics in 2000, probably because families often move after a first birth in the early child years and may therefore not be registered at the same practice during their subsequent birth.[179] Further support for this mechanism is that mothers in the cohort on average gave birth to 1.4 children during their follow up in the cohort, which is lower than the national average of 1.9. Similar with the older maternal age structure, more firstborn children in this cohort could also limit the generalisability of this cohort, if an interaction exist between parity and macrolides exposure on the association with adverse outcomes. Evidence is inconsistent for the effect of parity on neurodevelopmental disorders, [180] but nulliparity may be associated with an increased risk of some malformation such as hypospadias. Thus an effect modification of parity for the association between macrolides and outcomes could not be excluded which may limit the generalizability of the study. In a same manner with maternal age, I would also investigate whether maternal age modifies the

[2] The proportion of preterm birth was calculated by number of baby with gestational week <= 37 complete weeks (n=67334) - number of baby with 37 complete weeks (n=12883). The number of baby born at 37 complete gestational weeks (37*7 gestational days) seems large, but this is normal according to the distribution of gestational day, and was not induced by gestational age imputing (where I use 36 weeks as the gestational age for general premature marker).

association between macrolides prescribing and adverse child outcomes, in Chapter 7 (the cohort study).

Prevalence of potential risk factors of adverse child outcomes were described and compared with evidence from other sources (Table 4-5). Except for the risk factors discussed above (maternal age at pregnancy, pregnancy start year, parity and multiple births), other potential risk factors were largely comparable with evidence from previous UK studies. It is worth noticing that the prevalence of maternal anxiety and depression during pregnancy were slightly lower compared with other sources. The finding of under recording of antenatal and postnatal mental health in primary care are consistent with other research. A Previous study reported that less than 50% of postnatal depression cases were identified by primary healthcare professionals in routine clinical practice.[181] One reason of this could be that only around 25% of primary care trusts had a fully developed and implemented policy for antenatal and postnatal mental health.[182] However, since the assessment for anxiety and depression I applied in this database (i.e. >= 2 occurrences of diagnostic code, treatment code or symptom) was relatively specific, it is reasonable to postulate that the cases who were more serious were identified in the cohort. A more affluent population may also contribute to this lower prevalence of mental health problem. (Table 4-5)

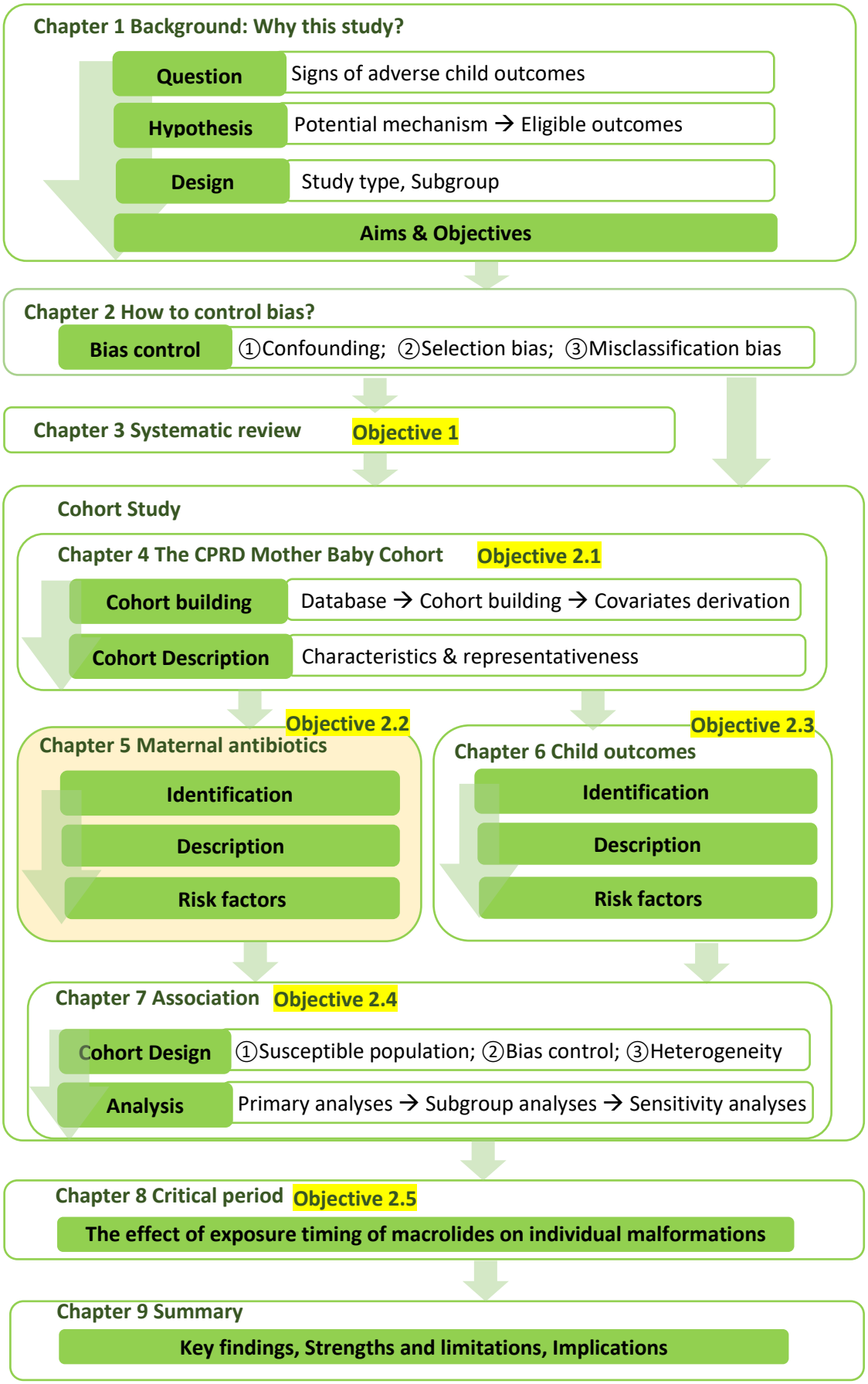
In summary, compared with the general population of pregnancies in the UK, this CPRD Mother Baby Cohort is largely representative in terms of maternal and birth characteristics, including gestational age (especially for preterm births), life style factors, and maternal conditions before and during pregnancy. This cohort involves more older mother and more firstborn children. These two factors will be adjusted for as covariates in statistical analyses. I will also examine whether they modify the association between macrolides and adverse child outcomes in Chapter 7 to assess the generalisability of this study.

Table 4-5. Distribution of potential risk factors for the CPRD Mother Baby Cohort.

Characteristics	Number of Children	Compared with evidence from other sources
Number of mothers	**514139**	
Maternal alcohol misuse (%)	23628 (4.6)	**Comparable.** NICE 2010: 4.5-6.6% alcohol abusers during pregnancy;[183] RCGP 2005-2017: 5.9%
Maternal illicit drug use (%)	4367 (0.8)	**Comparable.** NICE 2010: 1% cocaine or Heroin use during pregnancy;[183]
Maternal tobacco use (%)	153608 (29.9)	**Comparable.** Other studies using CPRD among general population (39.4% ever smokers 1993-
Maternal obesity (%)	56167 (10.9)	**Comparable.** Centre for Maternal and Child Enquiries (CMACE) UK: 8% maternal obesity[186]
Number of babies	**728921**	
Maternal age at pregnancy (%)		Section 3.2.1— maternal age **moderately older** than population
13-19	28272 (3.9)	
20-24	104365 (14.3)	
25-29	192245 (26.4)	
30-34	235317 (32.3)	
35-50	168722 (23.1)	
Pregnancy start year (%)		Section 3.2.1—more data from **recent years**
1990-1994	80403 (11.0)	
1995-1999	129860 (17.8)	
2000-2004	154684 (21.2)	
2005-2009	174720 (24.0)	
2010-2016	189254 (26.0)	
Maternal Hypertension (%)	48606 (6.7)	**Comparable.** NICE 2011: chronic hypertension 2%; gestational hypertension 4.2%- 7.9% during
Maternal Diabetes (%)	21149 (2.9)	**Comparable.** NICE 2015: 2–5% diabetes during pregnancy.[188]
Maternal Anxiety (%)	14608 (2.0)	**Mildly lower** than the prevalence of more serious cases. Systematic review: Generalised
Maternal Depression (%)	57567 (7.9)	**Mildly lower.** NICE 2014: 12.7%.[191] Avon Longitudinal Study using the Edinburgh Postnatal
Maternal Epilepsy (%)	4162 (0.6)	**Comparable.** RCOG 2016: 0.5-1% epilepsy during pregnancy[193]
Parity >=1 (%)	239225 (32.8)	Section 3.2.1—**more first-order** children
Multiple births (%)	20834 (2.9)	Section 3.2.1—**Comparable**.
Potentially fetal-damaging infection (%)	72660 (10.0)	**Comparable**. RCOG 2008: overall incidence of UTI: 8%[194]
Treatment of chronic medical conditions during pregnancy	89129 (12.2)	**Comparable.** 9%-15% using UK THIN database.
Female baby (%)	355008 (48.7)	**Comparable.**

*Proportion of maternal alcohol misuse, illicit drug use, tobacco use and obesity were calculated per mother. Maternal characteristics, except for age and start year, were measured using reported records. The conditions were assumed to be absence if not recorded.

Thesis Structure



Chapter 5 Maternal antibiotics prescribing during and prior to pregnancy

5.1 Background

5.1.1 Introduction

In Chapter 4, I began the ground work for conducting a cohort study to evaluate the association between macrolides exposure during pregnancy and adverse child outcomes. I developed the CPRD Mother Baby Cohort, the source population of the cohort study.

In this chapter, I explored whether the CPRD Mother Baby Cohort adequately captures the antibiotics (macrolides and penicillins) prescription during and prior to pregnancy in the general population, by evaluating the rates of antibiotic prescriptions among pregnant women. Prescriptions before pregnancy are also evaluated because they are used as the negative-control cohort (mentioned in Chapter 1).

I aim to understand the comparability (i.e. the distribution of potential confounders) between macrolides and penicillins groups. Among the confounders considered, the type of maternal infection could be an indication for macrolides or penicillins, thus is of special interest and will be explored. The remainder of this background provides a brief introduction on:

- Antibiotic prescribing for pregnant women and recording of antibiotic prescriptions in primary care; and
- Influencing factors, including infection type for antibiotics prescribing, maternal baseline characteristics and pregnancy-related factors, and how they may confound the relationship between antibiotic prescribing and child outcomes

5.1.2 Antibiotic prescribing in primary-care data

Oral antibiotics are the most frequently dispensed medications in pregnancy. One third of pregnant women received at least one antibiotic prescriptions during pregnancy in the UK.[9] The present pattern of routine antenatal care in the UK consists of one or two visits prior to 12 weeks of gestation, followed by further visits up to 41 weeks of gestation, provided by general practice and midwife as a shared care.[195] Complex cases are seen in hospital-based antenatal clinic. While antenatal care is often shared between general practice staff and midwives, the GP remains responsible for women's general medical care during pregnancy, including prescribing medicines due to the way pharmaceutical prescriptions are reimbursed in the UK.[196] There are exceptions, for example, women who previously had a baby affected by Group B Streptococcus (GBS) infection or with GBS colonisation during pregnancy, would normally be offered antibiotic

prophylaxis in labour.[197] Yet the vast majority of prescriptions before the intrapartum period are provided by general practice.[198]

Data of prescription event are well documented in CPRD, as they are generated within and automatically recorded into that patient's medical record while the prescription is issued. Prescriptions in CPRD are grouped by type according to the corresponding chapter, section, paragraph and sub-paragraph in the British National Formulary (BNF) and thus identification of antibiotic prescriptions is relatively straightforward. Though the quantity and product of prescription are automatically recorded, precise dose instructions and treatment duration are not always explicitly recorded.

5.1.3 Factors influencing antibiotic prescribing

Decisions about whom to treat and with which antibiotics are generally well established. Numerous guidelines for antibiotics prescribing are available for GPs, though none of the guidelines is specifically targeted at the pregnant woman. The safety information for pregnancy use is included in the BNF, which GPs use as a prescribing compendium. Evidence from systematic reviews provides strong evidence of maternal and fetal benefits of antibiotic treatment for a variety of conditions including gonorrhoea,[199] genital chlamydia,[200] syphilis,[201] pPROM,[202] UTIs,[203] asymptomatic bacteruria,[204,205] maternal GBS colonisation,[206] and lower genital tract infection.[207] According to a study using THIN 2013–2015, 50% of prescriptions could be linked to a clinical indication for antibiotics in the general population. Among them, 59% penicillins and 68% macrolides were prescribed for RTIs, while the corresponding proportions for urogenital tract infections were similar at 6% and 5%, respectively.[208] Another study based on THIN showed that penicillins seem more likely to be prescribed for urogenital infections during pregnancy as compared with macrolides (15% versus 5%).[9]

Antibiotic prescribing in the UK declined after reaching a peak in 1995 but remains constant in England, though the campaign of antimicrobial stewardship to decrease unnecessary use of antibiotics continues. In primary care practice, antibiotics are often prescribed for self-limiting and/or non-bacterial infections, and the unnecessary prescription may be driven by "a concern to meet patient expectations".[209] Population-based research has shown that maternal characteristics, such as young age and socio-economic deprivation, are associated with an increase in antibiotic prescribing.[9,210]

There is wide variation in antibiotic prescribing behaviour across general practices. A study based on THIN suggested that 47% of variation in prescription rates in the UK cannot be

explained by clinical risk factors (e.g. age distribution and prevalence of comorbidities). There was considerable between-practice variation associated with inappropriate prescribing and practice characteristics[211] (i.e. neighbourhood social-economic status, workload, local policy, and Influences of the pharmaceutical industry).[212] However, although the between-practice variance exist in "prescribing antibiotics or not", it was not clear whether there was also a between-practice variance in "prescribing macrolides or penicillins".

Indications and other factors influencing prescribing behaviour may potentially be related to adverse child outcomes. Understanding how penicillins and macrolides were prescribed will be critical to determine the appropriate analytical strategy for investigating associations between prenatal macrolides (versus penicillins) exposure and adverse child outcomes in the cohort study. Variation in penicillin and macrolide prescribing behaviour between general practices, if exists, also need to be considered to account for in order to evaluate the association above.

5.1.4 Objectives

This chapter aims to understand how penicillins and macrolides were prescribed during pregnancy. The understanding of antibiotic prescribing will inform the method of confounding control in the cohort study. Detailed objectives for this chapter are described below.

1. Explore patterns of antibiotic classes prescribed by deriving the prevalence by 1) calendar year and 2) month of exposure before and after Last menstrual period (LMP), according to 1) antibiotics class and 2) monotherapy or not (with combination or second-line prescription).
2. Explore patterns of infection types by deriving the prevalence of specific infection by 1) calendar year and 2) month of exposure before and after LMP.
3. Ascertain (a) the proportion of antibiotic prescriptions that are matched with a potential infection, by antibiotic class; and (b) the proportion of recorded infections that are matched with an antibiotic prescription, by infection type.
4. Identify other maternal risk factors (including a potential clustering in general practices) for antibiotic prescribing in pregnancy that might also potentially confound the association between antibiotic exposure (macrolides versus penicillins) and adverse child outcomes.

5.2 Methods

5.2.1 Population

Analyses in this chapter are conducted using the CPRD Mother Baby Cohort (described in Chapter 4), where the mothers' medical history from 1990 to 2015 was recorded. The cohort includes all livebirths registered at general practices within 6 month of birth and whose mother has a complete follow-up from 50 weeks before conception until delivery.

5.2.2 Variables of interest

5.2.2.1 Antibiotics

Antibiotic prescriptions were identified using 'Prodcode'. Women with one or more of these codes in their primary medical records were classified as exposed. I defined the antibiotics prescribed from 350 days to 1 day before the estimated last menstrual period (LMP) as "prescriptions before pregnancy", and antibiotics prescribed from the estimated LMP to the day before delivery as "prescriptions during pregnancy".

The prescription code list used to identify women prescribed antibiotics includes all antibiotics in Section 5.1 of the BNF. Those with topical formulations such as antibiotics used to treat acne (BNF paragraph 13.6.1) and conjunctivitis (BNF paragraph 11.3.1) are not included. The code list was developed using the mapping between BNF codes and "Prodcodes" provided by the R package "CALIBERcodelists".[213] I focused on five most common antibiotic classes according to BNF paragraph (i.e. headings under section), where antibiotic classes in other paragraphs were identified as "Others" (Table 5-1).

Table 5-1. Antibiotic classes and their corresponding BNF chapters.

Class	BNF Chapter
Penicillins	5.1.1: Penicillins
Cephalosporins	5.1.2: Cephalosporins, carbapenems, and other beta-lactams
Macrolides	5.1.5: Macrolides
Sulphonamides	5.1.8: Sulphonamides and trimethoprim
Nitrofurantoin	5.1.13: Urinary-tract infections
Others	Other paragraphs in 5.1: Tetracyclines, Aminoglycosides, Clindamycin, Some other antibacterials, Antituberculosis drugs, Antileprotic drugs, Metronidazole and tinidazole, and Quinolones.

I defined an antibiotic treatment as monotherapy if one or more consecutive prescriptions for a single antibiotic was separated by no more than 30 days, and were not interrupted, preceded or followed by prescriptions for other antibiotic drug substances or drug combinations within 30 days. Antibiotics not "monotherapy" therefore includes antibiotics prescribed as a drug

combination, as the second-line usage or with second-line usage. Table 5-2 shows examples for classification of “monotherapy” and “non-monotherapy” prescriptions.

Table 5-2. Examples of the classification of “Monotherapy” (assuming patient 999 has no other prescriptions in her record).

Patient ID	Date of prescription	Drug substance	Episode number	Monotherapy	Reason for ‘not monotherapy’
999	01/01/2010	Amoxicillin trihydrate	1	Yes	-
999	15/01/2010	Amoxicillin trihydrate	1	Yes	-
999	20/02/2010	Erythromycin	2	Yes	-
999	25/03/2010	Amoxicillin trihydrate	3	No	Drug combination
999	25/03/2010	Erythromycin	3	No	Drug combination
999	01/06/2010	Erythromycin	4	No	With second-line usage
999	18/06/2020	Erythromycin	4	No	With second-line usage
999	08/07/2020	Cefalexin	4	No	Second-line usage

5.2.2.2 Infections

Women who sought care from their GP for infections during pregnancy were identified using lists of Read codes for symptoms, diagnoses and tests. A woman was classified as exposed if she had one or more Read codes for infection.

I evaluated the comparability of the indications for macrolides and penicillins prescriptions in practice and its influence on the association between macrolide prescription in pregnancy and adverse child outcomes. I identified UTIs and STIs that are more likely to result in worse pregnancy outcomes.[203] Common infections were also identified by the target organ or system. In total, infections were classified as six types: RTIs, Genitourinary tract infections, STIs, gastrointestinal tract infections, head and neck infections, skin infections, and other infections. Systemic infections (e.g. sepsis) were included in the category of “other infections”. The common eye infection, conjunctivitis, was not included as it is mostly self-recovered or treated by topical antibiotics (instead of systematic use of antibiotics).

Code lists for each infection type were developed using published methods and reviewed by a clinician (RG).[214] Besides diagnoses (e.g. upper respiratory tract infection), a variety of codes can indicate a potential infection, including symptoms (e.g. cough), agent of infection (e.g. H1N1 swine flu), specific disease (e.g. Tuberculosis), positive culture results (e.g. urine culture-Bacteria OS), and specific antibiotic treatment (e.g. nitrofurantoin). The ten most common codes for each type of infection are listed in Appendix 5-1. Vomiting and nausea were not used as the indication for gastrointestinal infection as these symptoms are common during early pregnancy.

To avoid repeated recording, I classified infection episodes, instead of each record of infection. Infection episode was defined as one or more consecutive recordings for an infection type separated by no more than 30 days. If there was only one recording, an infection episode was assumed to last 7 days. For episodes with consecutive recording (within 30 days), the length of the episode was calculated as from the date of the first recording to the last recording of the episode plus 7 days. Each mother could have more than one episode.

5.2.2.3 Matching between antibiotic prescriptions and recorded infections

In CPRD, there is no structural entry areas for indications of each prescription. I linked antibiotic prescriptions and infection recordings using temporal criteria to evaluate (1) the constitution of infection type associated with an antibiotic class, and (2) the constitution of antibiotic classes associated with an infection type, before and during pregnancy in the CPRD mother-baby cohort.

Antibiotics prescribed within an infection episode were considered a matched antibiotic treatment (i.e. prescribing date on or after the date of the entry of infection record up to 6 days). When multiple infection episodes could be matched to one antibiotics, the infection that has the shortest time distance from the antibiotic prescription was selected. In other words, each antibiotic prescription was matched to a single infection at most, while an infection could be matched to multiple antibiotic prescriptions where plausible.

5.2.2.4 Potential risk factors for antibiotics prescribing

As discussed in Chapter 3, macrolides are widely used as alternatives for women with suspected allergy to penicillins. Therefore, comparison with penicillins could minimise confounding due to infection.[3] However, residual confounding may exist if macrolides were prescribed for specific indications (e.g. chlamydia). Also, there could be potential differences between mothers prescribed macrolides and penicillins during pregnancy, which could also be associated with adverse child outcomes. I therefore compared the distribution of a range of maternal and pregnancy-related risk factors between macrolides and penicillins: calendar year at delivery, maternal age at delivery, alcohol misuse, illicit drug use, smoking/tobacco use, obesity, hypertension, diabetes, epilepsy, depression, anxiety, maternal infection during pregnancy, treatment for chronic medical conditions during pregnancy, parity, and multiple birth. Details regarding these factors, including information on how these variables were derived is available in chapter 4.

5.2.3 Analysis

5.2.3.1 Antibiotic prescribing and infection recording

I performed the following descriptive analyses:

1. Antibiotics prescribing:
    a. over calendar year: 1) average number of prescriptions per pregnancy, 2) prevalence of pregnancies ever prescribed antibiotics and 3) proportions of each antibiotic classes by monotherapy or not;
    b. over number of months before and during pregnancy: 1) average number of prescriptions per pregnancy, 2) prevalence of pregnancies prescribed each antibiotic class and 3) proportions of each antibiotic classes by monotherapy or not;
    c. treatment duration (days) by antibiotic class.
2. Infection recording:
    a. over calendar year: 1) average number of infection episodes per pregnancy, 2) prevalence of pregnancies with infection and 3) proportions of each infection type;
    b. over number of months before and during pregnancy: 1) average number of infection episodes per pregnancy, 2) prevalence of pregnancies with infection and 3) proportions of each infection type.

5.2.3.2 Matching between antibiotic prescriptions and infection episodes

Following analyses were performed:

1. Proportions of antibiotic prescriptions unmatched to any infection episode, matched to any infection, and matched to each infection type, by antibiotic class.
2. Proportions of infections unmatched to any antibiotics, matched to any antibiotics, and matched to each antibiotic class, by infection type.

5.2.3.3 Differences between pregnancies prescribed macrolides and penicillins

To understand the differences between the mothers prescribed macrolides or penicillins (before or during pregnancy), I estimated 1) risk ratios (RRs) using log binomial regression models, to assess whether maternal and pregnancy-related factors were associated with the prescribing; and 2) standardised difference (std.diffs) in these factors between groups to evaluate how meaningful the difference is. Given the large sample size of the cohort, a null hypothesis (i.e. a factor is not associated with macrolides versus penicillin prescribing) may be rejected with a small between-group difference that is clinically meaningless. While the RR estimates provide

the information on effect size, I also calculated standardized differences (which is thus not affected by sample size) to illustrate the difference in means for each unit increase in pooled standard deviation. The standardized difference for a dichotomous factor between two groups is defined as:

$$standard\ difference = \frac{(\hat{p}_{mac} - \hat{p}_{pen})}{\sqrt{\frac{\hat{p}_{mac}(1-\hat{p}_{mac}) + \hat{p}_{pen}(1-\hat{p}_{pen})}{2}}},$$

where $\hat{p}_{mac}$ and $\hat{p}_{pen}$ denote the prevalence of the dichotomous factor in macrolides and penicillins group, respectively.[215] A standardized difference of 0.1 (10 per cent) is usually recognised as a meaningful difference in the factor.[215]

To understand how much of the overall variation in prescribing macrolides versus penicillins was explained by clustering of practices and mothers, I calculated the Intraclass Correlation Coefficient (ICC) for the clustering terms (practices or mothers) in empty random effect models on macrolides versus penicillins prescribing during pregnancy respectively. Clustering term with higher ICC would be accounted for in future models, by adopting robust standard errors for the RR estimates. To stay consistent with the cohort design, as mentioned beforehand, the analyses were performed within mothers who were prescribed one monotherapy of macrolides or penicillins, before or during pregnancy. To inform the comparability between the macrolides and penicillins groups, and to understand the characteristic of mothers prescribed antibiotics during pregnancy, I also compared the maternal and pregnancy-related characteristics between mothers prescribed macrolides and mothers not prescribed any antibiotics during pregnancy. The statistical analysis were performed using R Studio 1.2.1335.

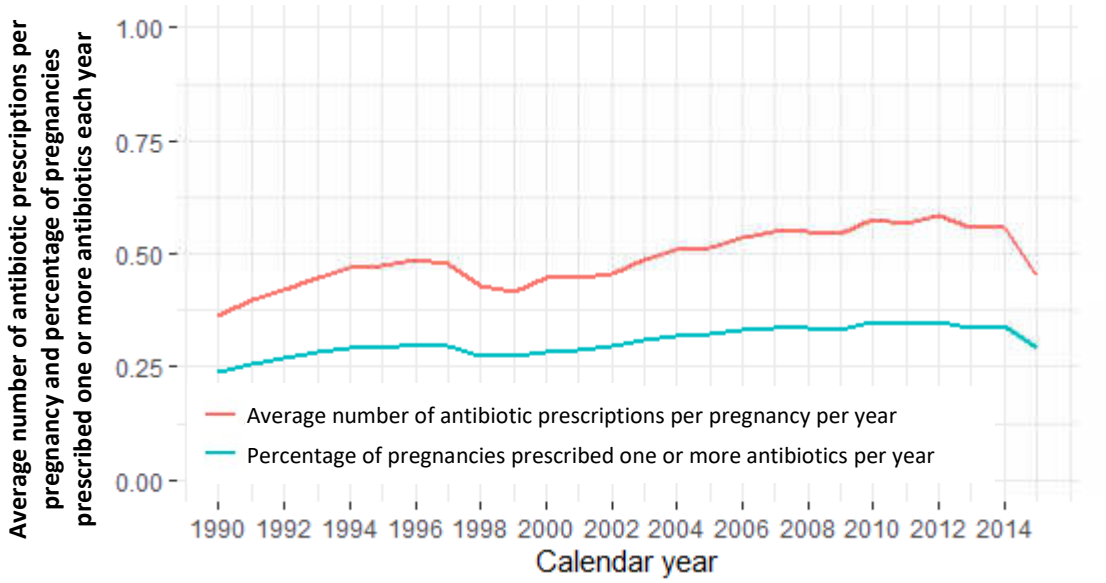
5.3 Results

5.3.1 Antibiotics prescribing

5.3.1.1 Overall proportions

Among the 718,400 pregnancies from 1990 to 2015 in the CPRD Mother Baby Cohort (728,921 children, Figure 4-1), a total of 357,675 antibiotic prescriptions were issued to mothers during pregnancy, with an average of 0.50 antibiotics per pregnancy. 31.0% of mothers were prescribed at least one antibiotic during pregnancy (Figure 5-1, Appendix 5-2).

Figure 5-1. Average number of antibiotic prescriptions per pregnancy per year, and percentage of pregnancies prescribed one or more antibiotics per year (denominator: number of pregnancies started this year) in the CPRD Mother Baby Cohort (1990-2015, n=718,400 pregnancies).[3]



Penicillins were the most frequently prescribed antibiotic in pregnancy (20.2% of pregnancies have prescribed at least one prescription for penicillin), followed by cephalosporins, carbapenems, or other beta-lactams (5.1%), macrolides (2.0%), sulphonamides and trimethroprim (1.3%) and nitrofurantoin (1.1%) (Appendix 5-2). Most antibiotics during pregnancy were prescribed as monotherapies (n = 285,059, 79.7%, Table 5-2). Compared with other antibiotic classes, penicillins were most likely to be prescribed as a first-line monotherapy (83.7%).

Table 5-2. Numbers (%) of first-line monotherapy during pregnancy in the CPRD Mother Baby Cohort (1990-2016, n=718,400 pregnancies).

Antibiotic classes	Monotherapy (%)	Drug combination or second-line usage (%)
Cephalosporins	49941 (74.38)	17199 (25.62)
Macrolides	18467 (70.61)	7685 (29.39)
Nitrofurantoin & Methenamine	9208 (68.07)	4319 (31.93)
Other	9802 (63.26)	5694 (36.74)
Penicillins	185283 (83.66)	36177 (16.34)
Sulphonamides	9577 (68.90)	4323 (31.10)

Most monotherapies had only one course of antibiotic prescription. Only 9% of monotherapies had more than one course of antibiotic prescriptions. Treatment duration was also calculated for monotherapy episodes (Table 5-3).

[3]Incidence calculated by pregnancy at any time of the year was lower than 31.0% which was calculated by pregnancy, as one pregnancy could be calculated twice in two years in the former incidence.

Table 5-3. Quartiles of treatment duration (days) for monotherapy episodes.

Class	Treatment duration (Q2 (Q1-Q3), day)
Cephalosporins	7 (5-7)
Macrolides	7 (7-7)
Nitrofurantoin & Methenamine	7 (5-7)
Others	7 (7-14)
Penicillins	7 (5-7)
Sulphonamides	5 (3-7)

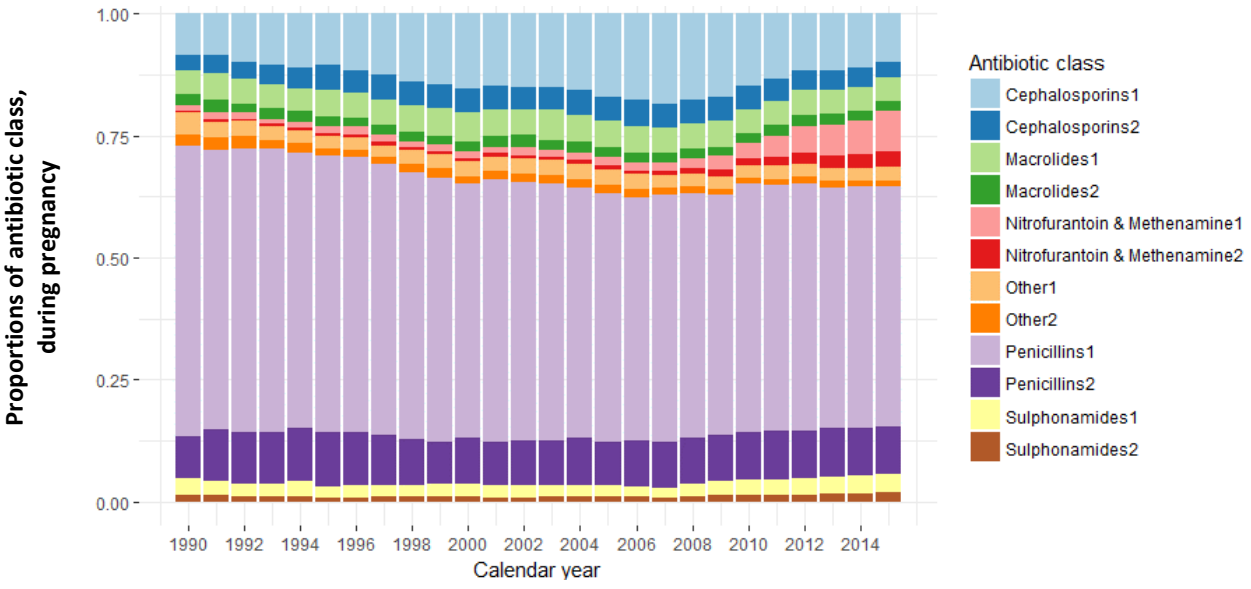
5.3.1.2 Trend over time

By calendar year

Both the proportion of pregnancies prescribed antibiotics and the number of prescription per pregnancy dropped around year 1999. While the number of prescriptions kept increasing afterwards, the proportion of women (pregnancies per se) prescribed any antibiotics during pregnancy remained relatively stable for the decade from 2005 to 2015 (Figure 5-1).

Composition of antibiotic classes change over the years. There was a steady decline in the proportion of penicillin in all antibiotics prescription from 1990 to 2015 (about 12%, Figure 5-2). Meanwhile, more cephalosporins were prescribed from 1990 to 2007 and more nitrofurantoin were prescribed after 2007, respectively (Figure 5-2).

Figure 5-2. Proportions of antibiotic classes prescribed during pregnancy, by calendar year (1 refers to "monotherapy" and 2 refers to "drug combinations or second-line usage") in the CPRD Mother Baby Cohort (1990-2015, n=718,400 pregnancies).



By month before and during pregnancy

Fewer mothers were prescribed antibiotics during the first three months of pregnancy, while the proportions of mothers prescribed antibiotics before pregnancy, and during mid- to late-pregnancy remained stable (Figure 5-3).

Figure 5-4 shows that more penicillins and cephalosporins, and fewer macrolides, nitrofurantoin, sulphonamides and other antibiotic classes were prescribed during pregnancy as compared with before pregnancy.

Figure 5-3. Average number of antibiotic prescriptions per pregnancy per month and percentage of pregnancies ever prescribed antibiotics per month, from 10 months before LMP to 9 months after LMP in the CPRD Mother Baby Cohort (1990-2015, n=718,400 pregnancies, denominator: women at the months before and after their LMP). Month 1: LMP to 30 gestational day (dashed line).

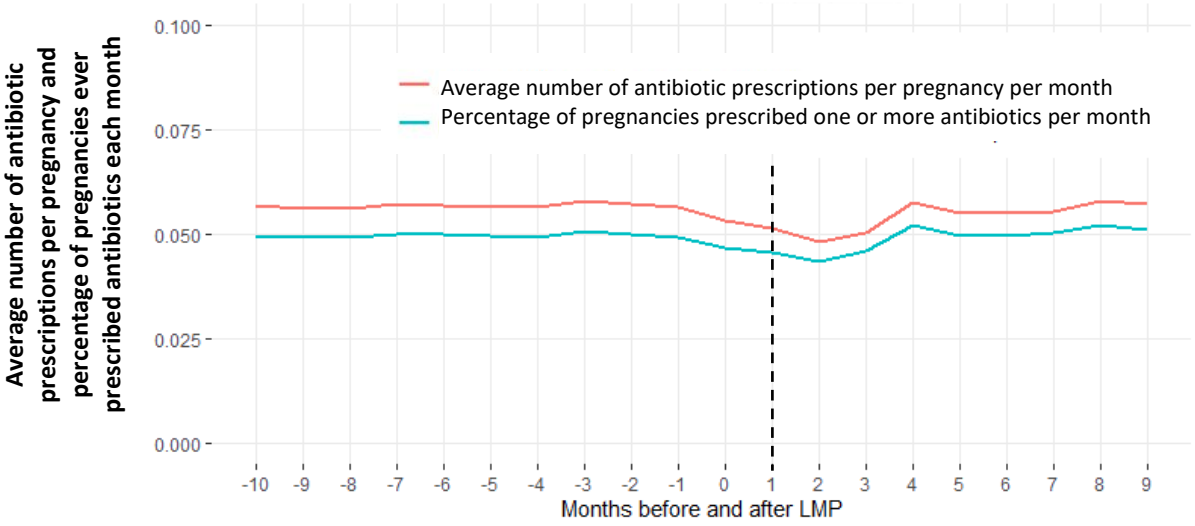
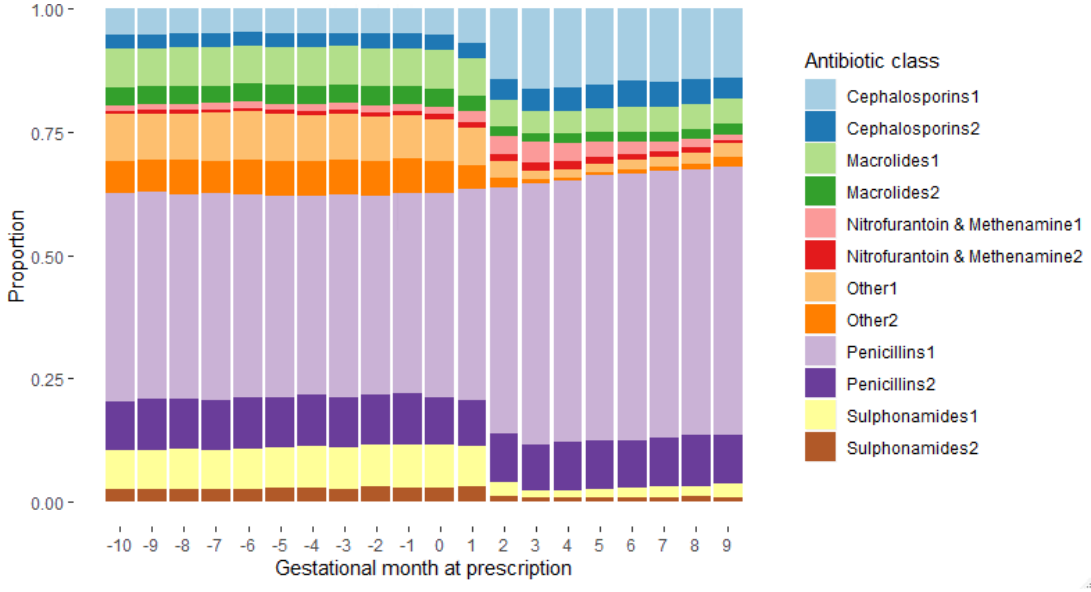


Figure 5-4. Proportions of antibiotic classes prescribed per month from 10 months before LMP to 9 months after LMP (1 refers to "monotherapy" and 2 refers to "drug combinations or second-line usage") in the CPRD Mother Baby Cohort (1990-2015, n=718,400 pregnancies). Month 1: LMP to 30 gestational day. Prescriptions at 11 months before LMP and at 10 months after LMP were trimmed.



5.3.2 Infection recording

5.3.2.1 Overall proportions

In total, 358,491 infection episodes (which could potentially be prescribed for systematic antibiotics) during pregnancy were recorded for 781,400 pregnancies in the cohort from 1990 to 2015. 36.1% of pregnancies had one or more infection episodes recorded. RTIs were the most common infections during pregnancy (22.6% pregnancies experienced at least one RTI during pregnancy), following by genitourinary tract infections (9.6%), gastrointestinal tract infections (4.8%), skin infections (5.1%), head and neck infections (2.4%), STIs (2.9%) and other infections (0.1%, including infection of central nervous system and infection of blood) (Appendix 5-4).

5.3.2.2 Trend over time

By calendar year

The trend of infection incidence during pregnancy over calendar years almost mirrors the shape with the trend in antibiotic prescriptions. The incidence of infection was lower between year 1998 and 2001 as compared with other years prior to and after this period. The composition of infection types during pregnancy stays stable throughout the years studied (Figure 5-5, Figure 5-6, Appendix 5-4).

By month before and during pregnancy

More infection episodes were recorded during pregnancy compared with before pregnancy. Compared with infections before pregnancy, there were more genitourinary tract infections during pregnancy (with an incidence of about 1.2% versus 0.7%) (Figure 5-7, Figure 5-8, Appendix 5-5).

Figure 5-5. Average number of infection episodes per pregnancy per year and percentage of pregnancies with one or more infections each year (denominator: number of pregnancies started this year) in the CPRD Mother Baby Cohort (1990-2015, n=718,400 pregnancies).

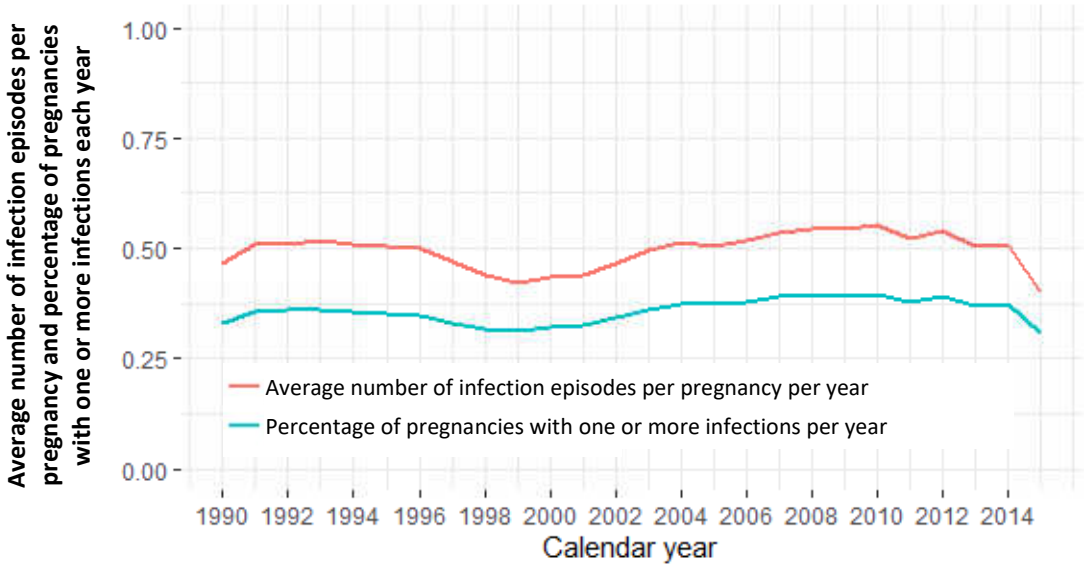


Figure 5-6. Proportions of infection types during pregnancy by calendar year in the CPRD Mother Baby Cohort (1990-2015, n=718,400 pregnancies).

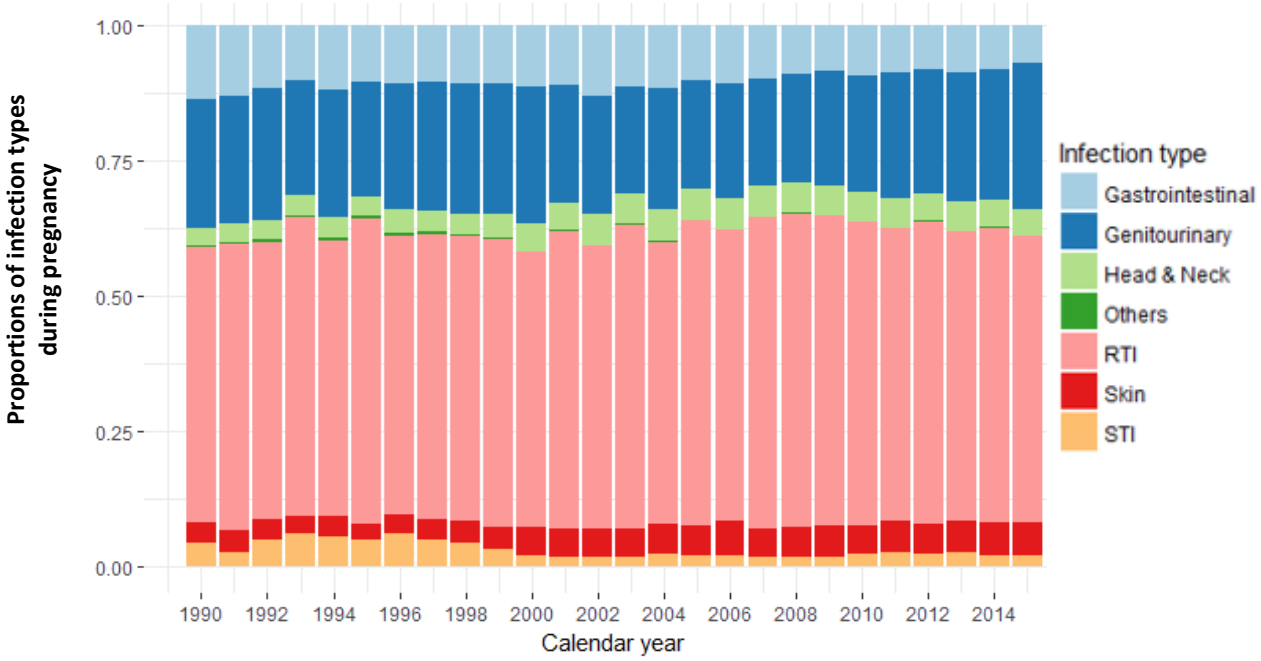


Figure 5-7. Average number of infection episodes per pregnancy per month and percentage of pregnancies with one or more infection episodes per month, from 10 months before LMP to 9 months after LMP (denominator: pregnancies in the month before and after LMP) in the CPRD Mother Baby Cohort (1990-2015, n=718,400 pregnancies. Month 1: LMP to 30 gestational day [dashed line]). Infections at 11 months before LMP and at 10 months after LMP were trimmed.

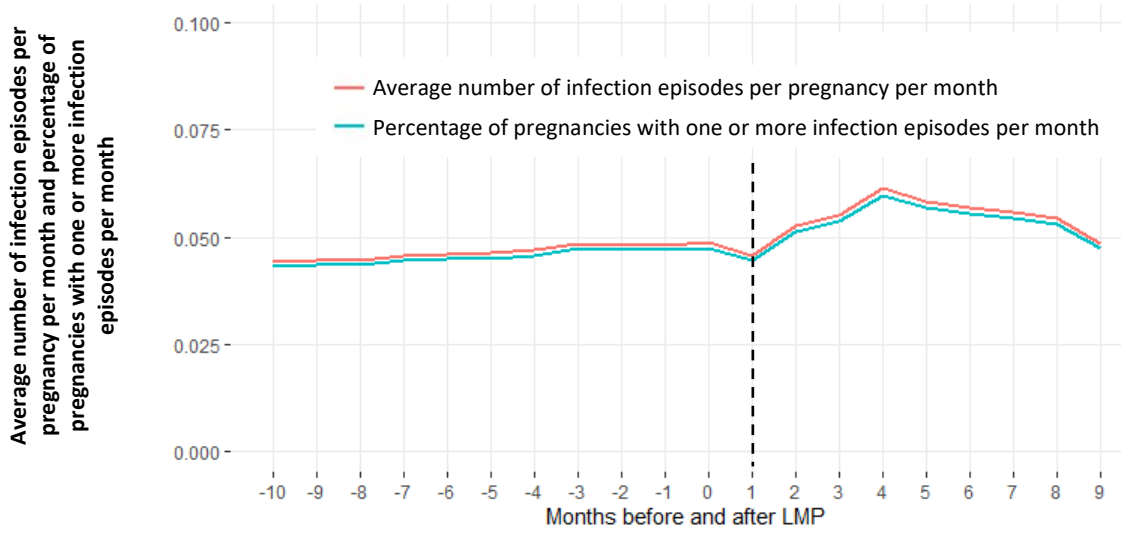
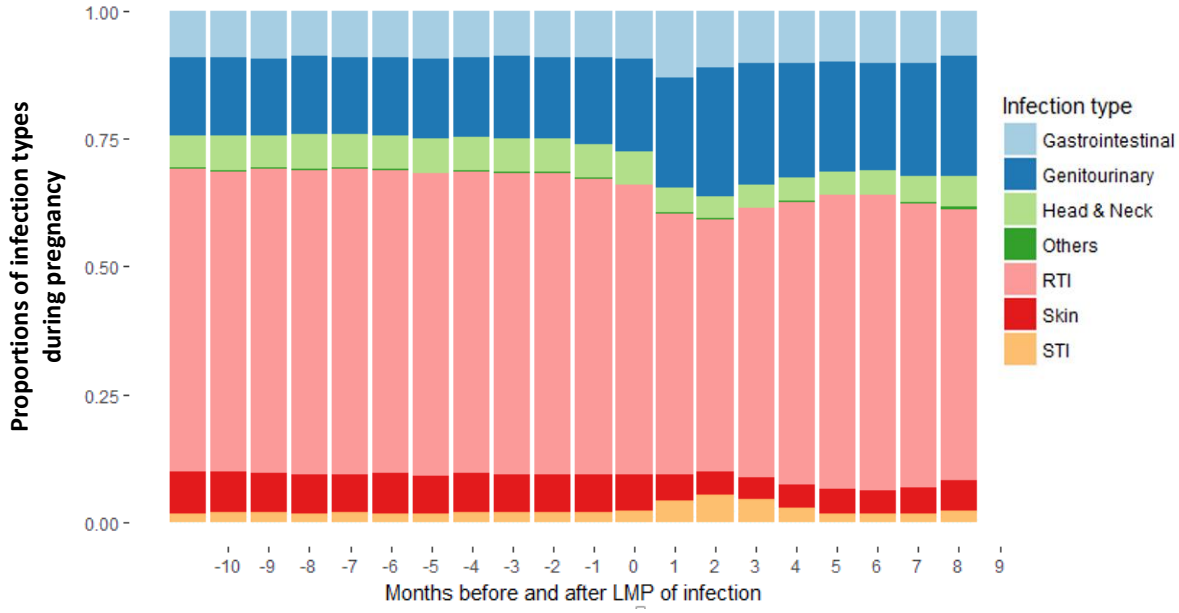


Figure 5-8. Proportions of infection types per month from 10 months before LMP to 9 months after LMP in the CPRD Mother Baby Cohort (1990-2015, n=718,400 pregnancies). Month 1: LMP to 30 gestational day. Infections at 11 months before LMP and at 10 months after LMP were trimmed.



5.3.3 Matching between antibiotics and infection episodes

Among the 718,400 pregnancies in CPRD Mother Baby Cohort, a total of 357,675 antibiotic prescriptions and 358,491 infection episodes were recorded during pregnancy from 1990 to 2015. It is possible to match 182,154 antibiotic prescriptions with an infection episode (recorded on or up to 6 days before the dates of the antibiotic prescription), which means 50.9% antibiotic prescription and 50.7%[4] infection episodes can be matched to an infection episodes or an antibiotic prescription (Table 5-4, Table 5-5).

For antibiotics and infections before pregnancy, there were 237,672 potential matches between infection episodes and antibiotic prescriptions, which were 50.2% of antibiotics prescribed (n=473,020) and 61.0% of infection episodes (n=389,322) recorded before pregnancy (Table 5-4, Table 5-5).

Almost all nitrofurantoin and other antibiotics in BNF chapter 5.1.13 were matched to potential genitourinary tract infection episodes (98.1% during pregnancy). This is expected because antibiotics in this chapter were exclusively prescribed for urinary tract infections, and thus all prescription of antibiotics in this chapter was identified as genitourinary tract infections automatically.[5] Penicillins (54% matched) and macrolides (53% matched) were more likely to be matched to potential infection episodes than other antibiotic classes (about 40% to 45%, Table 5-4).

RTIs were the most common indication for both penicillins and macrolides, which accounts for about three-quarters of all matched indications. It is worth noticing that the composition of infection types differed for penicillins prescriptions before and during pregnancy. For example, 5.8% penicillins prescriptions before pregnancy were matched to genitourinary tract infections, compared to 20.3% for prescriptions during pregnancy (Table 5-4).

Compared with penicillins, macrolides were less likely to be matched to genitourinary tract infections, especially during pregnancy (2.5% and 5.5% for macrolides prescriptions before and during pregnancy respectively). Other common indications for macrolides and penicillins include skin infections, head and neck infections (Table 5-4).

[4] The denominator for proportion of matched infection during pregnancy is 359,248, instead of 358,491 in appendix 5-4. Because when an infection episode before LMP was matched to antibiotic after LMP, the matching is counted as after LMP.

[5] The trivial unmatched Nitrofurantoin (about 2%) possibly appeared for prescriptions at 50 weeks before pregnancy where the previous medical history was not reviewed.

Table 5-4. Number of antibiotic prescription matched and unmatched with a potential infection. Proportions of antibiotics matched to individual infection types were calculated within prescriptions matched with an infection.

Antibiotic class	Before or during pregnancy	Antibiotic prescription matched with a potential infection								Unmatched antibiotic prescription
		Total	RTI	Genitourinary	STI	Skin	Head & Neck	Gastrointestinal	Others	
Macrolides	Before	27613 (50.94)	21461 (77.72)	689 (2.50)	473 (1.71)	2531 (9.17)	1939 (7.02)	471 (1.71)	49 (0.18)	26593 (49.06)
	During	13753 (52.59)	10327 (75.09)	762 (5.54)	354 (2.57)	1078 (7.84)	916 (6.66)	273 (1.99)	43 (0.31)	12399 (47.41)
Penicillins	Before	140871 (57.95)	107053 (75.99)	8124 (5.77)	154 (0.11)	14549 (10.33)	10128 (7.19)	581 (0.41)	282 (0.20)	102237 (42.05)
	During	118823 (53.65)	79034 (66.51)	24072 (20.26)	176 (0.15)	7920 (6.67)	6856 (5.77)	502 (0.42)	263 (0.22)	102637 (46.35)
Cephalosporins	Before	17381 (45.93)	7303 (42.02)	8488 (48.83)	22 (0.13)	496 (2.85)	873 (5.02)	169 (0.97)	30 (0.17)	20463 (54.07)
	During	26931 (40.11)	7225 (26.83)	18265 (67.82)	42 (0.16)	409 (1.52)	686 (2.55)	263 (0.98)	41 (0.15)	40209 (59.89)
Nitrofurantoin	Before	9787 (97.79)	20 (0.20)	9741 (99.53)	NA (NA)	<5 (-)	<5 (-)	18 (0.18)	NA (NA)	221 (2.21)
	During	13265 (98.06)	34 (0.26)	13179 (99.35)	<5 (-)	<5 (-)	- (-)	44 (0.33)	<5 (-)	262 (1.94)
Sulphonamides	Before	22896 (44.07)	2159 (9.43)	20119 (87.87)	48 (0.21)	103 (0.45)	235 (1.03)	222 (0.97)	10 (0.04)	29062 (55.93)
	During	5445 (39.17)	437 (8.03)	4879 (89.61)	- (-)	18 (0.33)	44 (0.81)	56 (1.03)	<5 (-)	8455 (60.83)
Other	Before	19124 (25.20)	9875 (51.64)	5422 (28.35)	818 (4.28)	1066 (5.57)	670 (3.50)	1152 (6.02)	121 (0.63)	56772 (74.80)
	During	3937 (25.41)	1035 (26.29)	2216 (56.29)	196 (4.98)	190 (4.83)	82 (2.08)	177 (4.50)	41 (1.04)	11559 (74.59)

*According to confidentiality preserving policy of Clinical Practice Research Datalink, "<5" is given when frequency cell contains less than five events and "-" is given to avoid deduction.

Table 5-5. Number of infection matched and unmatched with an antibiotic prescription. Proportions of infections matched to individual antibiotic class were calculated within infections matched to an antibiotic prescription.

Infection type	Before or during pregnancy	Infection matched with a potential antibiotic prescription							Unmatched infection
		Matched	Macrolides	Penicillins	Cephalosporins	Nitrofurantoin	Sulphonamides	Other	
RTI	Before	147871 (60.78)	21461 (14.51)	107053 (72.40)	7303 (4.94)	20 (0.01)	2159 (1.46)	9875 (6.68)	95427 (39.22)
	During	98092 (48.43)	10327 (10.53)	79034 (80.57)	7225 (7.37)	34 (0.03)	437 (0.45)	1035 (1.06)	104469 (51.57)
Genitourinary	Before	52583 (77.87)	689 (1.31)	8124 (15.45)	8488 (16.14)	9741 (18.52)	20119 (38.26)	5422 (10.31)	14946 (22.13)
	During	63373 (72.79)	762 (1.20)	24072 (37.98)	18265 (28.82)	13179 (20.80)	4879 (7.70)	2216 (3.50)	23693 (27.21)
STI	Before	1515 (21.05)	473 (31.22)	154 (10.17)	22 (1.45)	NA (NA)	48 (3.17)	818 (53.99)	5681 (78.95)
	During	780 (7.51)	354 (45.38)	176 (22.56)	42 (5.38)	<5 (-)	- (-)	196 (25.13)	9600 (92.49)
Skin	Before	18750 (58.84)	2531 (13.50)	14549 (77.59)	496 (2.65)	5 (0.03)	103 (0.55)	1066 (5.69)	13116 (41.16)
	During	9618 (49.82)	1078 (11.21)	7920 (82.35)	409 (4.25)	<5 (-)	- (-)	190 (1.98)	9686 (50.18)
Head & Neck	Before	13848 (51.23)	1939 (14.00)	10128 (73.14)	873 (6.30)	<5 (-)	- (-)	670 (4.84)	13181 (48.77)
	During	8584 (46.31)	916 (10.67)	6856 (79.87)	686 (7.99)	NA (NA)	44 (0.51)	82 (0.96)	9951 (53.69)
Gastrointestinal	Before	2613 (7.37)	471 (18.03)	581 (22.23)	169 (6.47)	18 (0.69)	222 (8.50)	1152 (44.09)	32838 (92.63)
	During	1315 (3.56)	273 (20.76)	502 (38.17)	263 (20.00)	44 (3.35)	56 (4.26)	177 (13.46)	35587 (96.44)
Others	Before	492 (52.28)	49 (9.96)	282 (57.32)	30 (6.10)	NA (NA)	10 (2.03)	121 (24.59)	449 (47.72)
	During	392 (44.70)	43 (10.97)	263 (67.09)	41 (10.46)	<5 (-)	<5 (-)	41 (10.46)	485 (55.30)

*According to confidentiality preserving policy of Clinical Practice Research Datalink, "<5" is given when frequency cell contains less than five events and "-" is given to avoid deduction.

The proportion of recorded infections that could be matched with antibiotics varied with the infection types. Genitourinary tract infections were most likely (72.8% during pregnancy) while gastrointestinal tract infections (3.6% during pregnancy) were least likely to be matched with a potential antibiotic treatment. Most RTIs during pregnancy (80.6%) were prescribed penicillins. For all genitourinary tract infections matched to antibiotics during pregnancy, 38.0% were prescribed penicillins and 28.9% prescribed cephalosporin, while only 1.2% were prescribed macrolides. Macrolides and penicillins were the two most common treatments matched to STIs.

5.3.4 Comparability between pregnancies prescribed macrolides and penicillins

A total of 9,719 pregnancies and 101,291 pregnancies were prescribed only one monotherapy of macrolides or penicillins during pregnancy in the CPRD Mother Baby Cohort, respectively. 14,820 and 93,003 pregnancies were prescribed one monotherapy of macrolides or penicillins from 50 weeks before pregnancy until LMP (Figure 5-9).

During pregnancy

The ICC for the clustering of "practices" and "mothers" was estimated to be 0.023 and 0.998 using empty random effect models, indicating that the between-practice variability in prescribing macrolides versus penicillins was negligible. Between-mothers variability accounts for the majority of total variability, which could be explained by the fact that 92% of the mothers had only one child included in the study cohorts (94,851 out of 103,353 mothers). Random effect models with within-mother clustering was thus not appropriate. Robust standard errors were instead applied to account for the clustering of siblings and multiple births within mothers in regression models in this chapter.

To better understanding the comparability between pregnancies prescribed macrolides and penicillins, I first compared pregnancies, where women were prescribed macrolides or no antibiotics. Compared with those without antibiotics, pregnant women prescribed macrolides (or penicillins) tended to be younger and in a more recent calendar year, have social or life style risk factors (drug use, tobacco use and obesity), have more clinical conditions during pregnancy (i.e. hypertension, anxiety, depression, genitourinary tract infections, STIs, treatment for chronic conditions), and previous births (Table 5-6).

Most of these differences were attenuated in analyses restricted to macrolides and penicillins. Although the associations of prescribing macrolides with maternal tobacco use, obesity and chronic treatment during pregnancy compared with mothers prescribing penicillins remained

significant, the strengths of these associations were modest. Maternal age was largely comparable between the two groups, although there were more mothers aged less than 20 years in the macrolides group than the penicillins group. Other measured maternal and pregnancy-related characteristics were comparable, including pregnancy start year, maternal alcohol misuse, drug use, maternal conditions including hypertension, diabetes, anxiety, depression and epilepsy, parity and multiple births. Nevertheless, a significant difference between macrolides and penicillins group was that fewer pregnancies in the macrolides group had a genitourinary tract infection recorded (4.1% vs 11.7%), and more had a STI recorded any time during pregnancy, compared with penicillins group (3.1% vs 1.3%).

Before pregnancy

Mothers prescribed macrolides before pregnancy experienced more instead of fewer genitourinary tract infections during pregnancy, as compared with penicillins group. Similarly, with associations in the cohort prescribed antibiotics during pregnancy, women prescribed macrolides before pregnancy were more likely to use tobacco, to be obese and to receive chronic treatment during pregnancy, though with less meaningful standardised differences (Table 5-6).

In summary, for pregnancies prescribed macrolides or penicillins during pregnancy, the most significant difference between the two groups was that macrolides prescribing was associated with a reduced prevalence of genitourinary tract infections and more STIs as compared with penicillins group. Macrolide prescribing was marginally associated with more maternal tobacco use and maternal obesity. For other factors, including maternal age and maternal conditions during pregnancy, mothers in the macrolides and penicillins group were comparable.

The maternal and pregnancy-related characteristics of pregnancies prescribed antibiotics during and before pregnancy were alike, except that mothers prescribed macrolides before pregnancy were slightly less likely to be teenager mothers and had experienced more instead of less genitourinary tract infections as compared with mothers prescribed penicillins.

Figure 5-9. Pregnancies prescribed only one monotherapy of macrolides before and during pregnancy.

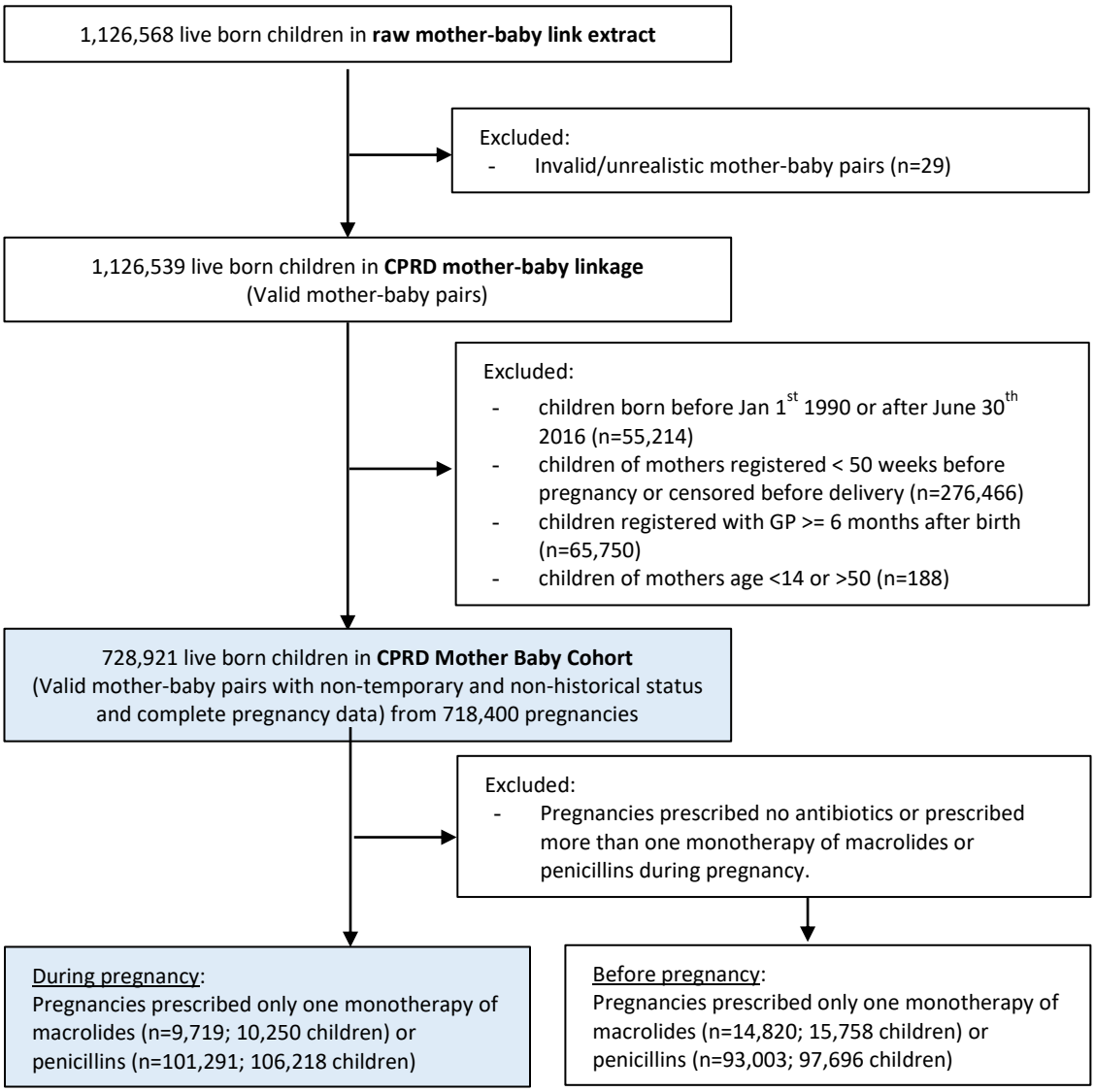


Table 5-6. Association of maternal and pregnancy factors with prescribing macrolides versus penicillins.

Maternal and pregnancy factors	During pregnancy							Before pregnancy			
				Mac VS Pen		Mac VS No-antibiotics					
	Macrolides number (%)	Penicillins number (%)	No antibiotics number (%)	Adj. RR [95% CI] for prescribing mac	Std.diff	Adj. RR [95% CI] for prescribing mac	Std.diff	Macrolides number (%)	Penicillins number (%)	Adj. RR [95% CI] for prescribing mac	Std. diff
Number of pregnancies	9719	101291	495100					14820	93003		
Length of follow-up in years*	17.2 (9.9-25.1)	18.0 (10.1-26.1)	18.0 (10.0-26.1)								
Maternal age at delivery in years					0.06		0.07				0.03
13-19	428 (4.4)	4197 (4.1)	17195 (3.5)	Reference		Reference		611 (4.1)	4110 (4.4)	Reference	
20-24	1369 (14.1)	14951 (14.8)	66059 (13.3)	0.90 [0.81, 1.00]		0.77 [0.69, 0.85]		2111 (14.2)	13599 (14.6)	1.05 [0.97, 1.15]	
25-29	2401 (24.7)	26839 (26.5)	129864 (26.2)	0.88 [0.80, 0.97]		0.71 [0.64, 0.79]		3853 (26.0)	24409 (26.2)	1.09 [1.01, 1.18]	
30-34	3121 (32.1)	31980 (31.6)	164559 (33.2)	0.95 [0.86, 1.04]		0.74 [0.67, 0.82]		4659 (31.4)	29624 (31.9)	1.10 [1.02, 1.20]	
35-50	2400 (24.7)	23324 (23.0)	117423 (23.7)	0.98 [0.88, 1.08]		0.77 [0.69, 0.85]		3586 (24.2)	21261 (22.9)	1.18 [1.08, 1.28]	
Pregnancy year at delivery					0.06		0.13				0.03
1990-1994	868 (8.9)	10346 (10.2)	58725 (11.9)	Reference		Reference		1369 (9.2)	8286 (8.9)	Reference	
1995-1999	1576 (16.2)	17791 (17.6)	90823 (18.3)	1.01 [0.94, 1.10]		1.08 [0.99, 1.17]		2483 (16.8)	16377 (17.6)	0.94 [0.89, 1.00]	
2000-2004	2085 (21.5)	20631 (20.4)	107958 (21.8)	1.12 [1.04, 1.21]		1.12 [1.03, 1.22]		3119 (21.0)	19573 (21.0)	0.98 [0.92, 1.04]	
2005-2009	2510 (25.8)	24873 (24.6)	115107 (23.2)	1.10 [1.02, 1.19]		1.23 [1.14, 1.33]		3768 (25.4)	23613 (25.4)	0.98 [0.93, 1.04]	
2010-2016	2680 (27.6)	27650 (27.3)	122487 (24.7)	1.04 [0.96, 1.12]		1.20 [1.10, 1.30]		4081 (27.5)	25154 (27.0)	1.00 [0.94, 1.06]	
Maternal alcohol misuse	488 (5.0)	4824 (4.8)	22907 (4.6)	1.01 [0.92, 1.10]	0.01	0.95 [0.87, 1.04]	0.02	739 (5.0)	4303 (4.6)	1.04 [0.98, 1.12]	0.02
Maternal illicit drug use	129 (1.3)	1042 (1.0)	3429 (0.7)	1.14 [0.97, 1.35]	0.03	1.24 [1.04, 1.47]	0.06	170 (1.1)	869 (0.9)	1.13 [0.99, 1.31]	0.02
Maternal obesity	1601 (16.5)	15098 (14.9)	55142 (11.1)	1.07 [1.01, 1.12]	0.04	1.34 [1.27, 1.41]	0.16	2414 (16.3)	14227 (15.3)	1.06 [1.02, 1.11]	0.03
Recent tobacco use	3320 (34.2)	32585 (32.2)	134338 (27.1)	1.07 [1.03, 1.11]	0.04	1.28 [1.23, 1.34]	0.15	4914 (33.2)	29821 (32.1)	1.04 [1.01, 1.07]	0.02
Hypertension during pregnancy	760 (7.8)	7357 (7.3)	30631 (6.2)	1.03 [0.96, 1.11]	0.02	1.09 [1.01, 1.17]	0.06	1098 (7.4)	6704 (7.2)	1.00 [0.94, 1.06]	0.01
Diabetes during pregnancy	358 (3.7)	3319 (3.3)	12651 (2.6)	1.03 [0.93, 1.14]	0.02	1.08 [0.97, 1.21]	0.07	483 (3.3)	3047 (3.3)	0.94 [0.86, 1.03]	0.00
Anxiety during pregnancy	297 (3.1)	2517 (2.5)	7141 (1.4)	1.11 [0.99, 1.24]	0.04	1.37 [1.22, 1.54]	0.11	382 (2.6)	2156 (2.3)	1.04 [0.94, 1.15]	0.02
Depression during pregnancy	1062 (10.9)	9813 (9.7)	30412 (6.1)	1.05 [0.99, 1.12]	0.04	1.35 [1.26, 1.44]	0.17	1424 (9.6)	8583 (9.2)	1.00 [0.95, 1.06]	0.01
Epilepsy during pregnancy	69 (0.7)	660 (0.7)	2426 (0.5)	1.01 [0.80, 1.26]	0.01	0.96 [0.76, 1.22]	0.03	92 (0.6)	584 (0.6)	0.94 [0.77, 1.14]	0.00
GUTIs during pregnancy	394 (4.1)	11808 (11.7)	6526 (1.3)	0.35 [0.31, 0.38]	0.29	2.86 [2.59, 3.16]	0.17	1477 (10.0)	8003 (8.6)	1.15 [1.09, 1.20]	0.05
STIs during pregnancy	304 (3.1)	1334 (1.3)	4999 (1.0)	2.23 [2.01, 2.47]	0.12	3.06 [2.74, 3.41]	0.15	227 (1.5)	1197 (1.3)	1.15 [1.02, 1.30]	0.02
Treatment of chronic medical conditions	1634 (16.8)	14703 (14.5)	47802 (9.7)	1.11 [1.05, 1.17]	0.06	1.54 [1.45, 1.63]	0.21	2214 (14.9)	12973 (13.9)	1.05 [1.00, 1.10]	0.03

Parity >=1	3520 (36.2)	36618 (36.2)	157628 (31.8)	0.97 [0.93, 1.01]	0.00	1.18 [1.13, 1.23]	0.09	5277 (35.6)	35447 (38.1)	0.90 [0.87, 0.93]	0.05
Multiple births	138 (1.4)	1357 (1.3)	7273 (1.5)	1.03 [0.88, 1.21]	0.01	1.00 [1.00, 1.00]	0.00	221 (1.5)	1241 (1.3)	1.08 [0.95, 1.22]	0.01
Intercept				0.09 [0.08, 0.10]		0.02 [0.02, 0.02]				0.13 [0.12, 0.14]	

*Q2(Q1-Q3). GUTIs: genitourinary tract infections. STIs: sexually transmitted infections. Mac: macrolides; Pen: penicillins; Std.diff: standardised difference.

5.4 Discussion

The overall objectives of this chapter was to explore the recording of antibiotic prescriptions and associated factors in primary care, to inform the comparability of pregnant women prescribed macrolides and penicillins, and thereby, the analyses of the association between child outcomes and macrolides versus penicillins. In this discussion, I compare the results with external data. In doing this, I show that antibiotic prescribing data recorded in UK primary care are comparable with studies from the UK (using different data sources) and around the world. I then summarise the strengths and limitations of this work. The chapter ends with a discussion of the implications of this work for the cohort study (Chapter 7).

5.4.1 Summary and comparison with external data

5.4.1.1 Antibiotics prescribing

Overall proportions

Nearly one-third (31%) of pregnancies in the CPRD Mother Baby Cohort were prescribed systematic antibiotics. This proportion was comparable with other studies from UK primary care. Two previous studies using the UK THIN database reported antibiotic prescriptions during pregnancy as 32.6% and 33% respectively.[9,56] The proportion of pregnancies prescribed antibiotics in north European countries (Norway, Sweden and Finland) was estimated to range from 24.1% to 35.2% based on dispensing data since 1990s.[56] Higher proportions of antibiotic prescribing during pregnancy were reported by studies from the United States (39.8%) and France (55.0%). Variance of the proportions between countries could be attributable to differences in antibiotics prescribing practices, the composition of care (primary care only or both primary care and secondary care), the composition of the cohort (all pregnancies versus only pregnancies resulting in live births), the definition of antibiotics (a board definition of anti-infectives or systemic antibacterials), and the types of data (prescribing versus dispensing).

The most common antibiotic class prescribed during pregnancy in this cohort was penicillins with an incidence of prescribing of 20.2% in pregnancies, which was consistent with other studies (proportion of pregnancy ranged from 15.6% to 23.2%).[9,56,210,216] A comparable incidence of pregnancies prescribed macrolides was observed between this study (2.0%) and previous studies using UK primary care databases (2-3%).[9,56]

Time trends

The proportion of pregnancy prescribed antibiotics declined around 1998 and remained relatively stable from 2005 to 2015, although the overall number of prescription (i.e. the average number of prescription per pregnancy) kept increasing slightly. Meanwhile, there was a steady decline in the proportion of penicillin in all antibiotic prescriptions from 1990 to 2015 (about 12%). Similar trends (declining proportions of antibiotics overall and of penicillins) have also been observed in general populations, probably due to the 1998 Department of Health report on antimicrobial resistance and the subsequent decrease in consultations and broad-spectrum antibiotic prescribing.[217] The recent increase in nitrofurantoin is in line with the recent changes of treatment guidelines, which now recommend nitrofurantoin as first-line therapy to treat UTI (previously mainly trimethoprim and cefalosporins).[218]

In terms of the trend over the months before and during pregnancy, fewer women were prescribed antibiotics during the first three months of pregnancy, while the numbers and incidence of antibiotic prescriptions after three months of pregnancy was similar to that before pregnancy. There was also an overall change in the antibiotics types: prescriptions seemed to shift from contraindicated or potentially harmful antibiotics (e.g. quinolone, tetracyclines, and sulfonamides) to relatively safe antibiotics during pregnancy (e.g. penicillins and cephalosporins). Similar findings were observed in many other studies in UK[9], Canada[219] and Germany[210].

5.4.1.2 Infection recording

Overall proportions

36.1% of pregnancies had at least one infection episode recorded. Among these, RTIs were the most common infection type (with an incidence of 22.6%), following by genitourinary infections with an incidence of 9.6% during pregnancy. Comparable evidence is scarce on recording of infections in primary care, particularly in pregnancy. One previous study using the THIN database reported that 46.7% women had at least one infection recorded during pregnancy from 1990 to 2010.[56] The lower incidence in my study could be explained by differences in the definition on infection. I excluded fungal infections that were rarely treated by antibiotics, and I regarded multiple recordings of the same infection within 7 days as one single infection episode, instead of individual infections. Nevertheless, the incidence of RTIs was comparable to the previous study that reported an incidence of 20.1%. The incidence of RTIs also compares to data in general population. NICE reported that a quarter of the general population in England and Wales will visit the GP for an RTI each year.[220] The incidence of genitourinary infection during pregnancy was on par with external data: incidence rates of 7.5% and 8.7% for urinary tract

infections were reported by studies using THIN and a nationally representative register of general practice in Netherlands, respectively.[221]

Time trends

The incidence and number of infections increased only slightly over time, and the composition of infections types remained largely unchanged. Compared with before pregnancy, there were more recordings of infection overall and genitourinary infections during pregnancy which peaked at around the 4th gestational month. This is consistent with women's increased contacts with GP and antenatal testing during pregnancy. The increased incidence of genitourinary tract infection could be due to the fact that some urinary infections such as cystitis are more common during pregnancy due to hormonal changes.[221]

5.4.1.3 Indications for antibiotics and antibiotics prescribing for infections

Identifying an infection indication was possible for half of all antibiotic prescriptions. The proportion matched (50%) is slightly lower compared with those from previous studies on pregnancy using THIN (54% to 56%), probably due to the narrower window of eligible matching in my study (7 days instead of 14 days).[9,56] A crude observation shows that matching between antibiotics and infections were consistent with prescribing guidelines. For example, antibiotics in BNF chapter 5.1.8 (Sulphonamides and trimethoprim) and 5.1.13 (Urinary-tract infections, including mainly nitrofurantoin and methenamine hippurate) were mainly matched with genitourinary tract infections. Some antibiotics cannot be matched to an indication, as GPs are not required to record the indication for antibiotic prescriptions.[222]

Although RTIs were the most common indications for both macrolides and penicillins, more penicillins were prescribed for genitourinary infections (20.3%) compared with macrolides (5.5%), as observed in another study using THIN.[9] This difference was less evident before pregnancy (5.8% of penicillins and 2.5% of macrolides were prescribed for genitourinary tract infections). In fact, as Table 5-5 showed, this could potentially explained by the trend that contraindicated or potentially harmful drugs for genitourinary infections were replaced by safer antibiotics drugs (penicillins and cephalosporins) during pregnancy.

Half of the infection episodes recorded in pregnancy were matched to antibiotic treatments. The seemed low matching rate reflects the fact that some infections may be not of bacteria-origin and thus not require antibiotic treatments.[223] Consistent with the previous studies of UK primary care in pregnancy and general population, high prescribing was observed for genitourinary tract infections.[9,223,224] The proportion of RTIs with antibiotics prescribed before

pregnancy in this study (60.8%) is comparable with a previous study using CPRD which reported proportions from 70.3% in 1997 to 60.7% in 2006 within general population.[225] Consistent with previous studies on antibiotic prescriptions during pregnancy, the proportions of infections linked to an antibiotic prescription were lower during pregnancy than before pregnancy.[210,221]

5.4.2 Comparability between mother prescribed macrolides and penicillins

5.4.2.1 Macrolides versus Penicillins: During pregnancy

Previously I assume that penicillin is a preferable comparator for macrolides, because macrolides are often prescribed as an alternative to penicillin in women with suspected penicillin allergy (Chapter 2). In this chapter, I evaluate the comparability between mothers prescribed macrolides and penicillins in detail, by comparing their indications, and maternal and pregnancy-related factors.

To summarise, mothers who were prescribed monotherapy macrolides and penicillins during pregnancy were largely comparable regarding the maternal characteristics measured in this study but differentiated in indications. Compared with mothers in the penicillins group:

1) Mothers in macrolides group were 65% less likely to have records of genitourinary tract infections during pregnancy. Specifically, based on indications matched to prescriptions, macrolides were less likely to be indicated for genitourinary tract infections (5.5% vs 20.2%), and more likely to be prescribed for RTIs (75.1% vs 66.5%), STIs (2.6% vs 0.2%), Skin infections (7.8% vs 6.7%), head and neck infections (6.7% vs 5.8%), and gastrointestinal infections (2.0% vs 0.4%).
2) Mothers in the macrolides group were more likely to have treatment for chronic conditions during pregnancy, and marginally more likely to use tobacco and to be obese.

5.4.2.2 During pregnancy versus before pregnancy

To evaluate the effect of unmeasured confounding between mothers prescribed macrolides and penicillin during pregnancy, I derived a cohort which included mothers who were prescribed monotherapy macrolides or penicillins before pregnancy. To reach this aim, it is expected that the difference between mothers prescribed macrolides and penicillins during pregnancy would be kept among mothers prescribed before pregnancy.

As expected, the differences between mothers prescribed macrolides and penicillins during pregnancy largely stayed among mothers prescribed before pregnancy. Mothers prescribed

macrolides before pregnancy were marginally more likely to have treatment for chronic conditions, to use tobacco and to have obesity compared with mothers prescribe penicillins. However, the proportion of pregnancies with genitourinary tract infections in macrolides group were higher than that in penicillins group, instead of lower within mothers prescribed during pregnancy.

No previous study has studied the factors associated with macrolides versus penicllins prescribing, although previous studies explored correlates of antibiotic prescribing (versus no antibiotics prescribing) during pregnancy. Consistent with this study, a UK THIN study found antibiotic prescribing was associated with a series of maternal characteristics, including obesity, use of illicit drugs, use of tobacco, alcohol misuse, chronic medical conditions, low social-economic status, and younger maternal age .[56] Another study using the UK THIN also reported increased antibiotics prescribing among mothers with young age (<20 versus 40+ years old, adjusted RR 1.33; 95% CI 1.24–1.42) and social deprivation (adjusted RR 1.25; 95% CI 1.21–1.30). Similarly, a study using German statutory insurance data showed that younger mothers, aged <21 years (adjusted OR 2.14; 95% CI 1.80–2.53) and being welfare recipient (adjusted OR 1.57; 95% CI 1.25–2.00), were associated with higher antibiotic use.[9,210]

5.4.3 Strengths and limitations

The strength of the CPRD database and CPRD Mother Baby Cohort have been discussed in Chapter 3. Strength of this particular study includes its large size (the previous largest similar study included 203,515 mother with singleton births), and the exploration of potential indications for each antibiotic prescription both during and before pregnancy. This study is also the first study that evaluated the antibiotic prescriptions during pregnancy as first-line monotherapies or drug combinations and second-line treatments.

As with other UK primary care databases, CPRD does not capture data on dispensing or whether the redeemed prescriptions were taken as instructed by the GP. Adherence to antibiotics prescribed in primary care is less well studied. A large survey led by the UK Department of Health reported that 11.3% British adults did not finish their last antibiotic course as prescribed by the GP.[226] However, the study was not focussed on pregnancy and pregnancy could make mothers either more or less likely to adhere to prescriptions instructed. A further problem of using prescription data is that the compliance may differ between macrolides and penicillins because the gastrointestinal side effects of erythromycin could reduce its compliance.

Because CPRD is a primary care database, antibiotics recorded outside primary care will be missed. Prophylactic antibiotics for Group B Streptococcus (GBS) are prescribed during labour in secondary care, with an estimated prevalence of 3.8% based on current recommendations in UK.[227] Other communities prescribing antibiotics outside primary care include dentist, out-of-hours prescribers and private practices. The English Surveillance Programme for Antimicrobial Utilisation and Resistance reported that only 6.2% of antibiotics were prescribed in other community settings (predominantly dentists) in the general population from 2010 to 2013.[198]

As a routine database collected for clinical management but not for research, CPRD does not capture all infections episodes, but only infections that presented to the GP. The infections seen and recorded in primary care are thus an underestimate of the true population burden. However, this underestimation may have limited influence on the cohort study of association in Chapter 7. To reduce potential indication bias in the cohort study, the goal is to capture potentially fetal damaging infection (genitourinary tract infections and STIs) during pregnancy, where the misclassification could be minimal. This is because genitourinary tract infections are perceived to be more dangerous by mothers and managed more aggressively in pregnancy than not in pregnancy, as has been shown by a previous study.[228] The validity of genitourinary tract infection recording was also reflected in this study, where genitourinary infection was observed to have the highest matching rate with an antibiotic prescription among the infection types. One limitation remains that data on infection severity is not well recorded in this routine health care database. Severity could potentially confound the association between antibiotic prescriptions and adverse child outcomes, and further sensitivity analysis is needed to evaluate its effect.

5.4.4 How this work informs my thesis

In this chapter, I have shown that data on antibiotics prescribing, indications and factors associated with prescribing during pregnancy in UK primary care database are comparable with those found in other studies from UK and other countries using different sources of data. This chapter also helps to inform the general comparability between the mothers prescribed macrolides and penicillins and differences in specific indications, thus provides valuable information regarding the design and analyses of the study in Chapter 7.

5.4.4.1 Comparability between mothers prescribed macrolides and penicillins

This chapter demonstrates that compared with mothers not prescribed antibiotics during pregnancy, mothers prescribed penicillins were more comparable to mothers prescribed macrolides during pregnancy, especially regarding factors such as maternal age, life style, and conditions during pregnancy. In terms of indications, though RTIs were the most common

indications for both macrolides and penicillins prescription, penicillins were more likely to be prescribed for genitourinary tract infections and less likely to be prescribed for STIs as compared with macrolides. These two infections, as well as other maternal and pregnancy factors warrants further investigations on their potential confounding effect for the association between macrolides use in pregnancy and adverse child outcomes.

5.4.4.2 Using negative control cohorts to evaluate the effect of unmeasured confounding

Potential unmeasured confounding could exist for the association between macrolide prescription in pregnancy and adverse child outcomes, for example, maternal social-economic status and genetic factors. This concern further demonstrates the necessity of building negative control cohorts to indirectly evaluate the effect of unmeasured confounders. If a harmful effect of macrolides was observed, not only for macrolides prescription during pregnancy, but also before pregnancy, it is reasonable to conclude that the observed harm was due to confounding between mothers prescribed macrolides and penicillins (e.g. social-economic status). A proper negative control cohort requires reproducing the condition (before pregnancy) that is likely to involve the same source of bias as in the original association (during pregnancy). The analysis in section 5.3.4 demonstrated that differences between the cohorts before pregnancy were similar during pregnancy, except that the direction of imbalance from genitourinary tract infection was inversed. Fortunately, this particular bias will overestimated (bias the RR away from 1) an expected null effect before pregnancy; therefore, the current negative control cohort is still proper (or even more convincing).

Another possible negative control cohort to evaluate the effect of unmeasured confounding is a sibling design. The risks of an adverse child outcome could be compared between siblings of children whose mother were prescribed macrolides during that pregnancy, and siblings of children whose mother were prescribed penicillins during that pregnancy (i.e. between siblings of children included in the two exposure groups in the main cohort study). This negative control cohort would provide an even stronger evidence for the effect of unmeasured confounding (as compared with the negative control cohort with prescriptions before pregnancy), because the children compared in the main cohort study and in the negative control cohort have the same mothers.

Therefore, for the cohort study in Chapter 7, two negative control cohorts are planned to evaluate unmeasured confounding: one among siblings of those children included in the main cohort study, another among macrolides versus penicillins prescribing before pregnancy.

5.4.4.3 Sensitivity analysis

As mentioned in Chapter 2, another method to deal with confounding is to restrict the analysis within mothers whose antibiotics were prescribed for RTIs. These mothers could be identified as those whose macrolides or penicillins were matched to an episode of RTI in this chapter. A sensitivity analysis stratified by number of antibiotics courses may be less informative, as most of the monotherapy macrolides and penicillins were prescribed for seven days, as was shown in Section 5.3.1..

5.5 Chapter appendix

Appendix 5-1. Common codes for identifying infection types.

Readco	Description	Count	Readcode	Description	Count
Gastrointestinal			**RTI**		
19F..11	Diarrhoea	15670	H05z.00	Upper respiratory	55082
19F..00	Diarrhoea symptoms	15532	171..00	Cough	53785
J521.11	Irritable bowel syndrome	12368	1C9..00	Sore throat symptom	45867
J43..11	Gastroenteritis	11717	H03..00	Acute tonsillitis	40303
19FZ.11	Diarrhoea & vomiting, symptom	6027	171..11	C/O - cough	37151
J155.00	Gastritis unspecified	2772	H05z.11	Upper respiratory tract	34463
19G..00	Diarrhoea and vomiting	2683	1C9..11	Throat soreness	26378
A07y00	Viral gastroenteritis	2664	H01..00	Acute sinusitis	25009
A083.11	Diarrhoea & vomiting -? infect	1980	H06z011	Chest infection	24669
19F2.00	Diarrhoea	1439	H01..11	Sinusitis	17380
Genitourinary			**Skin**		
K190z0	Urinary tract infection, site not	48173	M0...00	Skin and subcutaneous	6229
K15..00	Cystitis	36640	M05..00	Impetigo	5973
K190.00	Urinary tract infection, site not	33074	M01..00	Furuncle - boil	4869
-	Nitrofurantoin 50mg capsules	13002	AB0..11	Fungal infection of skin	3803
K421000	Vaginitis unspecified	4867	M07z.00	Local infection skin/subcut tissue NOS	3235
-	Nitrofurantoin 50mg tablets	4664	SP25500	Postoperative wound infection, unspecified	3021
K42190	Bacterial vaginitis	3302	M01z.00	Boil NOS	2965
-	Nitrofurantoin 100mg modified-release capsules	3006	M07z.11	Infected insect bite	2819
1AG..00	Recurrent urinary tract infections	2739	M0z..11	Infected sebaceous cyst	2810
K42191	Bacterial vaginosis	2576	M03z.00	Cellulitis and abscess NOS	2754
Head & Neck			**STI**		
F501.00	Infective otitis externa	12142	A56..00	Rubella	11257
F52z.00	Otitis media NOS	9087	4JK2200	HVS culture - trichomonas	5502
1C3..00	Earache symptoms	8677	A541.00	Genital herpes simplex	2185
F587.00	Otalgia	7663	A703.00	Viral (serum) hepatitis B	2083
F52..00	Suppurative and unspecified otitis	1908	A78A.00	Chlamydial infection	1078
F510.00	Acute non suppurative otitis	1682	A97..11	Syphilis	1074
F52z.11	Infection ear	1431	A7...00	Other viral and	585
F527.00	Acute right otitis media	1079	A7y..00	Other specified viral or chlamydial diseases	493
A76..00	Trachoma	1057	6794..00	Health ed. - rubella status	480
F526.00	Acute left otitis media	1011	K40y100	Female chlamydial pelvic	475
Others					
A....00	Infectious and parasitic diseases	1591			
A3BX20	Streptococc,group	217			
F011.00	Meningitis due to viral organisms	75			
A3BX70	Staphylococ	26			
F011z0	Meningitis - viral NOS	24			
N30200	Unspecified osteomyelitis of	15			
N010.1	Septic arthritis	14			
Az...00	Infectious and parasitic diseases	14			
F02..00	Meningitis of unspecified cause	14			
Q40400	Infectious granuloma	9			

Appendix 5-2. Numbers and prevalence (%) of antibiotics prescribed during pregnancy by calendar year (denominator: number of pregnancies started this year). The number 1 and 2 after each antibiotic class represent "monotherapy" and "drug combinations or second-line usage", respectively. Data was based on the CPRD Mother Baby Cohort (1990-2015, n=718,400 pregnancies).

Year	Total pregnancies	Total pregnancies prescribed AB	Total prescriptions	Cephalosporins1	Cephalosporins2	Macrolides1	Macrolides2	Nitrofurantoin1	Nitrofurantoin2	Other1	Other2	Penicillins1	Penicillins2	Sulphonamides1	Sulphonamides2
1990	15919	3783 (23.76)	5744 (36.08)	491 (3.08)	205 (1.29)	308 (1.93)	145 (0.91)	65 (0.41)	30 (0.19)	192 (1.21)	135 (0.85)	3363 (21.13)	571 (3.59)	164 (1.03)	75 (0.47)
1991	18044	4599 (25.49)	7208 (39.95)	646 (3.58)	265 (1.47)	361 (2.00)	149 (0.83)	108 (0.60)	40 (0.22)	228 (1.26)	190 (1.05)	4168 (23.10)	783 (4.34)	193 (1.07)	77 (0.43)
1992	19431	5201 (26.77)	8133 (41.86)	890 (4.58)	303 (1.56)	386 (1.99)	178 (0.92)	79 (0.41)	34 (0.17)	251 (1.29)	177 (0.91)	4662 (23.99)	866 (4.46)	204 (1.05)	103 (0.53)
1993	21282	6014 (28.26)	9557 (44.91)	1008 (4.74)	392 (1.84)	476 (2.24)	216 (1.01)	103 (0.48)	47 (0.22)	263 (1.24)	187 (0.88)	5505 (25.87)	977 (4.59)	284 (1.33)	99 (0.47)
1994	22386	6560 (29.30)	10510 (46.95)	1084 (4.84)	496 (2.22)	532 (2.38)	256 (1.14)	125 (0.56)	77 (0.34)	261 (1.17)	145 (0.65)	5988 (26.75)	1155 (5.16)	279 (1.25)	112 (0.50)
1995	23402	6839 (29.22)	11046 (47.20)	1240 (5.30)	533 (2.28)	560 (2.39)	206 (0.88)	143 (0.61)	69 (0.29)	259 (1.11)	155 (0.66)	6337 (27.08)	1187 (5.07)	272 (1.16)	85 (0.36)
1996	25912	7774 (30.00)	12598 (48.62)	1502 (5.80)	590 (2.28)	629 (2.43)	250 (0.96)	207 (0.80)	97 (0.37)	292 (1.13)	202 (0.78)	7071 (27.29)	1357 (5.24)	292 (1.13)	109 (0.42)
1997	27197	8055 (29.62)	12943 (47.59)	1745 (6.42)	685 (2.52)	649 (2.39)	294 (1.08)	161 (0.59)	102 (0.38)	289 (1.06)	201 (0.74)	7069 (25.99)	1305 (4.80)	308 (1.13)	135 (0.50)
1998	27680	7633 (27.58)	11875 (42.90)	1730 (6.25)	575 (2.08)	628 (2.27)	221 (0.80)	159 (0.57)	81 (0.29)	354 (1.28)	226 (0.82)	6410 (23.16)	1070 (3.87)	295 (1.07)	126 (0.46)
1999	28317	7696 (27.18)	11795 (41.65)	1737 (6.13)	564 (1.99)	715 (2.52)	215 (0.76)	148 (0.52)	63 (0.22)	344 (1.21)	189 (0.67)	6345 (22.41)	1013 (3.58)	340 (1.20)	122 (0.43)
2000	28982	8240 (28.43)	12906 (44.53)	1993 (6.88)	607 (2.09)	714 (2.46)	308 (1.06)	163 (0.56)	82 (0.28)	397 (1.37)	186 (0.64)	6869 (23.70)	1170 (4.04)	277 (0.96)	140 (0.48)
2001	29429	8440 (28.68)	13165 (44.73)	1997 (6.79)	616 (2.09)	693 (2.35)	294 (1.00)	186 (0.63)	110 (0.37)	387 (1.32)	211 (0.72)	6977 (23.71)	1209 (4.11)	360 (1.22)	125 (0.42)
2002	31472	9268 (29.45)	14393 (45.73)	2178 (6.92)	658 (2.09)	827 (2.63)	337 (1.07)	222 (0.71)	103 (0.33)	458 (1.46)	220 (0.70)	7592 (24.12)	1314 (4.18)	349 (1.11)	135 (0.43)
2003	32300	9985 (30.91)	15783 (48.86)	2322 (7.19)	765 (2.37)	904 (2.80)	344 (1.07)	234 (0.72)	121 (0.37)	498 (1.54)	277 (0.86)	8338 (25.81)	1446 (4.48)	365 (1.13)	169 (0.52)
2004	33446	10703 (32.00)	17002 (50.83)	2815 (8.42)	839 (2.51)	957 (2.86)	338 (1.01)	261 (0.78)	127 (0.38)	534 (1.60)	274 (0.82)	8667 (25.91)	1629 (4.87)	387 (1.16)	174 (0.52)
2005	34081	10968 (32.18)	17497 (51.34)	3057 (8.97)	877 (2.57)	907 (2.66)	327 (0.96)	283 (0.83)	134 (0.39)	567 (1.66)	303 (0.89)	8900 (26.11)	1568 (4.60)	404 (1.19)	170 (0.50)
2006	34372	11357 (33.04)	18370 (53.44)	3301 (9.60)	1000 (2.91)	991 (2.88)	384 (1.12)	303 (0.88)	148 (0.43)	495 (1.44)	306 (0.89)	9120 (26.53)	1740 (5.06)	400 (1.16)	182 (0.53)
2007	34956	11804 (33.77)	19167 (54.83)	3533 (10.11)	966 (2.76)	945 (2.70)	388 (1.11)	365 (1.04)	168 (0.48)	485 (1.39)	266 (0.76)	9681 (27.69)	1802 (5.16)	392 (1.12)	176 (0.50)
2008	34885	11767 (33.73)	19159 (54.92)	3397 (9.74)	950 (2.72)	984 (2.82)	395 (1.13)	434 (1.24)	218 (0.62)	495 (1.42)	260 (0.75)	9486 (27.19)	1792 (5.14)	514 (1.47)	234 (0.67)
2009	35180	11728 (33.34)	19102 (54.30)	3108 (8.83)	915 (2.60)	985 (2.80)	348 (0.99)	551 (1.57)	280 (0.80)	524 (1.49)	246 (0.70)	9530 (27.09)	1796 (5.11)	548 (1.56)	271 (0.77)
2010	34466	12057 (34.98)	19891 (57.71)	2844 (8.25)	923 (2.68)	980 (2.84)	413 (1.20)	640 (1.86)	343 (1.00)	435 (1.26)	279 (0.81)	10169 (29.50)	2002 (5.81)	593 (1.72)	270 (0.78)
2011	34071	11728 (34.42)	19340 (56.76)	2501 (7.34)	771 (2.26)	974 (2.86)	432 (1.27)	914 (2.68)	390 (1.14)	530 (1.56)	272 (0.80)	9697 (28.46)	1929 (5.66)	642 (1.88)	288 (0.85)
2012	30933	10773 (34.83)	18062 (58.39)	2044 (6.61)	732 (2.37)	903 (2.92)	450 (1.45)	1030 (3.33)	394 (1.27)	449 (1.45)	280 (0.91)	9097 (29.41)	1819 (5.88)	608 (1.97)	256 (0.83)
2013	27066	9128 (33.72)	15098 (55.78)	1729 (6.39)	597 (2.21)	766 (2.83)	328 (1.21)	1027 (3.79)	397 (1.47)	398 (1.47)	184 (0.68)	7344 (27.13)	1525 (5.63)	567 (2.09)	236 (0.87)
2014	22429	7600 (33.88)	12467 (55.58)	1298 (5.79)	467 (2.08)	635 (2.83)	228 (1.02)	943 (4.20)	361 (1.61)	365 (1.63)	162 (0.72)	6120 (27.29)	1189 (5.30)	452 (2.02)	247 (1.10)
2015	10762	3137 (29.15)	4864 (45.20)	504 (4.68)	155 (1.44)	226 (2.10)	73 (0.68)	496 (4.61)	161 (1.50)	155 (1.44)	58 (0.54)	2279 (21.18)	462 (4.29)	202 (1.88)	93 (0.86)
Total	718400	222837 (31.02)	357675 (49.79)	32817 (4.57)	3538 (0.49)	13105 (1.82)	1563 (0.22)	6571 (0.91)	971 (0.14)	7495 (1.04)	2099 (0.29)	133528 (18.59	11882 (1.65)	7497 (1.04)	1771 (0.25)

Appendix 5-3. Numbers and prevalence (%) of antibiotic prescriptions, by month before and after Last Menstrual Period (denominator: pregnancies at risk that month). The number 1 and 2 after each antibiotic class represent "monotherapy" and "drug combinations or second-line usage", respectively. Data was based on the CPRD Mother Baby Cohort (1990-2015, n=718,400 pregnancies).

Month	Total pregnancies	Total pregnancies prescribed AB	Total prescriptions	Cephalosporins1	Cephalosporins2	Macrolides1	Macrolides2	Nitrofurantoin1	Nitrofurantoin2	Other1	Other2	Penicillins1	Penicillins2	Sulphonamides1	Sulphonamides2
-10	718400	35792 (4.98)	40796 (5.68)	2195 (0.31)	1146 (0.16)	3199 (0.45)	1460 (0.20)	467 (0.07)	309 (0.04)	3882 (0.54)	2648 (0.37)	17203 (2.39)	4033 (0.56)	3156 (0.44)	1098 (0.15)
-9	718400	35522 (4.94)	40371 (5.62)	2124 (0.30)	1157 (0.16)	3116 (0.43)	1470 (0.20)	464 (0.06)	345 (0.05)	3773 (0.53)	2582 (0.36)	16950 (2.36)	4162 (0.58)	3220 (0.45)	1008 (0.14)
-8	718400	35326 (4.92)	40261 (5.60)	2081 (0.29)	1111 (0.15)	3169 (0.44)	1488 (0.21)	451 (0.06)	306 (0.04)	3774 (0.53)	2770 (0.39)	16735 (2.33)	4059 (0.57)	3272 (0.46)	1045 (0.15)
-7	718400	36021 (5.01)	41070 (5.72)	2065 (0.29)	1133 (0.16)	3324 (0.46)	1405 (0.20)	488 (0.07)	310 (0.04)	3954 (0.55)	2752 (0.38)	17145 (2.39)	4191 (0.58)	3243 (0.45)	1060 (0.15)
-6	718400	35846 (4.99)	40978 (5.70)	1996 (0.28)	1159 (0.16)	3099 (0.43)	1523 (0.21)	495 (0.07)	307 (0.04)	3976 (0.55)	2914 (0.41)	16842 (2.34)	4291 (0.60)	3311 (0.46)	1065 (0.15)
-5	718400	35618 (4.96)	40671 (5.66)	2034 (0.28)	1144 (0.16)	3176 (0.44)	1555 (0.22)	506 (0.07)	347 (0.05)	3847 (0.54)	2878 (0.40)	16538 (2.30)	4162 (0.58)	3350 (0.47)	1134 (0.16)
-4	718400	35461 (4.94)	40549 (5.64)	2027 (0.28)	1147 (0.16)	3237 (0.45)	1514 (0.21)	546 (0.08)	340 (0.05)	3758 (0.52)	2802 (0.39)	16370 (2.28)	4165 (0.58)	3509 (0.49)	1134 (0.16)
-3	718400	36400 (5.07)	41622 (5.79)	2074 (0.29)	1136 (0.16)	3221 (0.45)	1578 (0.22)	566 (0.08)	373 (0.05)	3787 (0.53)	2985 (0.42)	17068 (2.38)	4193 (0.58)	3525 (0.49)	1116 (0.16)
-2	718400	35888 (5.00)	41184 (5.73)	2117 (0.29)	1259 (0.18)	3179 (0.44)	1559 (0.22)	549 (0.08)	349 (0.05)	3726 (0.52)	2924 (0.41)	16517 (2.30)	4240 (0.59)	3505 (0.49)	1260 (0.18)
-1	718400	35454 (4.94)	40514 (5.64)	2064 (0.29)	1204 (0.17)	3098 (0.43)	1495 (0.21)	551 (0.08)	396 (0.06)	3526 (0.49)	2827 (0.39)	16451 (2.29)	4153 (0.58)	3555 (0.49)	1194 (0.17)
0	718400	33671 (4.69)	38319 (5.33)	2105 (0.29)	1138 (0.16)	3034 (0.42)	1346 (0.19)	610 (0.08)	422 (0.06)	3233 (0.45)	2467 (0.34)	15808 (2.20)	3703 (0.52)	3313 (0.46)	1140 (0.16)
1	718400	32758 (4.56)	36964 (5.15)	2648 (0.37)	1124 (0.16)	2794 (0.39)	1192 (0.17)	774 (0.11)	414 (0.06)	2777 (0.39)	1864 (0.26)	15762 (2.19)	3419 (0.48)	3066 (0.43)	1130 (0.16)
2	718400	31145 (4.34)	34502 (4.80)	4937 (0.69)	1515 (0.21)	1788 (0.25)	722 (0.10)	1236 (0.17)	494 (0.07)	1175 (0.16)	701 (0.10)	17169 (2.39)	3368 (0.47)	955 (0.13)	442 (0.06)
3	718400	33148 (4.61)	36311 (5.05)	5891 (0.82)	1734 (0.24)	1615 (0.22)	615 (0.09)	1486 (0.21)	586 (0.08)	681 (0.09)	314 (0.04)	19187 (2.67)	3376 (0.47)	543 (0.08)	283 (0.04)
4	718400	37495 (5.22)	41382 (5.76)	6597 (0.92)	2010 (0.28)	1910 (0.27)	772 (0.11)	1572 (0.22)	614 (0.09)	718 (0.10)	317 (0.04)	21854 (3.04)	4006 (0.56)	664 (0.09)	348 (0.05)
5	718400	35763 (4.98)	39493 (5.50)	6078 (0.85)	1946 (0.27)	1919 (0.27)	774 (0.11)	1155 (0.16)	553 (0.08)	700 (0.10)	280 (0.04)	21160 (2.95)	3888 (0.54)	696 (0.10)	344 (0.05)
6	718400	35717 (4.97)	39526 (5.50)	5839 (0.81)	2026 (0.28)	2042 (0.28)	788 (0.11)	1028 (0.14)	453 (0.06)	741 (0.10)	287 (0.04)	21387 (2.98)	3853 (0.54)	738 (0.10)	344 (0.05)
7	716165	35999 (5.03)	39769 (5.55)	5934 (0.83)	1973 (0.28)	2026 (0.28)	778 (0.11)	853 (0.12)	434 (0.06)	800 (0.11)	304 (0.04)	21545 (3.01)	3904 (0.55)	823 (0.11)	395 (0.06)
8	711115	37126 (5.22)	41272 (5.80)	5937 (0.83)	2080 (0.29)	2087 (0.29)	863 (0.12)	705 (0.10)	388 (0.05)	947 (0.13)	522 (0.07)	22150 (3.11)	4249 (0.60)	905 (0.13)	439 (0.06)
9	698813	35614 (5.10)	39941 (5.72)	5591 (0.80)	1724 (0.25)	2024 (0.29)	855 (0.12)	491 (0.07)	221 (0.03)	1102 (0.16)	798 (0.11)	21695 (3.10)	3984 (0.57)	1045 (0.15)	411 (0.06)

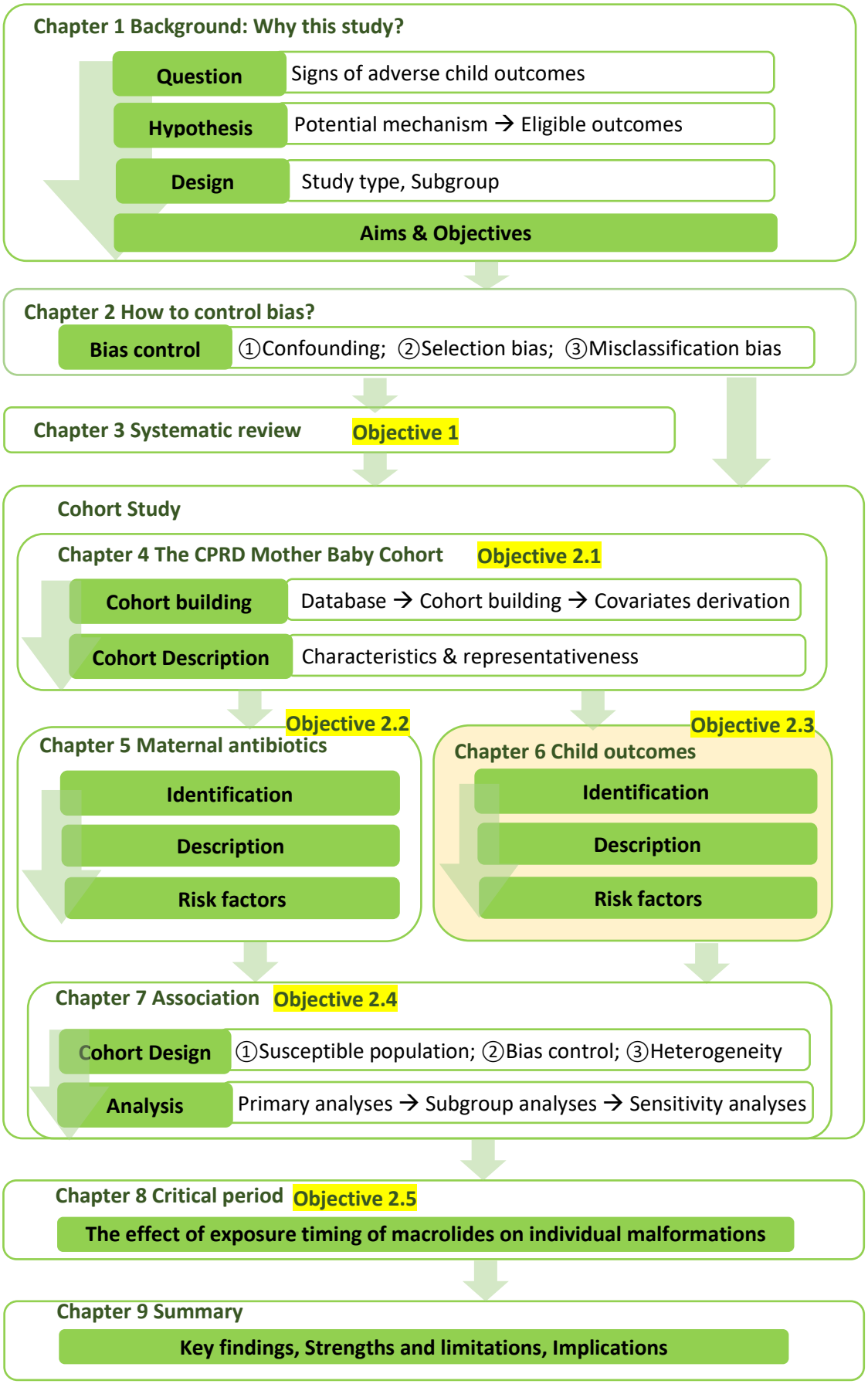
Appendix 5-4. Numbers and prevalence (%) of infection episodes during pregnancy by calendar year (denominator: pregnancies started this year). The counts for individual infection type was de-duplicated by pregnancy. Data was based on the CPRD Mother Baby Cohort (1990-2015, n=718,400 pregnancies).

Year	Total Pregnancies	Total pregnancies having infection	Total infections	Gastrointestinal	Genitourinary	Head & Neck	Others	RTI	Skin	STI
1990	15919	5285 (33.20)	7428 (46.66)	924 (5.80)	1766 (11.09)	283 (1.78)	19 (0.12)	3878 (24.36)	319 (2.00)	239 (1.50)
1991	18044	6450 (35.75)	9180 (50.88)	1178 (6.53)	2209 (12.24)	314 (1.74)	35 (0.19)	4845 (26.85)	343 (1.90)	256 (1.42)
1992	19431	6966 (35.85)	9882 (50.86)	1141 (5.87)	2380 (12.25)	327 (1.68)	36 (0.19)	5059 (26.04)	359 (1.85)	580 (2.98)
1993	21282	7662 (36.00)	10997 (51.67)	1217 (5.72)	2392 (11.24)	395 (1.86)	59 (0.28)	5946 (27.94)	362 (1.70)	626 (2.94)
1994	22386	7983 (35.66)	11418 (51.01)	1281 (5.72)	2528 (11.29)	410 (1.83)	52 (0.23)	6182 (27.62)	378 (1.69)	587 (2.62)
1995	23402	8200 (35.04)	11815 (50.49)	1266 (5.41)	2613 (11.17)	450 (1.92)	64 (0.27)	6370 (27.22)	385 (1.65)	667 (2.85)
1996	25912	9011 (34.78)	13002 (50.18)	1346 (5.19)	2981 (11.50)	517 (2.00)	59 (0.23)	6863 (26.49)	488 (1.88)	748 (2.89)
1997	27197	8937 (32.86)	12753 (46.89)	1401 (5.15)	3139 (11.54)	442 (1.63)	64 (0.24)	6614 (24.32)	488 (1.79)	605 (2.22)
1998	27680	8746 (31.60)	12119 (43.78)	1321 (4.77)	2931 (10.59)	468 (1.69)	45 (0.16)	6320 (22.83)	506 (1.83)	528 (1.91)
1999	28317	8816 (31.13)	11927 (42.12)	1280 (4.52)	2881 (10.17)	541 (1.91)	26 (0.09)	6304 (22.26)	562 (1.98)	333 (1.18)
2000	28982	9325 (32.18)	12586 (43.43)	1356 (4.68)	2928 (10.10)	616 (2.13)	21 (0.07)	6811 (23.50)	636 (2.19)	218 (0.75)
2001	29429	9553 (32.46)	12934 (43.95)	1536 (5.22)	2931 (9.96)	682 (2.32)	14 (0.05)	6845 (23.26)	699 (2.38)	227 (0.77)
2002	31472	10793 (34.29)	14595 (46.37)	1875 (5.96)	3034 (9.64)	879 (2.79)	15 (0.05)	7751 (24.63)	772 (2.45)	269 (0.85)
2003	32300	11675 (36.15)	16048 (49.68)	1843 (5.71)	3329 (10.31)	906 (2.80)	20 (0.06)	8757 (27.11)	875 (2.71)	318 (0.98)
2004	33446	12554 (37.54)	17161 (51.31)	1830 (5.47)	3620 (10.82)	985 (2.95)	32 (0.10)	9354 (27.97)	926 (2.77)	414 (1.24)
2005	34081	12735 (37.37)	17262 (50.65)	1837 (5.39)	3519 (10.33)	1003 (2.94)	17 (0.05)	9536 (27.98)	1027 (3.01)	323 (0.95)
2006	34372	13049 (37.96)	17820 (51.84)	1806 (5.25)	3603 (10.48)	1009 (2.94)	28 (0.08)	9902 (28.81)	1082 (3.15)	390 (1.13)
2007	34956	13657 (39.07)	18692 (53.47)	1959 (5.60)	3794 (10.85)	1092 (3.12)	26 (0.07)	10425 (29.82)	1055 (3.02)	341 (0.98)
2008	34885	13845 (39.69)	19028 (54.54)	1689 (4.84)	3921 (11.24)	1080 (3.10)	28 (0.08)	10838 (31.07)	1094 (3.14)	378 (1.08)
2009	35180	13815 (39.27)	19168 (54.49)	1754 (4.99)	4095 (11.64)	1016 (2.89)	47 (0.13)	10786 (30.66)	1073 (3.05)	397 (1.13)
2010	34466	13695 (39.73)	18995 (55.11)	1663 (4.83)	3958 (11.48)	1027 (2.98)	24 (0.07)	10761 (31.22)	1071 (3.11)	491 (1.42)
2011	34071	12841 (37.69)	17730 (52.04)	1529 (4.49)	4058 (11.91)	938 (2.75)	35 (0.10)	9731 (28.56)	966 (2.84)	473 (1.39)
2012	30933	12056 (38.97)	16655 (53.84)	1386 (4.48)	3848 (12.44)	817 (2.64)	25 (0.08)	9198 (29.74)	980 (3.17)	401 (1.30)
2013	27066	10008 (36.98)	13685 (50.56)	1153 (4.26)	3397 (12.55)	739 (2.73)	20 (0.07)	7182 (26.54)	832 (3.07)	362 (1.34)
2014	22429	8378 (37.35)	11419 (50.91)	929 (4.14)	2842 (12.67)	547 (2.44)	18 (0.08)	6187 (27.58)	664 (2.96)	232 (1.03)
2015	10762	3801 (35.32)	5161 (47.96)	360 (3.35)	1443 (13.41)	276 (2.56)	9 (0.08)	2637 (24.50)	310 (2.88)	126 (1.17)
Total	718400	259349 (36.10)	358602 (49.92)	34793 (4.84)	69069 (9.61)	16358 (2.28)	817 (0.11)	162617 (22.64)	17136 (2.39)	9943 (1.38)

Appendix 5-5. Numbers and prevalence (%) of infection episodes at month before and after Last Menstrual Period (denominator: pregnancies at risk that month). Data was based on the CPRD Mother Baby Cohort (1990-2015, n=718,400 pregnancies).

Month	Total Pregnancies	Total pregnancies having infection	Total infections	Gastrointestinal	Genitourinary	Head & Neck	Others	RTI	Skin	STI
-10	718400	31163 (4.34)	31970 (4.45)	2930 (0.41)	4853 (0.68)	1994 (0.28)	70 (0.01)	18940 (2.64)	2580 (0.36)	603 (0.08)
-9	718400	31384 (4.37)	32177 (4.48)	2963 (0.41)	4927 (0.69)	2094 (0.29)	68 (0.01)	18967 (2.64)	2544 (0.35)	614 (0.09)
-8	718400	31573 (4.39)	32326 (4.50)	2940 (0.41)	4891 (0.68)	2014 (0.28)	63 (0.01)	19328 (2.69)	2473 (0.34)	617 (0.09)
-7	718400	32163 (4.48)	33031 (4.60)	2930 (0.41)	5004 (0.70)	2244 (0.31)	75 (0.01)	19682 (2.74)	2509 (0.35)	587 (0.08)
-6	718400	32375 (4.51)	33235 (4.63)	2987 (0.42)	5009 (0.70)	2112 (0.29)	82 (0.01)	19983 (2.78)	2428 (0.34)	634 (0.09)
-5	718400	32490 (4.52)	33328 (4.64)	3019 (0.42)	5078 (0.71)	2193 (0.31)	75 (0.01)	19777 (2.75)	2572 (0.36)	614 (0.09)
-4	718400	32963 (4.59)	33779 (4.70)	3133 (0.44)	5270 (0.73)	2265 (0.32)	72 (0.01)	19911 (2.77)	2512 (0.35)	616 (0.09)
-3	718400	34030 (4.74)	34982 (4.87)	3106 (0.43)	5477 (0.76)	2303 (0.32)	90 (0.01)	20646 (2.87)	2658 (0.37)	702 (0.10)
-2	718400	33816 (4.71)	34714 (4.83)	3033 (0.42)	5567 (0.77)	2318 (0.32)	84 (0.01)	20533 (2.86)	2502 (0.35)	677 (0.09)
-1	718400	33744 (4.70)	34627 (4.82)	3076 (0.43)	5558 (0.77)	2268 (0.32)	72 (0.01)	20427 (2.84)	2571 (0.36)	655 (0.09)
0	718400	34153 (4.75)	35024 (4.88)	3154 (0.44)	5936 (0.83)	2292 (0.32)	75 (0.01)	20251 (2.82)	2618 (0.36)	698 (0.10)
1	718400	32030 (4.46)	32810 (4.57)	3055 (0.43)	6030 (0.84)	2065 (0.29)	66 (0.01)	18506 (2.58)	2321 (0.32)	767 (0.11)
2	718400	36856 (5.13)	37839 (5.27)	4940 (0.69)	8244 (1.15)	1802 (0.25)	71 (0.01)	19195 (2.67)	1886 (0.26)	1701 (0.24)
3	718400	38612 (5.37)	39631 (5.52)	4377 (0.61)	9891 (1.38)	1742 (0.24)	85 (0.01)	19675 (2.74)	1794 (0.25)	2067 (0.29)
4	718400	42813 (5.96)	44062 (6.13)	4468 (0.62)	10544 (1.47)	1936 (0.27)	59 (0.01)	23172 (3.23)	1928 (0.27)	1955 (0.27)
5	718400	40845 (5.69)	41806 (5.82)	4223 (0.59)	9406 (1.31)	1864 (0.26)	73 (0.01)	23227 (3.23)	1907 (0.27)	1106 (0.15)
6	718400	39937 (5.56)	40970 (5.70)	4077 (0.57)	8777 (1.22)	1843 (0.26)	79 (0.01)	23569 (3.28)	1932 (0.27)	693 (0.10)
7	716165	39113 (5.46)	40103 (5.60)	4041 (0.56)	8473 (1.18)	1868 (0.26)	84 (0.01)	23142 (3.23)	1880 (0.26)	615 (0.09)
8	711115	38019 (5.35)	39049 (5.49)	3921 (0.55)	8692 (1.22)	1961 (0.28)	139 (0.02)	21743 (3.06)	1938 (0.27)	655 (0.09)
9	698813	33395 (4.78)	34224 (4.90)	3013 (0.43)	8029 (1.15)	2069 (0.30)	138 (0.02)	18166 (2.60)	2066 (0.30)	743 (0.11)

Thesis Structure



Chapter 6 Adverse Children Outcomes in CPRD

6.1 Background

6.1.1 Introduction

In Chapter 5, I started the ground work for the cohort study to investigate the association between macrolides prescription during pregnancy and adverse child outcomes. I did this by describing the antibiotic prescriptions by classes, calendar year and month before and during pregnancy, and by exploring the underlying indications and other risk factors related with macrolides versus penicillin prescribing. In this chapter, I focus on evaluating the adverse child outcomes recorded in CPRD. Before performing the cohort study, I need to understand how these outcomes could be identified from the primary care database, and to demonstrate that these cases can be identified with a certain degree of accuracy. I also investigate the potential confounders to be able to control for them in the following association analyses in Chapter 7.

In Chapter 1, I specify the outcomes of interest in this study as those that could potentially result from short-term fetal hypoxia, including fetal death, major malformation and neurodevelopmental disorders (Table 1-3). While fetal death was studied in the systematic review (Chapter 3), fetal death will not be studied in the cohort study and in this chapter. Conducting a cohort study based on pregnancies instead of live births in CPRD would be difficult, since miscarriage is not well captured by CPRD.[56] Miscarriage may not be recorded in primary care records as miscarriage may occur before pregnancy booking with the GP. If miscarriage occurs after pregnancy booking, the GP may not be aware of the reason for not continuing pregnancy follow up. Furthermore, women may leave their GP-practice at any time during pregnancy (or shortly after delivery). In both these examples, information will be missing from the GP records on the outcome of that pregnancy (miscarriage, stillbirth and early infant death). A previous research using THIN suggested that 60% of the known pregnancies have unknown pregnancy outcomes.[56] A recent study using CPRD identified a rate of miscarriage that was more consistent with population figures, but the rate (12.4%) was calculated within pregnancies with known outcomes.[170]

This cohort is therefore restricted to live births that were registered with the same primary care practice as the mother. The adverse child outcome of interest, are those where short-term fetal hypoxia is know from clinical or animal studies to a contributory cause. Outcomes include: any major malformation, system-specific major malformations, cerebral palsy, epilepsy, ADHD and ASD.

6.1.2 Clinical follow-up of adverse child outcomes in CPRD in primary care settings

In the UK, primary care practice is ideally positioned for monitoring the care requirements of children with complex conditions such as congenital malformation. Primary care provides first-contact, longitudinal and comprehensive care, and refers children to specialist services.[229]

In chapter 2, I defined cerebral palsy (CP), epilepsy, ADHD and ASD as chronic neurological conditions requiring ongoing care and management. Although this care may often occur outside of primary-care, the GP is at the heart of the process; providing universal care, coordinating referrals, and prescribing treatment – all of which will be recorded in patients' medical records. Children with cerebral palsy receive specialist management via a local integrated multidisciplinary team, but the GPs is responsible for coordinating care, managing or referring to specialists and providing support to carers.[230] GPs are usually the first point of contact for common issues including problems with eating and drinking, concerns about speech, nutritional status, drooling, pain, sleep disturbances, gastro-oesophageal reflux disease and constipation, which are complications of cerebral palsy. Besides, subsequent prescription initiated by a specialist should be prescribed by GPs as part of a shared care arrangement.[230]

For children with epilepsy, the initial diagnosis and AED treatment starts in secondary-care according to NICE guidelines. Information about diagnosis will always be fed back to the GP via letters from the specialists. Due to restricted hospital prescribing budgets, all follow-on AED prescriptions are issued in primary care.[111] The GP contract from 2004 includes quality markers, and hence a financial incentive, for the management of epilepsy in primary care.[231]

In the UK, adolescents with ADHD are managed by child and adolescent mental health services or by paediatric services. The NICE guideline requires that the diagnosis and treatment should be initiated by specialists, and the GPs continue the treatment in addition to monitoring the effectiveness and adverse effects of treatment.[232] For some children with only moderate impairment to their ability to function socially and at school, ADHD can also be managed initially by GPs with self-help, simple behavioural management or parent support programmes.[232]

The NICE guideline requires the child or young person with suspected ASD to be referred to a multidisciplinary team who can confirm the diagnosis.[233] GPs are responsible for a routine review to ensure that the child with ASD and their family are coping well and receiving appropriate medical, educational, and social support.

In summary, children with major malformations and neurodevelopmental disorders need complex longitudinal care. Although the secondary care usually confirms the diagnoses and initiates the treatment, primary care stays as the centre of the management, by providing repeated prescriptions, referring to specialists and providing support to carers, to cope with the changing prognosis or care needs of children.

6.1.3 Recording of adverse child outcomes in CPRD

In UK general practice, health records are maintained as part of patient care using Read code and Prod code. While BNF codes can be explicitly mapped with Prod codes (the coding system for prescriptions in CPRD), one diagnosis could be recorded as one in dozens of potential correlated read codes. For example, glandular hypospadias could be recorded as "Hypospadias, glandular", "hypospadias, glanular", "Hypospadias", or "hooded penis". Recording habits may vary among practitioners, in terms of the particular Read codes they choose, their thoroughness, and timing of their recording. Therefore, specific code lists need to be developed for each outcome taking account of variation in coding to optimise sensitivity and specificity.

The coding cluster, or algorithm, used to define an adverse child outcome, needs to be tailored to the research question. My main measure of association will be relative risks (or hazard ratios) to compare outcome occurrence between children of mothers prescribed macrolides and penicillins. To minimise bias, I need the case identification to be specific, while sensitivity is less important. Low sensitivity does not bias the relative risk provided it is non-differential. However, low specificity often underestimates the relative risk due to a large number of false positive outcomes in the non-exposure group.[234] Intuitively, diagnosis would be the most specific index for case identification among various medical events (e.g. symptoms, diagnosis and treatment) recorded in primary care database. In some circumstances, GPs enter a Read code as a "working diagnosis" as the confirmation diagnoses of outcomes of interest often take place in secondary care and may be delayed. Despite these problems numerous validation studies have reporting high positive predictive values (PPVs) of diagnoses recorded in the CPRD for different outcomes.[165,166,235-237] For example, two studies that validated coding for major cardiovascular malformation, reported that 93% to 94% of the cases could be confirmed.[238]

A previous studies suggested that the diagnoses of neurodevelopmental disorders were under-recorded in primary care compared with prospective cohort studies.[239] The reason for the under-recording remains unclear. A possible reason is that GPs may be more likely to record symptom-related codes than specific diagnoses.[239] In this case, identification of children with

neurodevelopmental disorders in CPRD could benefit from using not only code for diagnoses, and codes for symptoms, treatment and management.

6.1.4 Specific Objectives

The main objective for this chapter was to identify and validate the indicators for each interested adverse child outcome in the CPRD Mother Baby Cohort. The findings of this work were perceived to inform the cohort study on the association between macrolides use during pregnancy and adverse child outcomes. The specific objectives were:

- Develop indicators for each adverse child outcome in CPRD.
- Validation of the identified outcomes:
    - Calculate the cumulative incidence, overall and specific to sex and birth year;
    - Compare the incidence estimates with existing evidence, and summarise previous validation studies of these outcomes.
- Identify maternal and pregnancy-related risk factors for adverse child outcomes that could potentially confound the association between prenatal macrolides (versus penicillins) prescribing and adverse child outcomes.

6.2 Methods

6.2.1 Study population

The study population includes all children and adolescents up to age 14 years who were included in the CPRD Mother Baby Cohort (n=728,921, described in Chapter 4), where the children's medical history from 1990 to 2016 was recorded. The cohort includes all livebirths registered at general practices within 6 month of birth and whose mother has a complete follow-up from 50 week before conception until delivery. Children were followed from birth to 14 years, death, or end of follow-up (June 2016), whichever came first.

6.2.2 Eligibility criteria for outcomes

6.2.2.1 Consideration on definition of adverse child outcomes

While the definition of neurodevelopmental disorders (cerebral palsy, epilepsy, ADHD and ASD) were explicit as previously mentioned, there were several ways to define major malformations. This cohort investigates the major malformations first as a group (any major malformation) and then as organ-system-specific malformations (e.g. cardiovascular malformation), instead of as individual malformation (e.g. ventricular septal defect).

To maintain high specificity and thereby, avoid dilution bias, the ideal definition for the malformations would be individual malformations for which the aetiological mechanism is known to involve fetal hypoxia. However, practical issues make this definition unfeasible. The major concern is the type II error, which is critical to drug safety studies. Definition of malformations according to specific aetiological mechanisms will end up with few cases in each group of malformations. This classification would thus lead to a major loss in statistical power caused by the sparsity of cases and discarded information. This definition would also make an investigation on patterns by trimester and macrolides subtype nearly implausible even though CPRD is one of the largest datasets for malformation aetiology studies in the world.

A compromise would be to identify homogenous categories to preserve internal homogeneity while maximising the statistical power. This leads to another challenge in categorising cases, due to incomplete knowledge on mechanisms. In addition, most non-chromosomal malformations have multifactorial unknown causes. It is often difficult to attribute a malformation to a single risk factor and exclude other mechanisms. Therefore, bias is also inevitable in practice. An equally important problem inherent to this method is the risk of a fishing expedition - meaning searching for significant associations. Phased analyses are needed as an endeavour to minimise fishing, by systematically pre-specify outcomes and analyses.

To define major malformations, I systematically applied the widely used classification approach, European Concerted Action of Congenital Anomalies and Twins (EUROCAT), with explicit classification and coding guidance for malformations. The EUROCAT approach groups together malformations that share aetiologic or clinical characteristics. There is a balance to be achieved a) between grouping together heterogeneous sets of anomalies and finely "splitting" b) between creating groups based on great precision of diagnosis and coding groups that take into account medical records in practice.[240] While less specific, this method reduces type II error, is plausible and avoids a fishing expedition.

I used the EUROCAT classification to exclude malformations with a specific known cause (other than fetal hypoxia). I excluded malformation due to maternal infections, fetal alcohol syndrome and chromosomal malformations from groupings for system-specific malformations and any major malformation. . Additionally, I excluded musculoskeletal malformations (e.g. club foot, knock-knee and hip dislocation), as they are not reliably recorded in GP records (Table 6-1).[241]

6.2.2.2 Power criteria

Among the outcomes of interest for the cohort study, I conducted further power calculations to include only outcomes for which there was enough statistical power to identify a potential risk of macrolide exposure. Specifically, I restricted the cohort study to outcomes for which there was >= 80% statistical power to detect a 2-fold relative risk increase at a 5% level of significance for macrolides exposure during the whole pregnancy.

Baseline risks of CP, epilepsy, ADHD, and ASD were calculated based on literature review and preliminary analyses. For malformations, I systematically applied the definition and classification of EUROCAT, while the prevalence of major malformations (any and system-specific) were based on the EUROCAT estimates.[242] I calculated the sample size for the macrolides group for each outcome, based on a cohort of about 10,000 pregnancies exposed to monotherapy macrolides (data from preliminary analyses Section 5.3.1). Therefore outcomes that required more than 10,000 samples in the macrolides group were excluded and will not be evaluated as system-specific malformations in this study (i.e. eye malformation, ear and face malformation, orofacial cleft, respiratory malformation, abdominal wall malformation and other malformation). Power calculations were performed using R package “pwr”. (Figure 6-1 and Table 6-1)

Finally, 10 specific outcomes were eligible for the cohort study, including any major malformation, nervous system malformation, cardiovascular malformation, gastrointestinal malformation, genital malformation, urinary malformation, cerebral palsy, epilepsy, ADHD and ASD (Table 6-1).

Figure 6-1. Sample size estimation for macrolides group, with n (penicillins) =100,000, Significant level=0.05, power=80%, risk ratio=2. Outcomes require more than 10,000 samples in macrolides group were excluded.

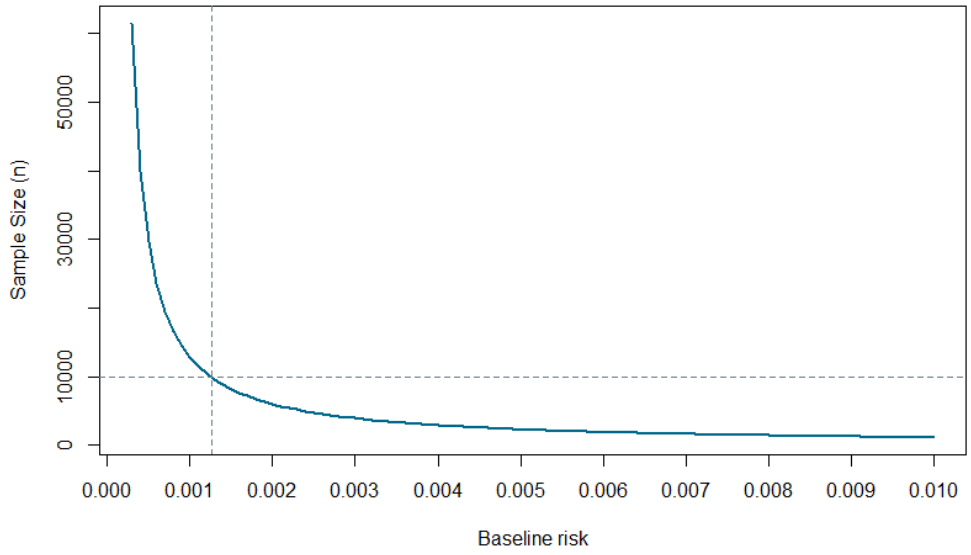


Table 6-1. Eligible outcomes in the cohort study.

No.	Outcome	Baseline Risk	Eligible		Reason for non-eligible
			as one outcome	as "any major malformation"	
1	Any major malformation	0.02	Yes	-	-
2	Cardiovascular malformation	0.007	Yes	Yes	-
3	Genital tract malformation	0.002	Yes	Yes	-
4	Urinary malformation	0.003	Yes	Yes	-
5	Gastrointestinal malformation	0.002	Yes	Yes	-
6	Nervous system malformation	0.002	Yes	Yes	-
-	Eye malformation	0.0003	No	Yes	Limited power
-	Ear and face malformation	0.0001	No	Yes	Limited power
-	Orofacial cleft	0.001	No	Yes	Limited power
-	Respiratory system malformation	0.0004	No	Yes	Limited power
-	Abdominal wall malformation	0.0005	No	Yes	Limited power
-	Other malformation	<0.001	No	Yes	Limited power
-	Musculoskeletal malformations	0.004	No	No	Unreliable recording in CPRD
-	All other malformation categories in EUROCAT	-	No	No	Known causes other than fetal hypoxia
7	Cerebral palsy	0.002	Yes	-	-
8	Epilepsy	0.006	Yes	-	-
9	ADHD	0.01	Yes	-	-
10	ASD	0.01	Yes	-	-

6.2.3 Case identification

6.2.3.1 Congenital major malformation

Previous validation studies have demonstrated that diagnostic codes of congenital malformation in CPRD are highly specific and associated with a high positive predicative value (PPV).[166,236,243] Besides the good validity, CPRD is also believed to serve as a more complete source of background prevalence of malformation diagnoses compared with national malformation registries (UK include the National Congenital Anomaly System (NCAS) and the EUROCAT).[166,243] Therefore, in this study, I use diagnostic codes as the index for malformation cases. For cleft lip and cleft palate, procedures were also used as alternative indicators due to the high specificity of such codes (e.g. "Repair of cleft palate").

Codes for major malformations were identified from a child's primary care records up to 14 years of age, using Read codes which were mapped to the tenth edition of the International Classification of Diseases (ICD–10) code lists provided by EUROCAT.[240] Minor malformations were excluded according to the EUROCAT coding guidance. EUROCAT revised its list of minor anomalies at 2005, and I applied the updated "Excluded minor anomalies post-2005" list in this study (Table 6-2).The mapping from ICD-10 code to Read code was performed using R package "CALIBERcodelists".

6.2.3.2 Cerebral palsy

Identification of cerebral palsy in CPRD was challenging and was achieved using an algorithm based on the random forest approach. Cerebral palsy is a complex condition that can manifest in different ways at different ages. Previous studies have shown that cerebral palsy is under-recorded in CPRD and THIN.[56,244] Nevertheless, treatment and symptoms coded in the database can also provide information specifically associated with diagnosis. Though neurological expertise could identify likely cerebral palsy cases by reviewing medical history of children based on the experience, this is unrealistic given the large size of data in my study. I therefore developed an algorithm that automatically predict likely cases which were then validated by clinical expert review. The detailed approach is included in Section 6.5. The cases were identified from child records up to 14 years old.

6.2.3.3 Epilepsy

I used a previously validated coding cluster to identify epilepsy. The incomplete recording of the epilepsy diagnosis in children has been reported by previous studies.[245] An explanation is that GPs may feel reluctant to label a child with epilepsy when the diagnosis is uncertain, given the complexity for diagnosis and the social stigma for people with epilepsy. While some medical conditions (e.g. generalized convulsive movements) can cause symptoms similar to epilepsy, AEDs are rarely used to treat children with conditions other than epilepsy in UK.[245] Previous validation studies have shown that multiple prescriptions of AEDs were associated with high specificity, and an algorithm to identify epilepsy using a combination of epilepsy diagnosis and repeated AEDs may best balance the sensitivity and specificity.[245,246] I therefore identified epilepsy cases using the criteria of one diagnosis or repeated AEDs prescription within 4 month (Table 6-2). The cases were identified from child records up to 14 years old.

6.2.3.4 ADHD

The diagnosis of ADHD is particularly challenging since the symptoms of ADHD differ both according to subtypes (inattentive or hyperactive) and in extent, as there is no simple test available to determine whether a child has ADHD or not.[232] For mild ADHD, GPs can initiate the management, and treatment with medication may not be needed. I was unable to find any validation study of diagnostic codes for ADHD in UK primary care database. I therefore devised a pragmatic case definition, which favoured high specificity for a condition that is very heterogeneous. My case definition required at least two occurrences of diagnosis and/or prescription within 4 month (two diagnoses, two prescriptions, or one diagnoses and one prescription). For cases where there was no prescription for an ADHD medication, the requirement of two or more diagnoses was used to avoid selecting for patients with only a provisional diagnosis recorded by the GP. In a sense, the criteria identifies patients at the more severe end of the spectrum of ADHD. A similar identification criterion has been applied in a previous study using CPRD, which reported a prevalence comparable to that from government-sponsored audit of ADHD services in Scotland (Table 6-2).[247] The cases were identified from child records up to 14 years old.

6.2.3.5 ASD

I identified ASD cases using diagnostic codes which have been validated by two previously studies by reviewing full photocopied medical records of children.[248,249] These studies also found consistent characteristics of patients compared with published studies in other populations of children with ASD, including the high proportion of males, age at diagnosis, maternal epilepsy, and nulliparity.[248,249] The quality and specificity of the diagnosis of autism in the GPRD have also

been validated based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria.[250,251] For the sensitivity of autism diagnoses in CPRD, while there was a study suggested an under-recording compared with screening studies, other studies proposed that studies based on screening questionnaires typically misidentify substantial numbers of children who have other difficulties but not ASD and contribute to over-diagnosis.[252] In this study, I used the diagnostic codes shown in table 6.2, up to 14 years of age, to identify children with ASD.

Table 6-2. Codes for identification of adverse child outcomes.

Outcome	Case identification*
Major congenital malformation	Any major system specific malformation according to the EUROCAT classification. I use Read code list mapped to ICD 10 codes Chapter Q. Exclude: 1) minor anomalies post-2005; 2) musculoskeletal malformations; 3) malformations caused by known chromosomal abnormalities and teratogens (i.e. Teratogenic syndromes with malformations, Fetal alcohol syndrome, Valproate syndrome, Maternal infections resulting in malformations, Genetic syndromes + microdeletions, Chromosomal malformations)
Nervous system	Read code mapped from ICD 10 (Q00-Q07, exclude Q0461, Q0782)
Cardiovascular	1 Read code mapped from ICD 10 (Q20-Q26, exclude Q2111, Q250 if GA <37 weeks, Q2541, Q256 if GA<37 weeks, Q261)
Gastrointestinal	Read code mapped from ICD 10 (Q38-Q45, Q790, exclude Q381, Q382, Q3850, Q400, Q401, Q4021, Q430, Q4320, Q4381, Q4382)
Genital	Read code mapped from ICD 10 (Q50-Q52, Q54-Q56, exclude Q523, Q525, Q527, Q5520, Q5521)
Urinary-renal	Read code mapped from ICD 10 (Q60-Q64, Q794, exclude Q610, Q627, Q633)
Cerebral palsy	Besides cases identified by >= 1 diagnostic code specifying cerebral palsy, I identified cerebral palsy cases from informative prescription or Read codes using the Random Forest approach and a logistic prediction model. Samples of cases were validated by a paediatric-neurologist (Dr. Finbar O'Callaghan) blind to prenatal antibiotics exposure.[253] (Section 6.5)
Epilepsy	2 prescriptions of antiepileptic drug (AED, identified based on British National Formula Chapter 4.8) within 4 months or >= 1 diagnosis [56]
Attention deficit hyperactivity disorder (ADHD)	>= 2 occurrence of prescriptions for ADHD (identified based on British National Formula Chapter 4.4) or diagnoses (attention deficit hyperactivity disorder, hyperkinetic disorders, hyperkinetic syndrome, hyperkinetic reaction of childhood or adolescence, overactive child syndrome and disturbance of activity and attention) within 4 month [254]
Autism spectrum disorder (ASD)	At least 1 diagnostic code ((infantile or childhood) autism, Asperger's syndrome, Rett's syndrome, Heller's syndrome, Autistic spectrum disorder, disintegrative disorder, and other pervasive developmental disorders) [255]

*The mapping from ICD 10 code to Read code was performed using R package "CALIBERcodelists". EUROCAT revised its list of minor anomalies at 2005, and I applied the updated "Excluded minor anomalies post-2005" list in this study.

6.2.4 Validation

6.2.4.1 Estimating recorded incidence

I estimated the cumulative incidence of newly recorded outcomes at ages one, five, and fourteen. Kaplan-Meier (KM) failure curves were generated using the CPRD Mother Baby Cohort

to estimate the cumulative incidence risk of the 10 adverse child outcomes by year of age with 95% confidence intervals (CI). Year of birth effects were assessed by plotting KM failure curves for children born in four five-year and one 7-year birth groups (1990-1994, 1995-1999, 2000-2004, 2005-2009 and 2010-2016). The cumulative incidences were also estimated using sub-cohort by sex. The log-rank test was used to test the equality of failure function estimates between the five birth year groups, and between male and female.

To externally validate the outcome indicators, I compare the estimated incidence with estimates from prospective cohorts, surveys and registries where robust case definitions and multisource ascertainment were applied. I also compared the estimated incidence in this study with other studies using administrative database, including databases from the UK and from other countries.

6.2.4.2 Associations between outcomes and potential risk factors

To further evaluate the validity of the outcomes, I tested for the associations between each outcome and potential risk factors. I chose risk factors which could realistically be identified using primary-care data. The risk factors included those have been tested for associations with maternal macrolides versus penicillins prescription in Chapter 5, including maternal age, pregnancy calendar year, life style factors (drug use, obesity and recent tobacco use), maternal hypertension, anxiety, depression, epilepsy, and conditions during pregnancy (genitourinary tract infections, sexually transmitted infections (STIs), and treatment for chronic conditions), and previous births. I also test the associations of outcomes with neonatal factors, including sex of baby, multiple births status (singleton or multiple births), and gestational age. These neonatal factors were known to be associated with most adverse child outcomes interested in the cohort study. I tested these factors to compare the direction and magnitude of the association with evidence from previous literature. Maternal age, pregnancy calendar year and gestational age were multi-categorical in the model, and all other factors were binary (see Section 4.4).

For malformations, I calculated absolute risks (per 1,000 children) and risk ratios (RR) with 95% confidence intervals (CI) using log-binomial models. For neurodevelopmental disorders where the follow-up time was censored, absolute rates (per 1,000 Person Year) and hazard ratios (HR) with 95% CIs were estimated using Cox proportional hazard models. Standard differences between cases and non-cases were calculated, and multivariate models were conducted. I tested the proportional hazard assumption using Schoenfeld residuals after all Cox proportional hazards models. The outcome for Cox proportional hazard model was time from birth to the first new recording of each neurodevelopmental disorder, with right-censoring occurring among

children who were event-free at the end of follow-up time. Robust standard errors were applied to account for the clustering of siblings and multiple births within mothers in the regression models.

6.3 Results

6.3.1 Cumulative incidence risk

6.3.1.1 Major malformation

In total, 14,365 (22.4‰) children in the CPRD Mother Baby Cohort born between 1990 and 2016 were identified with major malformation by age 14. The cumulative incidence risks by age, gender and birth year are shown in Table 6-3. According to the cumulative incidence curves (Figure 6-2), most major malformations were diagnosed by the age of 3 years and the cumulative incidence began to plateau thereafter. 13,162 major malformations were identified by age 3 years, which accounted for 92% of major malformations identified by age of 14. There was a significant difference between the incidence curves for the five birth-year periods (Figure 6-2). The probability of a malformation diagnosis at any time point increased in more recent years.

Among the system-specific malformation, the most common ones were cardiovascular malformations (a maximum cumulative incidence of 8.4 (95% CI 8.1-8.6) per 1,000 children, accounting for 36.6% of all major malformation) and genital tract malformations (a maximum cumulative incidence of 3.9 (95% CI 3.7-4.0) per 1,000 children, accounting for 17.9% of all major malformation, followed by urinary malformation, gastrointestinal malformation and nervous system malformation. The trend of diagnosis mostly by age 3 was also observed for each of the system-specific malformation. Significantly increasing incidence by study years was observed for cardiovascular malformations, genital tract malformations and gastrointestinal malformation. The cumulative incidences for rarer malformations, nervous system malformation and urinary malformation, were not found to be significantly different among the birth-year periods. While the incidence of cardiovascular, nervous system and gastrointestinal malformations were comparable between boys and girls, genital tract malformation was found mostly among boys. The effect of sex will be evaluated further in Chapter 7 (Table 6-3).

Table 6-3. Cumulative incidence for adverse child outcomes by age, sex and year of birth (per 1,000 children).

Any major malformation		No. Cases	Cum Incidence		
			by age 1	by age 7	by age 14
Total		14365	16.9 (16.6-17.2)	20.6 (20.2-20.9)	22.4 (22.0-22.8)
Gender	Male	8774	20.3 (19.8-20.7)	24.5 (24.0-25.1)	26.4 (25.8-27.0)
	Female	5591	12.0 (11.6-12.4)	16.6 (16.2-17.1)	18.5 (18.0-19.0)
Year	1990-1994	1605	15.0 (14.1-15.8)	19.1 (18.1-20.1)	21.1 (20.1-22.1)
	1995-1999	2549	15.1 (14.4-15.7)	19.3 (18.5-20.1)	21.1 (20.3-22.0)
	2000-2004	3239	17.5 (16.8-18.2)	21.2 (20.5-22.0)	23.2 (22.3-24.0)
	2005-2009	3599	18.3 (17.6-18.9)	21.9 (21.2-22.6)	-
	2010-2016	3373	17.5 (16.9-18.1)	-	-
Nervous system malformation		**No. Cases**	**by age 1**	**by age 7**	**by age 14**
Total		801	0.8 (0.7-0.9)	1.2 (1.1-1.2)	1.4 (1.3-1.5)
Gender	Male	433	0.8 (0.8-0.9)	1.2 (1.1-1.4)	1.5 (1.3-1.6)
	Female	368	0.8 (0.7-0.9)	1.1 (1.0-1.2)	1.3 (1.2-1.5)
Year	1990-1994	114	1.0 (0.8-1.2)	1.3 (1.1-1.6)	1.5 (1.2-1.8)
	1995-1999	160	0.8 (0.6-0.9)	1.1 (0.9-1.3)	1.4 (1.2-1.6)
	2000-2004	212	1.0 (0.8-1.1)	1.4 (1.2-1.5)	-
	2005-2009	179	0.7 (0.6-0.9)	1.1 (1.0-1.3)	-
	2010-2016	136	0.7 (0.6-0.8)	-	-
Cardiovascular malformation		**No. Cases**	**by age 1**	**by age 7**	**by age 14**
Total		5258	6.6 (6.4-6.8)	7.9 (7.7-8.1)	8.4 (8.1-8.6)
Gender	Male	2628	6.5 (6.2-6.7)	7.7 (7.4-7.9)	8.1 (7.8-8.5)
	Female	2630	6.8 (6.5-7.1)	8.1 (7.8-8.5)	8.6 (8.3-8.9)
Year	1990-1994	523	5.6 (5.0-6.1)	7.0 (6.4-7.6)	7.6 (6.9-8.2)
	1995-1999	898	6.1 (5.7-6.5)	7.6 (7.1-8.1)	8.1 (7.5-8.6)
	2000-2004	1155	6.8 (6.4-7.3)	8.0 (7.6-8.5)	8.4 (7.9-8.9)
	2005-2009	1347	7.1 (6.7-7.5)	8.3 (7.8-8.7)	-
	2010-2016	1335	6.9 (6.5-7.3)	-	-
Gastrointestinal malformation		**No. Cases**	**by age 1**	**by age 7**	**by age 14**
Total		917	1.0 (0.9-1.1)	1.3 (1.2-1.4)	1.5 (1.4-1.6)
Gender	Male	526	1.2 (1.1-1.3)	1.4 (1.3-1.6)	1.6 (1.5-1.8)
	Female	391	0.8 (0.8-0.9)	1.1 (1.0-1.2)	1.4 (1.2-1.6)
Year	1990-1994	95	0.6 (0.5-0.8)	0.9 (0.7-1.1)	1.3 (1.0-1.6)
	1995-1999	157	0.8 (0.7-1.0)	1.1 (1.0-1.3)	1.3 (1.1-1.5)
	2000-2004	223	1.2 (1.0-1.3)	1.4 (1.2-1.6)	-
	2005-2009	222	1.1 (1.0-1.3)	1.3 (1.1-1.5)	-
	2010-2016	220	1.1 (1.0-1.3)	-	-
Genital tract malformation		**No. Cases**	**by age 1**	**by age 7**	**by age 14**
Total		2566	3.2 (3.0-3.3)	3.6 (3.5-3.8)	3.9 (3.7-4.0)
Gender	Male	2429	5.9 (5.7-6.2)	6.7 (6.4-7.0)	7.0 (6.7-7.3)
	Female	137	0.3 (0.2-0.3)	0.4 (0.3-0.4)	0.6 (0.4-0.7)
Year	1990-1994	266	2.8 (2.4-3.2)	3.2 (2.8-3.6)	3.4 (3.0-3.8)
	1995-1999	401	2.5 (2.3-2.8)	3.1 (2.8-3.4)	3.2 (2.9-3.5)
	2000-2004	592	3.3 (3.0-3.6)	3.8 (3.5-4.1)	4.3 (3.9-4.7)
	2005-2009	673	3.6 (3.3-3.9)	4.0 (3.7-4.3)	-
	2010-2016	634	3.3 (3.1-3.6)	-	-
Urinary tract malformation		**No. Cases**	**by age 1**	**by age 7**	**by age 14**
Total		1271	1.4 (1.3-1.5)	1.8 (1.7-1.9)	2.0 (1.9-2.2)
Gender	Male	850	1.9 (1.8-2.1)	2.4 (2.20-2.5)	2.6 (2.4-2.8)
	Female	421	0.9 (0.8-1.0)	1.2 (1.1-1.3)	1.4 (1.3-1.6)
Year	1990-1994	135	1.0 (0.8-1.3)	1.6 (1.3-1.9)	1.8 (1.5-2.1)
	1995-1999	242	1.4 (1.2-1.6)	1.8 (1.6-2.0)	2.0 (1.7-2.3)
	2000-2004	317	1.7 (1.5-1.9)	2.0 (1.8-2.2)	-
	2005-2009	302	1.5 (1.3-1.7)	1.8 (1.6-2.0)	-

	2010-2016	275	1.4 (1.2-1.6)	-	-
Cerebral palsy		**No. Cases**	**by age 1**	**by age 7**	**by age 14**
Total		1436	1.1 (1.0-1.2)	2.2 (2.1-2.3)	2.7 (2.5-2.8)
Gender	Male	862	1.3 (1.2-1.4)	2.6 (2.4-2.8)	3.1 (2.9-3.3)
	Female	574	0.9 (0.8-1.0)	1.8 (1.6-1.9)	2.2 (2.0-2.4)
Year	1990-1994	227	1.4 (1.1-1.7)	2.4 (2.1-2.8)	2.9 (2.5-3.3)
	1995-1999	337	1.3 (1.1-1.5)	2.4 (2.1-2.6)	2.8 (2.5-3.1)
	2000-2004	390	1.3 (1.1-1.4)	2.6 (2.3-2.8)	3.1 (2.8-3.4)
	2005-2009	333	1.2 (1.0-1.3)	2.2 (2.0-2.5)	-
	2010-2016	149	0.6 (0.5-0.7)	-	-
Epilepsy		**No. Cases**	**by age 1**	**by age 7**	**by age 14**
Total		5274	2.4 (2.3-2.5)	6.7 (6.4-6.9)	11.4 (11.0-11.7)
Gender	Male	2811	2.5 (2.4- 2.7)	7.0 (6.7- 7.3)	11.9 (11.4-12.4)
	Female	2463	2.2 (2.1- 2.4)	6.3 (5.9- 6.6)	10.8 (10.2-11.3)
Year	1990-1994	1270	2.9 (2.5- 3.3)	7.9 (7.2- 8.5)	13.0 (12.2-13.9)
	1995-1999	1430	2.7 (2.4- 3.0)	6.8 (6.4- 7.3)	11.7 (11.1-12.4)
	2000-2004	1159	2.7 (2.4- 2.9)	6.6 (6.2- 7.1)	10.9 (10.2-11.7)
	2005-2009	913	2.1 (1.9- 2.4)	6.5 (6.0- 6.9)	-
	2010-2016	502	1.9 (1.7- 2.1)	-	-
ADHD		**No. Cases**	**by age 1**	**by age 7**	**by age 14**
Total		3540	0.0 (0.0-0.0)	3.7 (3.5-3.9)	11.8 (11.4-12.2)
Gender	Male	2966	0.0 (0.0- 0.1)	6.2 (5.8- 6.5)	19.4 (18.7-20.2)
	Female	574	0.0 (0.0-0.0)	1.1 (0.9-1.2)	3.8 (3.5-4.2)
Year	1990-1994	729	0.2 (0.1- 0.3)	3.4 (2.9- 3.8)	9.8 (9.1-10.6)
	1995-1999	1104	0.0 (0.0- 0.0)	3.3 (3.0- 3.7)	11.0 (10.3-11.7)
	2000-2004	1127	0.0 (0.0- 0.1)	4.1 (3.8- 4.5)	13.4 (12.6-14.3)
	2005-2009	570	0.0 (0.0- 0.1)	4.4 (4.0- 4.9)	-
	2010-2016	10	-	-	-
ASD		**No. Cases**	**by age 1**	**by age 7**	**by age 14**
Total		6016	0.1 (0.1-0.1)	8.5 (8.2-8.8)	16.5 (16.1-17.0)
Gender	Male	4933	0.2 (0.1- 0.2)	13.8 (13.3-14.30)	26.5 (25.7-27.3)
	Female	1083	0.0 (0.0-0.1)	2.9 (2.7-3.1)	6.0 (5.6-6.4)
Year	1990-1994	554	0.1 (0.0- 0.1)	2.7 (2.3- 3.1)	6.5 (5.9- 7.2)
	1995-1999	1478	0.1 (0.1- 0.2)	6.3 (5.8- 6.7)	13.7 (12.9-14.4)
	2000-2004	1878	0.2 (0.1- 0.2)	8.8 (8.2- 9.3)	20.9 (19.8-22.0)
	2005-2009	1594	0.1 (0.0- 0.1)	12.8 (12.1-13.5)	-
	2010-2016	512	0.0 (0.0- 0.1)	-	-

6.3.1.2 Neurodevelopmental disorders

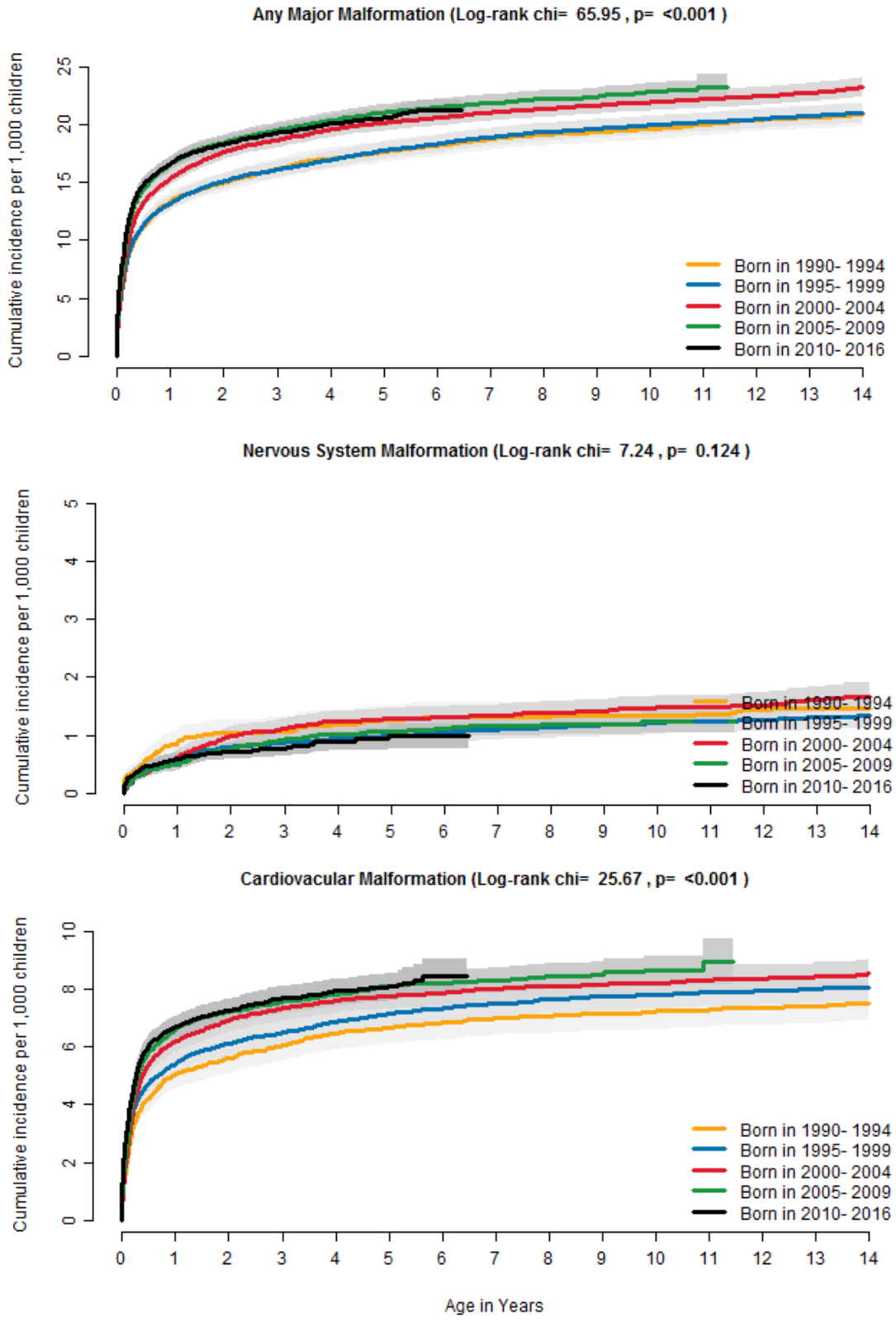
Among the four neurodevelopmental disorders, cerebral palsy had the lowest cumulative incidence of 2.7 (95% CI 2.5-2.8) per 1,000 children by age 14, identified using both diagnoses and prediction model based on selected codes of treatments, symptoms, and management.(Section 6.5) Most of the cerebral palsy cases were identified by the age of 7. Although there was a significant difference in incidence of cerebral palsy among the birth-year periods (log-rank test, $\chi2=30.81$, $p <0.001$), no obvious linear trend in calendar year was observed (Table 6-3, Figure 6-2).

In total, 5274 children in the cohort were identified with epilepsy defined by at least 2 prescriptions of AEDs or one diagnosis. The cumulative incidence of epilepsy keeps increasing to 11.4 (95% CI 11.0-11.7) per 1,000 children by age 14. There was no significant difference in incidence curves between the five birth-year periods.

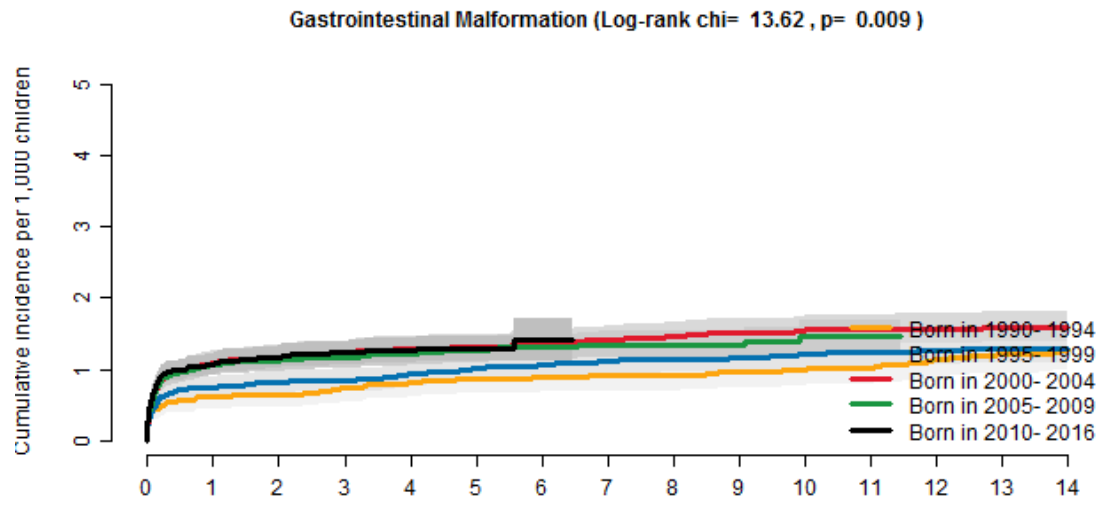
3540 children in the cohort were identified with ADHD defined by at least two occurrences of diagnosis or prescription in CPRD database. ADHD began to be diagnosed or treated round age 5, and the cumulative incidence increased rapidly afterwards to 11.8 (95% CI 11.4-12.2) per 1,000 children by age 14. The incidence of ADHD increased significantly during the study period, from 9.8 per 1,000 children during 1990-1994 to above 15 per 1,000 children during 2005 -2009.

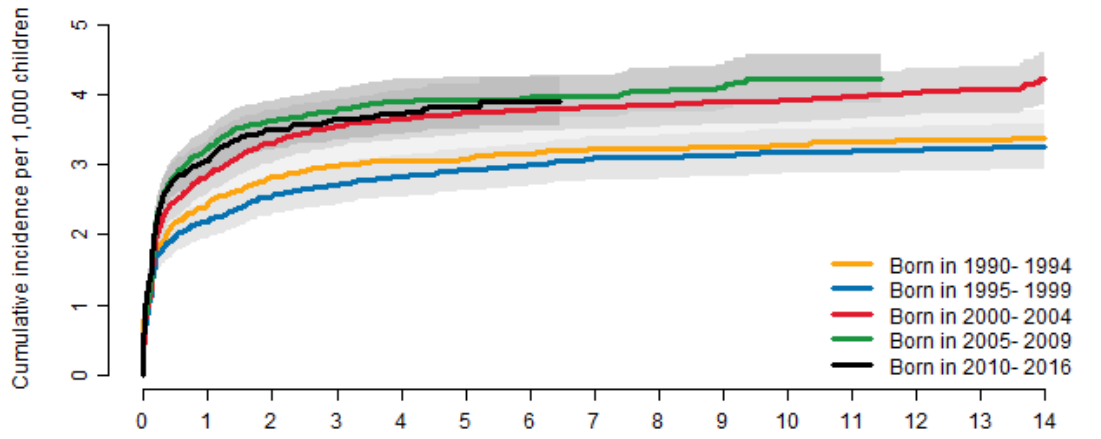
ASD began to be diagnosed at around age 2, and new cases kept being identified to a cumulative incidence of 16.5 (95% CI 16.1-17.0) per 1,000 children by age 14. The incidence of ASD diagnosis increased significantly through the study years, from 6.5 per 1,000 children during 1990-1994 to above 20.9 per 1000 per children by age 14 during 2010-2016 (log-rank test, $\chi 2=1345.4$, $p <0.001$). Both ADHD and ASD were more common in boys.

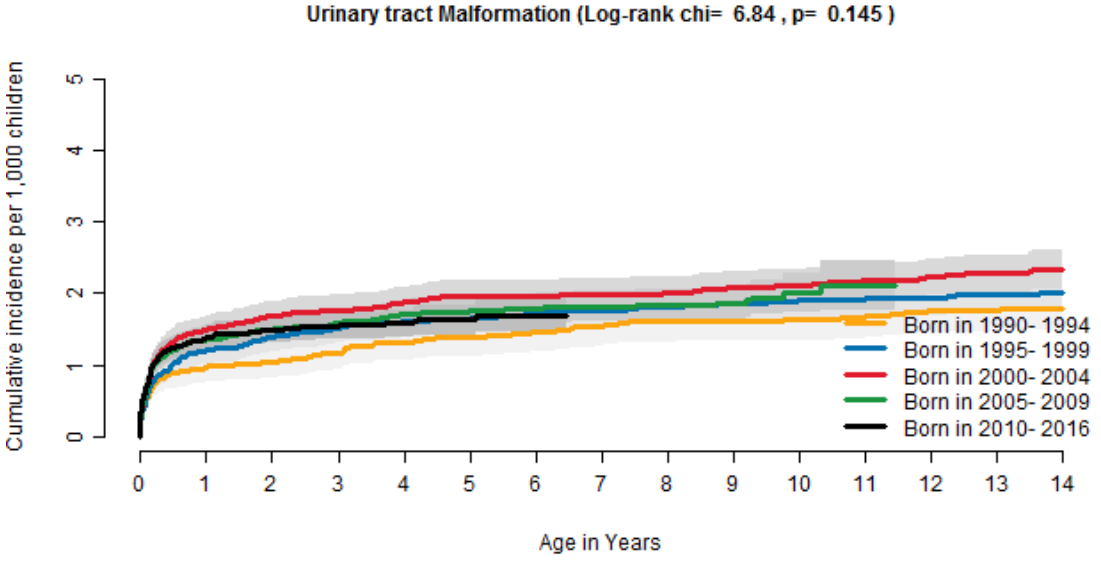
Figure 6-2. Kaplan-Meier failure estimates for adverse child outcomes, by birth year period.



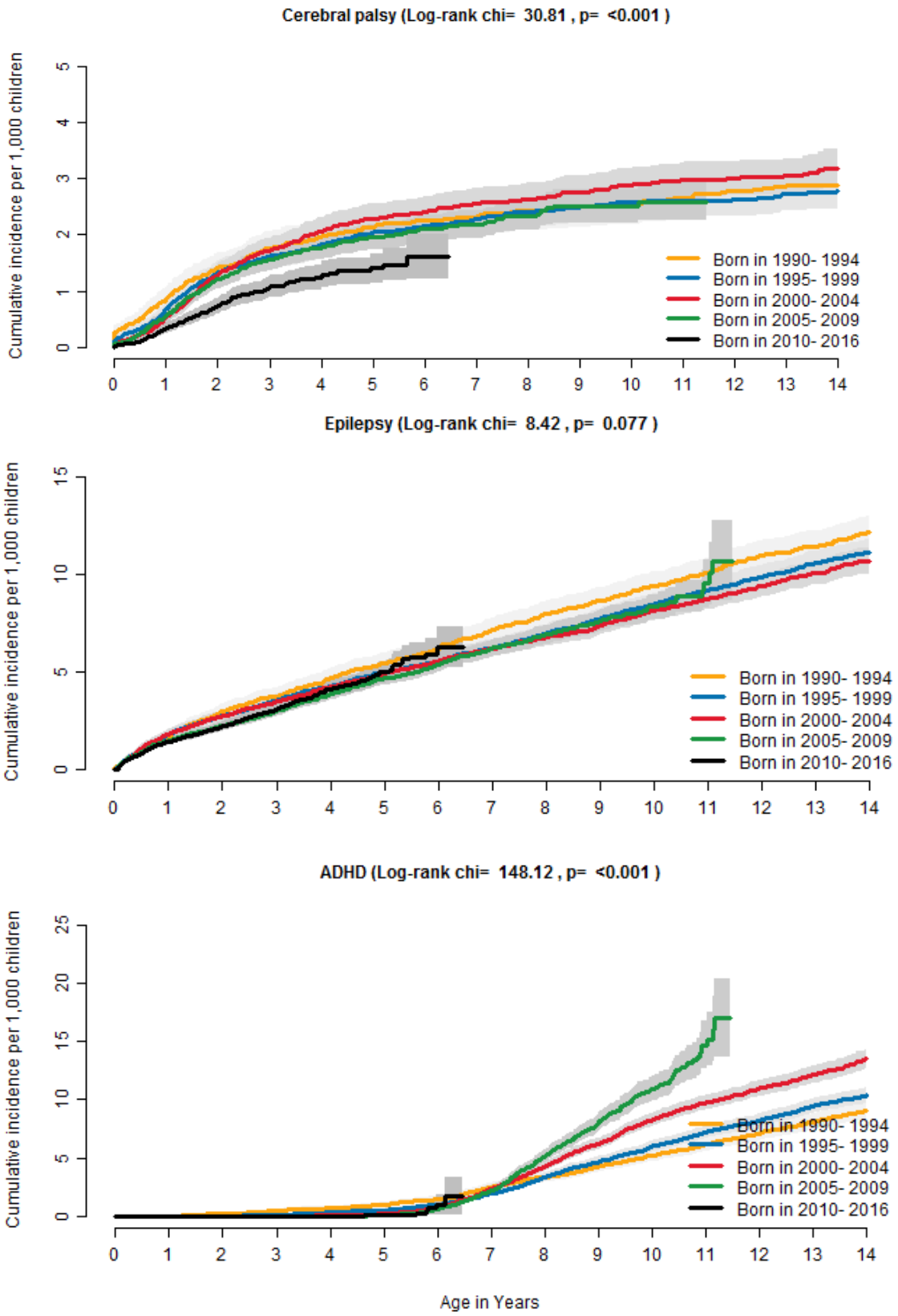
(Continued)


Gastrointestinal Malformation (Log-rank chi= 13.62 , p= 0.009)
Cumulative incidence per 1,000 children
0
1
2
3
4
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Born in 1990- 1994
Born in 1995- 1999
Born in 2000- 2004
Born in 2005- 2009
Born in 2010- 2016

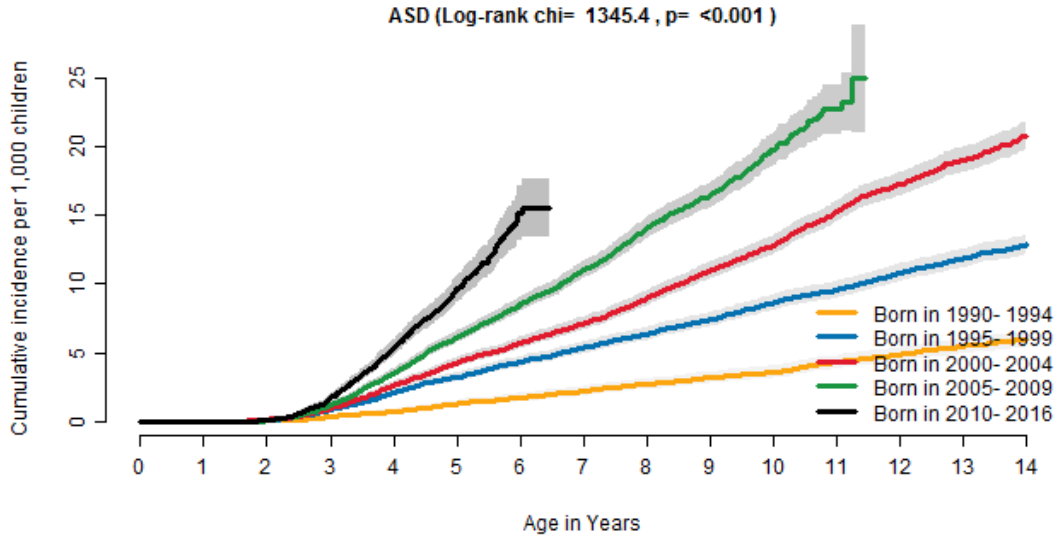

Genital tract Malformation (Log-rank chi= 33.25 , p= <0.001)
Cumulative incidence per 1,000 children
0
1
2
3
4
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Born in 1990- 1994
Born in 1995- 1999
Born in 2000- 2004
Born in 2005- 2009
Born in 2010- 2016


Urinary tract Malformation (Log-rank chi= 6.84 , p= 0.145)
Cumulative incidence per 1,000 children
0
1
2
3
4
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Born in 1990- 1994
Born in 1995- 1999
Born in 2000- 2004
Born in 2005- 2009
Born in 2010- 2016
Age in Years

(Continued)



(Continued)



6.3.2 Association with risk factors

In this section I described the association of these ten child adverse outcomes with potential risk factors. For major malformation, I set a cut-off of age 3 (before 4 year-old) and only evaluate malformation cases recorded in medical history by age 3. According to the cumulative incidence shown in the previous section, most of major malformation events could be identified using medical history by age 3 (before 4 years old). For neurodevelopmental disorders, age 14 were used as the cut-off to exclude outcomes with potentially post-neonatal aetiologies.

6.3.2.1 Major malformation

The risk of any major malformation increased significantly with younger gestational age, where the risk within extremely preterm births was 5 times of that within children born at ≥ 39 GW. Other major risk factors included male gender (RR 1.49; 95% CI 1.45-1.56), maternal epilepsy in pregnancy (RR 1.39; 95% CI 1.16-1.66), maternal diabetes in pregnancy (RR 1.31; 95% CI 1.20-1.43) and multiple births (RR 1.30; 95% CI 1.20-1.41). Other factors that also statistically associated with increased risk of major malformation included: maternal age of 13-19 (as compared with maternal age of 25-29), pregnancy year at delivery between 1995 and 2009 (as compared with 1990-1994), maternal illicit drug use, obesity, hypertension during pregnancy, anxiety during pregnancy, genitourinary tract infection, and treatment for chronic conditions in pregnancy (Appendix 6-1).

Younger gestational age was associated with increased risks of all the five system-specific malformations, although the risk ratios for urinary malformation included one due to limited number in the extremely preterm group (23-27 GW). A male predominance was observed for four system-specific malformation except for neurological malformation, and especially for

genital malformation, where a risk ratio (male versus female) of 19.6 (95% CI 16.3-23.7) was observed. The associations of other factors were less consistent among malformations. For example, maternal diabetes during pregnancy was associated with an increased risk of nervous system malformation (1.46; 95% CI 1.00-2.16), cardiovascular malformation (1.64; 95% CI 1.45-1.86) and urinary malformation (1.49; 95% 1.12-1.99), but not associated with risk of gastrointestinal malformation and genital malformation. Maternal epilepsy during pregnancy was found to be associated with increased risk of nervous system malformation (2.07; 95% CI 1.09-3.92) and cardiovascular malformation (1.53; 95% CI 1.53-2.01), but not with other three system-specific malformations. Among children whose mother experienced genitourinary tract infection during pregnancy, an increased risk of cardiovascular malformation (1.15; 95% CI 1.05-1.26), gastrointestinal malformation (1.35; 95% CI 1.05-1.68) and urinary malformation (1.22; 95% CI 1.01-1.48) was observed. Multiple births were associated with risk of cardiovascular malformation (1.33; 95% CI 1.17-1.52) and genital tract malformation (1.44; 95% 1.19-1.75). Beside the factors mentioned, an increased risk of cardiovascular malformation was also associated with illicit drug use, obesity, and treatment for chronic conditions during pregnancy; genital malformation was associated with hypertension; and urinary malformation was associated with alcohol misuse. Factors not associated with any of the malformations include maternal tobacco use, anxiety, depression, STIs during pregnancy, and parity ≥ 1 (Appendix 6-1-Appendix 6-6).

6.3.2.2 Neurodevelopmental disorders

Significant male predominance has been observed in all of the four neurodevelopmental disorders (cerebral palsy: 1.43 [95% CI 1.28-1.59]; epilepsy 1.12 [95% CI 1.06-1.19]; ADHD 5.26 [95% CI 4.76-5.88]; ASD 4.55 [95% CI 4.35-5.00]). Other risk factors common to the four neurodevelopmental disorders include maternal depression (cerebral palsy: 1.46 [95% CI 1.21-1.75]; epilepsy 1.20 [95% CI 1.08-1.34]; ADHD 1.80 [95% CI 1.60-2.02]; ASD 1.36 [95% CI 1.24-1.49]) and treatment for chronic conditions during pregnancy (cerebral palsy: 1.22 [95% CI 1.04-1.44]; epilepsy 1.18 [95% CI 1.07-1.30]; ADHD 1.28 [95% CI 1.14-1.43]; ASD 1.24 [95% CI 1.15-1.35]). A clear increasing trend of incidence along calendar years was present for epilepsy, ADHD and ASD (Appendix 6-7-Appendix 6-10).

Younger gestational age (≥ 39 GW versus 23-29 GW) was a significant risk factor for both cerebral palsy (12.50; 95% 10.00-16.67) and epilepsy (2.44; 95% 1.89-3.13), but not for ADHD or ASD. Risk factors other than younger gestational age, male gender, maternal depression and epilepsy were not associated with risk of cerebral palsy. An increased hazard of epilepsy was observed in children born to mother with younger maternal age, illicit drug use, obesity, maternal epilepsy

(2.67; 95% 2.14-3.34, the highest hazard ratio observed for epilepsy) and genitourinary tract infection during pregnancy (Appendix 6-7-Appendix 6-8).

For ADHD, risk factors included male gender (as the most significant risk factor), maternal depression, and younger mothers, illicit drug use, tobacco use, obesity, genitourinary tract infection, treatment for chronic conditions and parity ≥ 1. The risk of ADHD also increased each year. ASD was associated with male gender, more recent year of pregnancy, tobacco use, obesity, hypertension, diabetes, anxiety, epilepsy, depression, treatment for chronic conditions and parity ≥ 1 (Appendix 6-9-Appendix 6-10).

Factors not associated with any of the four neurodevelopmental disorders include maternal alcohol misuse (>=14 units of alcohol per week), STIs during pregnancy and multiple births.

6.4 Discussion

The overall aim of this chapter was to identify and validate the indicators for each adverse child outcome in the CPRD Mother Baby Cohort. These indicators will be used in the following chapters to investigate associations between macrolide vs penicillin antibiotic prescribing during pregnancy and adverse child outcomes. A further aim of this chapter was to evaluate potential confounders for the association.

In this discussion, I first compare the cumulative incidence or prevalence of the ten adverse child outcomes with external evidence including prospective studies (registries and surveys) and other administrative databases including primary care databases. This comparison provides a crude evaluation of the sensitivity of the indicators. Second, I evaluate the specificity of the indicators using evidence from previous validation studies using CPRD. I then describe whether the identified outcomes were associated with known risk factors, and compare the strength of the associations observed using the indicators with those reported by previous research. This comparison provides an external evaluation of the validity of the indicators. Third, I summarise the validity of the outcome indicators. The discussion ends up with describing how the findings in this chapter inform the analyses of the association between macrolides prescription during pregnancy and adverse child outcomes, including the control of confounding.

6.4.1 Comparing cumulative incidence with external data

6.4.1.1 Major malformations

In this chapter, I estimated the maximum cumulative incidence of newly recorded major malformations, which is a proxy measure for malformation birth prevalence. Estimates of cumulative prevalence for any major malformation and the five system-specific malformations, are listed in Table 6-4. I compared the malformation prevalence in the CPRD Mother Baby Cohort with those reported by the European Concerted Action of Congenital Anomalies and Twins (EUROCAT), one of the two major national registries for birth defects in UK. Another national registry is the National Congenital Anomaly System (NCAS), which stopped generating reports in 2010. NCAS was known to have a lower ascertainment rate and to report consistently lower prevalence rates compared with EUROCAT.[256] The EUROCAT system began in 1979 and is a European network of population-based registries for the epidemiologic surveillance of congenital anomalies. EUROCAT covers approximately 35% of the UK births.[257] It records congenital anomalies for live births, fetal deaths, and induced abortions. EUROCAT uses a detailed classification and ICD-10 coding system based on organ system as well as an exclusion list for minor malformation, which is also applied in this thesis. EUROCAT receives voluntary reports from an extensive network of health professionals including obstetricians, midwives, neonatal nurses, geneticists, pathologists, ultrasonographers, obstetricians, and paediatricians.[257] I also listed the prevalence reported by previous studies using CPRD where available to check whether the prevalence were consistent (Table 6-4).

Sensitivity. Compared with EUROCAT UK registries, the cumulative prevalence estimates of major malformations estimated in the CPRD Mother Baby Cohort were slightly higher (Table 6-4). For example, the prevalence for any major malformation in this study and in EUROCAT was 20.6 and 15.3 per 1000 livebirths, respectively. For cardiovascular malformation, the prevalence was 7.9 and 4.3 per 1000 livebirths, respectively. This discrepancy across all malformations could reflect improved ascertainment in the CPRD database compared with EUROCAT. Better ascertainment could be explained by the fact that CPRD records clinical follow up for all registered patients.[258] In contrast, EUROCAT is based on voluntary reports and active follow-up which is subject to attrition. For example, less than 2% malformation cases in EUROCAT were identified after age 1.[259] In contrast, 25% of the malformation diagnoses in the study using CPRD were made after the children's first birthday. However, the prevalence of any major malformation identified by age 1 year was consistent between the two data sources (16.9 in this studies versus 15.3 in EUROCAT). The higher prevalence of malformation recording in primary care has been reported by a number of other studies. These confirmed the value of primary

care data as a source to investigate congenital malformation.[236,243,258,260] The CPRD cohort reports a similar prevalence of cardiovascular malformations to regional registry databases.[261,262]

Prevalence rates were much higher in CPRD than EUROCAT for one system-specific malformation included in this study: genital malformation. The prevalence of genital malformation reported in this study is about 3 times of that reported in EUROCAT (3.6 versus 1.3 per 1000). This higher prevalence has also been observed in a previous study using THIN.[258] Genital malformations mainly comprise hypospadias (88%). A possible explanation of the much lower rate of recording in EUROCAT, is that before 2015, this registry classified mild hypospadias as a minor malformation and excluded them from major malformation related to genital malformations.[258] The classification of hypospadias as major or minor anomalies remains controversial. Researchers and surgeons have argued that all hypospadias cases are likely to share the same aetiology and many require surgery, classification should therefore, not distinguish between major and minor cases. In response, since 2015, the EUROCAT registry no longer differentiates between severe and mild hypospadias and classifies all hypospadias as major malformation, as applied in my CPRD cohort.[240]

Secondary care data was unlikely to add cases, because both national malformation registries and primary care incorporate hospital and specialist visits summaries. To our knowledge, the prevalence of non-chromosomal malformation has not been studied using the secondary care data alone in the UK. Only one study was found to link primary care data with hospital data and study malformations from each data source. In the study using Born in Bradford (BiB) prospective birth cohort, 51% of all malformation events were missed by hospital data before age 1 and were only recorded in the primary care database.[229]

Specificity. Recording of Cong malformation in CPRD is highly specific, leading to a high PPV. Several studies have evaluated the PPV of congenital malformation identified using Read codes in CPRD with manual review of care trajectories or comparison with a reference datasets.[130,166,236,243] A systematic review reported a pooled median PPV of 93.5% (range: 71%-100%) for malformations based on three CPRD studies. Two studies that validated major cardiovascular malformation reported that 93% to 94% of the cases could be confirmed using photocopied medical records or questionnaires from GP based on 906 cases from 1996 to 2010.[166,236] For hypospadias, the positive predictive value was reported to be 96% in a CPRD study by reviewing photocopied medical records.[243] Evidence for the PPV of other system specific malformations is limited.

Table 6-4. A comparison between the prevalence of child outcomes in this study and prevalence reported in literature.

Outcome	Prevalence in literature		Prevalence estimated in the target population of this study by age 7*
	Database, Year	Prevalence	
Major congenital malformation	EUROCAT UK registries, 1990-2015 [263]	15.3 per 1000 live birth	20.6 per 1000
Cardiovascular	EUROCAT UK registries, 1990-2015 [263]	4.3 per 1000 live birth	7.9 per 1000
	Northern Health Region of England, The Regional Paediatric Cardiology Database 1995-2000 [261]	7.4 per 1000 live birth	
	CPRD, 2001-2003 [262]	5.1 to 8.3 per 1000 from ages 1 to age 6	
Gastrointestinal	EUROCAT UK registries, 1990-2015 [263]	1.4 per 1000 live birth	1.3 per 1000
Nervous system	EUROCAT UK registries, 1990-2015 [263]	0.8 per 1000 live birth	1.2 per 1000
Genital	EUROCAT UK registries, 1990-2015 [263]	1.3 per 1000 live birth	3.6 per 1000
	Scotland hospital admissions, 1994 [264]	4.6 per 1000 live birth	
Urinary-renal	EUROCAT UK registries, 1990-2015 [263]	2.3 per 1000 live birth	1.8 per 1000
Cerebral palsy	Manually reviewed case from North of England Cerebral Palsy Survey, 1991-2000 [265]	2.5 per 1000 neonatal survivors	1.8 per 1000
	Surveillance of Cerebral Palsy in Europe, 2002 [266]	2.08 per 1000 livebirths	
	THIN, using indicators of different sensitivity, 2015[56]	1.4 to 3.1 per 1000 by age 12	
Epilepsy	Prospective cohort (British national child development study), 1998 [267]	8.4 per 1000 age 23	6.2 per 1000
	NICE 2012 [231]	7-8 per 1000 all age	
	THIN, using indicators of different sensitivity, 2015[56]	6.4 to 10.1 per 1000 by age 10	
Attention deficit hyperactivity disorder (ADHD)	CPRD, 2004-2013 [268]	8.7 per 1000 male all age; 1.2 per 1000 female all age	7.5 per 1000 male; 1.4 per 1000 female
	NICE 2011[269]	15 per 1000 boys (NICE commented that "prevalence estimates vary widely across Studies")	
Autism spectrum disorder (ASD)	Manually reviewed case from Special Educational Needs (SEN) register, 2003-2004 [270]	9.4-9.9 per 1000 age 5-7	7.7 per 1000
	NICE 2011[233]	10 per 1,000 children	

* EUROCAT: European Surveillance of Congenital Anomalies; CPRD: The Clinical Practice Research Datalink; CPRD: Clinical Practice Research Datalink; THIN: The Health Improvement Network [THIN], similar with CPRD but covers different population; NICE: National Institute for Health and Clinical Excellence (guidance).

6.4.1.2 Neurodevelopmental disorders

The cumulative incidence (prevalence at birth) of neurodevelopmental disorders were slightly lower than those reported by prospective studies (e.g. surveys and registries), but comparable with those reported using primary care databases (e.g. CPRD and THIN). As mentioned in Section 6.1.3, under-recording of diagnoses of neurodevelopmental disorders in primary care has been reported in previous studies.[239,245,271] Compared with malformation, this problem could be specific to neurodevelopmental disorders, due to their subtle and heterogeneous phenotypes and the time required to make a diagnosis. There may also be reluctance by clinicians to record a neurodevelopmental condition until the diagnosis is certain as communication with parents about a neurodevelopmental diagnosis for their child, is an important part of the relationship with healthcare services. In contrast, congenital malformations may be obvious, and require an early explanation, therapeutic input, and surveillance reporting. On the other hand, prospective studies, such as surveys, reported higher rates than CPRD, based on screening and actively assess using specific diagnostic criteria (e.g. DSM-IV).[272,273] However, for neurodevelopmental disorders such as ADHD and ASD, prevalence estimates from prospective studies vary widely, probably due to different diagnostic criteria and thresholds applied for individual symptoms (Table 6-4).[112,233]

To improve the sensitivity of case identification, studies based on primary care database often include treatments or symptoms additional to diagnostic codes. While very sensitive approaches are possible (e.g. to define a case with at least one diagnosis or one prescription or one symptom), this reduces specificity. In this thesis, I favoured specific identification criteria for the four neurodevelopmental disorders. As previously mentioned, while a non-differential sensitivity does not bias the relative risk, a non-differential specificity often underestimates the relative risk due to the larger numbers of false-positive in the non-exposure group.[234] For example, the study of Meeraus et al. proposed three indicators for epilepsy in children, from the most specific to the most sensitive including: 1) at least two prescriptions of AEDs within 4 month; 2) 1) or a diagnosis of epilepsy; and 3) 1) or 2) or a symptom of epilepsy. These indicators resulted in prevalence rates ranging from 6.4 to 10.1 per 1,000 children by age of 10, and a PPV of 94% for the most sensitive indicator 3).[245] This thesis applied the second indicator to identify epilepsy cases, as epileptic symptoms (e.g. seizures) could reflect non-epileptic febrile seizures, which are relatively common. In a sense, this indicator also identifies patients at the more severe end of the spectrum of epilepsy. Similar logic was applied to cerebral palsy, ADHD and ASD, where more specific identification approaches are preferred although with imperfect sensitivity.

6.4.2 Associations with known risk factors

6.4.2.1 Major malformations

As a further measure of validation, I tested whether the malformations identified in this study were associated with known (yellow) risk (or protective) factors, by comparing the RR estimates with evidence from previous literature (Table 6-5). As few studies reported risk factors for nervous system malformation and gastrointestinal malformation, I listed the results for any major malformation, cardiovascular malformation and hypospadias as examples. Hypospadias accounts for most cases of genital malformation (88% in this study), and most of the literature studied reported hypospadias instead of "genital malformation". I therefore report evidence for hypospadias to compare with the RRs of genital malformation in this study.

Recognised risk factors for "any major malformation" include maternal obesity, hypertension, diabetes, AEDs exposure during pregnancy, multiple births, TORCHs and prematurity. Reassuringly, six of the seven factors were associated with any major malformation in this study, though the RR was attenuated. No association was observed with maternal recording of STIs, which could be due to the inclusion of specific STIs in the algorithm for this variable that have not been shown to consistently be associated with major malformation. For example, TORCH infections are known to be teratogenic, but there is no evidence that hepatitis B and chlamydia infections are teratogenic.[274] The small number of exposed children precluded individual analyses for each of the maternal infections. Another explanation is that there was simply insufficient power for this particular analysis.

Table 6-5. Comparison of potential and known risk factors for major malformations identified in this study and in previous research.

Potential risk factors	Any major malformation		Cardiovascular malformation		Hypospadias	
	RR in literature	RR in this study	RR in literature	RR in this study	RR in literature	RR in this study*
Maternal age at pregnancy	Inconsistent[275,276]	-	3.95 (1.70-9.17)[277] >=40y vs 20-24y	1.18 (1.09-1.28) >=35y vs 25-29y	-	-
Pregnancy start year (%)	Decreasing from 1990 to 1997 and increasing from 1997 to 2015[263]	1.09 (1.02-1.16); 2005-2009 vs 1990-1994	Increasing (~1% per year)[263]	1.06 (0.96-1.18) 2005-09 vs 1990-94	Increasing (~1% per year)[263]	1.21 (1.04-1.41) 2005-09 vs 1990-94
Maternal alcohol misuse	Heterogonous and inconsistent[278]	-	Inconsistent [137]	-	Not associated[279]	0.94 (0.77-1.14)
Maternal illicit drug use	Heterogonous and inconsistent[280]	-	1.9-2.4[137]	1.29 (1.01-1.65)	-	-
Recent maternal tobacco use	Heterogonous and inconsistent[278]	-	Inconsistent [137]	-	Not associated[279]	0.90 (0.82-0.99)
Maternal obesity	1.12-1.37 fold [281]	1.07 (1.02-1.13)	1.30 (1.12-1.51)[282]	1.12 (1.03-1.21)	2.6 (1.2-5.7)[283]	1.05 (0.93-1.18)
Hypertension	1.2-1.3 fold [284]	1.12 (1.05-1.19)	1.4 (1.2-1.7)[285]	1.08 (0.98-1.20)	2.0 (1.1-3.7)[283]	1.20 (1.04-1.39)
Diabetes	2-9 fold [286]	1.31 (1.20-1.43)	3.8 (3.0-4.9)[287]	1.64 (1.44-1.85)	Not associated	1.02 (0.82-1.28)
Anxiety	Heterogonous and inconsistent[288] , maybe associated with lower birthweight and premature babies	-	-	-	-	-
Depression		-	Inconsistent results on the effect of SSRIs[289]	-	-	-
Epilepsy	Risk from AEDs: 3.26 (2.15–4.93)	1.39 (1.16-1.66)	Risk from AEDs: 4.2[137]	1.53 (1.17-2.01)	Risk from AED Valproic acid: 4.8 (2.9- 8.1)[290]	1.28 (0.81-2.03)
Parity ≥ 1	Heterogonous	-	Not associated[291]	0.97 (0.92-1.03)	0.8 (0.7-0.9)[292]	1.04 (0.95-1.13)
Multiple births	1.7 (1.5-2.0)[293]	1.30 (1.20-1.41)	1.59 (1.42–1.79)[294]	1.33 (1.12-1.52)	2.5 (1.1-6.1) [295]	1.44 (1.19-1.75)
Genitourinary tract infection	Inconsistent[296,297]	-	Inconsistent[296,297]	-	-	-
Sexually Transmitted Infection	TORCH are teratogenic[274]	0.88 (0.74-1.03)	Rubella and Zika virus infection	0.89 (0.68-1.16)	-	-
Treatment of chronic medical conditions	-	-	1.59 (1.54–1.63)[298]	1.23 (1.13-1.33)	-	-
Male baby	Heterogonous	-	Heterogonous	-	Only in boys	19.63 (16.25-23.73)
Gestational weeks	3.2-4.8 fold preterm vs term[299]	2.14-4.76	2.4 fold preterm vs term[300]	2.29-1.63	9.1 (3.3-25)[283] Preterm vs term	1.38-2.16

*Factors recognised as known risk (or protective) factors by literatures were highlighted with yellow background. Factors recognised as not associated with the outcome were highlighted with grey background. For some other factors, there were less than two available studies (noted as "-") or inconsistent evidence, where the RRs in this study were not shown or compared. RR: risk ratios. VS: versus. AEDs: antiepileptic drugs.

Factors associated with increased risks of cardiovascular malformation or hypospadias were reported in two expert reviews.[137,279] Findings from my study of CPRD were consistent with previous reported associations for cardiovascular malformations, where the known risk factors include older maternal age, illicit drug use, obesity, hypertension, diabetes, AEDs exposure during pregnancy, multiple births, certain STIs, chronic maternal conditions, and prematurity.[137] Most of these known factors for cardiovascular malformation were also reported to be associated with hypospadias, except for maternal diabetes.[279] The risk of hypospadias was increased in children of nulliparous mothers. The strength of the associations observed in my study for hypospadias was weaker than in previous researches. A possible explanation is that the effect has been diluted by measuring genital malformation in both genders in this study, instead of measuring hypospadias in only boys in literature.

6.4.2.2 Neurodevelopmental disorders

In contrast to risk factors for major malformations, the associations between neurodevelopmental disorders and maternal chronic conditions (e.g. diabetes and hypertension) are less marked or consistent in literatures. Recognised prenatal and perinatal risk factors for cerebral palsy identified in my CPRD study were preterm birth, multiple births, intrauterine infection (e.g. chorioamnionitis), specific STIs and male gender. The magnitude of these associations were similar to previous reports for preterm birth and male gender. The strength of association for multiple births, genitourinary tract infection and STIs were weaker as compared with literature. There are two possible explanations. First, genitourinary tract infections and STIs may include common infections that have not been consistently recognised as risk factors for cerebral palsy, such as asymptomatic bacteriuria, asymptomatic bacterial vaginosis, hepatitis B and chlamydia infections.[301] Second, the small number of outcomes may reduce the power to detect an association (Table 6-6).

According to previous literature, the most significant known prenatal-perinatal risk factors for epilepsy in children include maternal epilepsy and prematurity. I observed similar associations in this study. Other risk factors recognised by previous studies include younger maternal age, maternal obesity and STIs during pregnancy. The association with STIs has the similar weakened association as observed with malformation and cerebral palsy. A declining in epilepsy incidence from mid-1990s has been identified, which was consistent with previous findings (Table 6-6).[245,302]

As expected, a significant male predominance was observed for both ADHD and ASD. Consistent with previous reports, the increased risk of ADHD was associated with maternal life style (e.g.

maternal alcohol, drug and tobacco use), maternal stress, male gender and preterm births. In contrast, life style factors were not associated with increased risks of ASD, in both literatures and my study. Known risk factors for ASD instead include maternal obesity, diabetes, and maternal exposure during pregnancy to the antidepressant selective serotonin reuptake inhibitors (SSRIs) and the anticonvulsant Valproate, and to genitourinary infections. The differential patterns of risk factors between ADHD and ASD observed in previous studies were also observed in this study. However, two out of the eight known risk factors for ADHD and two out of the nine known risk factors for ASD were not statistically significant (Table 6-7).

Table 6-6. Comparison of potential and known risk factors for major malformations identified in this study and in previous research.

Potential risk factors	Cerebral palsy		Epilepsy	
	HR or RR in literature	HR in this study	HR or RR in literature	HR in this study
Maternal age at pregnancy	Inconsistent[303,304]		1.14 (1.04-1.24) <20y vs 25-29y	1.21 (1.05-1.39) <20y vs 25-29y
Pregnancy start year (%)	Stable across study years[56]	Stable across study years	Decline from mid 1990s [245,302]	0.80 (0.72-0.89) 2005-09 vs 1990-94
Maternal alcohol misuse	-		Inconsistent[305,306]	
Maternal illicit drug use	Inconsistent[56,304]		-	
Recent maternal tobacco use	Inconsistent[56,304]		Inconsistent[305,306]	
Maternal obesity	Inconsistent[307,308]		1.2-1.8[309]	1.23 (1.12-1.35)
Parity ≥ 1	Inconsistent[303]		-	
Multiple births	1.3-6 [304,310]	1.17 (0.94-1.46)	-	
Hypertension	Inconsistent[301,303]		Preeclampsia: 1.2-2.6[311,312]	1.08 (0.96-1.21)
Diabetes	Inconsistent[303,122]		-	
Anxiety	Inconsistent, maybe associated with poorer long-term outcomes of children, including lower birthweight, premature babies, internalising and externalising disorders in children.[182]			
Depression				
Epilepsy	Inconsistent[303,122]		Associated 6.4 (3.3-10.7) [313]	2.67 (2.13-3.35)
Genitourinary tract infection	Intrauterine infection: 4 fold[310]	1.18 (0.99-1.41)	Inconsistent[25,231]	
Sexually Transmitted Infection	Associated (rubella, toxoplasmosis, cytomegalovirus, herpes simplex)[230]	0.83 (0.51-1.37)	Toxoplasmosis, Cytomegalovirus, Herpes simplex[313]	0.85 (0.65-1.11)
Treatment of chronic medical conditions	Inconsistent[303]		-	
Male baby	1.3[310]	1.41 (1.28-1.58)	Inconsistent	
Gestational weeks	~4 fold for 32-36 GW; ~30 fold for <27 GW[310]	5.13-12.47 fold	1.7-5 fold [309,313]	1.52-2.44 fold

*Factors recognised as known risk (or protective) factors by literatures were highlighted with yellow background. Factors recognised as not associated with the outcome were highlighted with grey background. For some other factors, there were less than two available studies (noted as "-"). RR: risk ratios. VS: versus. GW: gestational week.

Table 6-7. Comparison of the potential risk factor for ADHD and ASD identified in this study and in previous literature.

Potential risk factors	ADHD		ASD	
	RR in literature	RR in this study	RR in literature	RR in this study
Maternal age at pregnancy	Inconsistent[314]		Associated (higher risk in higher maternal age group)[315]	1.04 (0.96-1.12) >=35y vs 25-29y
Pregnancy start year (%)	Increasing from 1998 to 2007[254]	1.45 (1.28-1.64); 2005-2009 vs 1990-1994	Increased during 1990s and steady in 2000s[252]	4.45 (3.97-4.99) 2005-09 vs 1990-94
Maternal alcohol misuse	2.2-2.4 fold[316]	1.11 (0.94-1.31)	Not associated	0.93 (0.82-1.05)
Maternal illicit drug use	Associated[269]	1.57 (1.17-2.09)	-	
Recent maternal tobacco use	1.5-3.0 fold[316]	1.56 (1.45-1.68)	Inconsistent[315]	
Maternal obesity	Inconsistent[317,318]		1.36[315,319]	1.42 (1.31-1.53)
Parity ≥ 1	-		Inconsistent[315]	
Multiple births	Not associated[320,128]	0.80 (0.63-1.02)	Not associated[320,128]	0.92 (0.79-1.08)
Hypertension	Inconsistent[314]		Inconsistent[315]	
Diabetes	Inconsistent[321]		1.48[315,136]	1.23 (1.06-1.43)
Anxiety	Associated (maternal stress)[269]	1.21 (0.98-1.49)	SSRIs: 1.52[319]	1.25 (1.07-1.47)
Depression		1.80 (1.61-2.01)		1.36 (1.24-1.49)
Epilepsy	Inconsistent		Valproate: 7.4[319]	1.36 (1.03-1.79)
Genitourinary tract infection	-		Associated[315]	1.08 (0.99-1.18)
Sexually Transmitted Infection	-		Inconsistent[315]	
Treatment of chronic medical conditions	-		-	
Male baby	1.6-3 fold risk in male children[269]	5.26 (4.79-5.77)	Male predominance: 4 fold[114]	4.57 (4.27-4.89)
Gestational weeks	2.2-4.0 fold risk in preterm[322]	1.24-1.28	Inconsistent[315]	

*Hypospadias account for most genital malformation events (88% in this study), and most of the literature studied hypospadias instead of “genital malformation”. Therefore evidence for hypospadias is used here to compare with the results of genital malformation in this study. RR: risk ratios. VS: versus. AEDs: antiepileptic drugs.4.2.2 Neurodevelopmental disorders

6.4.3 Summary on definition and validation of outcomes indicators

The comparisons with external evidence show that diagnostic codes used to identify major malformations in the CPRD studies had with high sensitivity and specificity, according to the slightly higher prevalence as compared with the national registry, high PPVs reported by previous validation studies, and a profile of risk factors generally comparable with literature.

Among the four neurodevelopmental disorders, cerebral palsy, epilepsy and ASD identified in this study achieve prevalence comparable with external evidence from surveys and studies using primary care data. The prevalence of ADHD could be lower than that estimated in surveys but comparable with that estimated using primary care data. The complex nature of neurodevelopmental disorders and a following tendency of under-recording diagnoses justified identification approaches incorporating diagnoses and other specific codes such as treatments and symptoms. The high PPVs of the identification approaches for these neurodevelopmental disorders (except for cerebral palsy) has been demonstrated by previous validation studies. In the next section I use internal validation for cerebral palsy.

One disadvantage, however, is that the CPRD mother-baby linkage would not be possible to ascertain malformations that result in pregnancy termination or death before the child can be registered with the GP. However, the prevalence of malformation measured at age 1 was comparable between this study and EUROCAT registry (as mentioned in Section 6.3.1), suggesting a comparable prevalence in live-births and a limited influence of death before registering with GPs. There is still a potential live-birth bias due to restricting the analyses to live-births. The live-birth bias is with unknown direction and will be evaluated using a quantitative bias analysis in Chapter 7.

6.4.4 How this chapter inform my thesis

The work conducted in this chapter informs the validity of the outcome definition, which in turn affects the validity of the association analyses and control of confounding, which are described in the following chapter.

The derivation and validation of outcomes in this chapter, shows that prevalence rates and associations with risk factors are consistent with previous literature. However, outcomes recorded in primary care data may be misclassified. Such misclassification is likely to be non-differential, meaning not conditioned on the type of antibiotic exposure in pregnancy, and would be expected to bias the relative risks/hazards towards null (see Section 6.1.3). In the next chapter, I quantify the underestimation of effect due to misclassification in a sensitivity analysis.

The sensitivity analysis captures the missing signals for an adverse effect of prescriptions which is important for a drug safety study.

The validation of risk factors for adverse child outcomes reported in this chapter will be used to develop a propensity score- to balance potential confounders between macrolides and penicillins groups in the cohort study in Chapter 7. In order to form an optimal propensity score, I include not only known confounders (associated with both exposure and outcomes), but factors only associated with outcomes and not necessarily with macrolides (versus penicillins) prescriptions.[323] Therefore most of the risk factors tested in this chapter will be included in the model for calculating the propensity score, except baby gender and gestational age. Baby gender could be a potential effect modifier for the associations between macrolides exposure and adverse outcomes. Gestational age will not be adjusted for as it is considered as a mediator on the causal between fetal damage and cerebral palsy or a descending proxy for an intermediate variable (e.g. cerebral damages), rather than a risk factor for adverse child outcomes. I did not include postnatal risk factors for adverse child outcomes, such as head trauma, meningitis and encephalitis, as covariates in the study, as these events may also mediate causal pathways from prenatal insult to adverse child outcomes. Nevertheless, the prevalence of these events was relatively low.

6.5 A machine learning approach to identify cases of cerebral palsy using the UK primary care database

6.5.1 Introduction

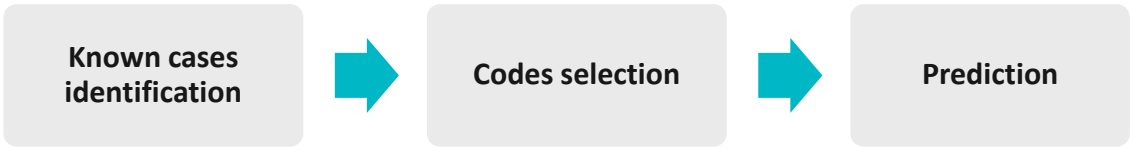
Cerebral palsy (CP) is a complex condition that can manifest in different ways, including disorders of sensation, cognition, behaviour, epilepsy, and musculoskeletal problems. Given this complexity, NICE guidelines suggest that a formal diagnosis of CP should be initiated by specialists and patients should be managed by a multi-disciplinary team, including primary and secondary care. This arrangement for specialist care may partly account for under-recording of CP in primary care databases.[56,244] A further reason may be that GPs tend to record symptom-related or treatment codes, rather than specific diagnoses for CP. In this study, the prevalence of recorded CP diagnosis was1.4 per 1000 people under 25 years old, which is much lower than the generally recognised prevalence ranging from 2 to 3 per 1000 in developed countries. I therefore developed a machine learning approach to improve identification of CP in CPRD.

To identify under-recorded CP cases from CPRD, I included symptoms, treatments and managements that are associated with CP diagnosis. For example, symptoms include spasticity, dyskinesia and ataxia are common in CP cases, while some otherwise uncommon drugs, e.g. Baclofen, tizanidine and diazepam are frequently prescribed for patients with CP. However, these symptoms and treatments can also be recorded in children without CP. An algorithm which can automatically identify the predicative variables and a subsequent prediction model is needed. This appendix describes how I develop an algorithm to identify highly predictive diagnosis, treatment or symptom codes and thus predict under-recorded CP cases in CPRD. This approach was based on the random forest method. An abstract for this approach is available online.[253]

6.5.2 Methods and Results

The case identification approach can be summarised as the following four steps: 1) **Known cases identification**. Some CP cases could be readily identified ("known cases") using a predefined diagnostic code list; 2) **Selection of predictors.** A comparison of the medical history between known cases and the remaining "not known cases" could identify potential predictors for CP diagnoses using the random forest method; 3) **Prediction.** Using the identified predictors from Step 2, I built a logistic prediction model. This simple model provides the likelihood of being a case for each child, thereby facilitating the classification of a large number of children according to CP status (Figure 6-3).

Figure 6-3. Four steps of the approach to identify under-recorded cerebral palsy cases.



Step 1: Known cases identification

The known cases were identified using 43 diagnostic codes for cerebral palsy, which were previously derived by Meeraus et al, using clinical review of all primary care trajectories for possible cases.[25] Among the CPRD Mother Baby Cohort (n=728,921), patients with at least one of the selected diagnostic codes were classified as known cerebral palsy cases (n=956).

Step 2: Codes selection

2.1: Preliminary selection of predictors

There are more than 63,000 distinct read codes and prescription codes available in the medical history of children in the CPRD Mother Baby Cohort. Most of codes were not associated with cerebral palsy, so only those potentially relevant codes were considered. I first selected relevant codes by comparing the relative frequencies of each code *i* in the database within known cerebral palsy cases with the relative frequencies of each code *i* within all children who were not known cases (controls). Next, calculated the strength of the association between the codes and cerebral palsy diagnoses was evaluated using two values, one as the difference and another as the ratio between the two relative frequencies:

$$FreqDiff_i = \left(\frac{Freq_i}{\sum_i^n Freq_i}\middle|Cases\right) - \left(\frac{Freq_i}{\sum_i^n Freq_i}\middle|Controls\right), where\ n\ is\ the\ number\ of\ codes$$

$$FreqRatio_i = \left(\frac{Freq_i}{\sum_i^n Freq_i}\middle|Cases\right) \div \left(\frac{Freq_i}{\sum_i^n Freq_i}\middle|Controls\right), where\ n\ is\ the\ number\ of\ codes$$

2766 codes with a more extreme value of FreqDiff or FreqRatio (FreqDiff>0.00001 or FreqRatio>1 or FreqRatio<0.5) were selected as preliminarily predicators. The cut-off was set according to the distribution of FreqDiff and FreqRatio and the clinical significance of the codes.

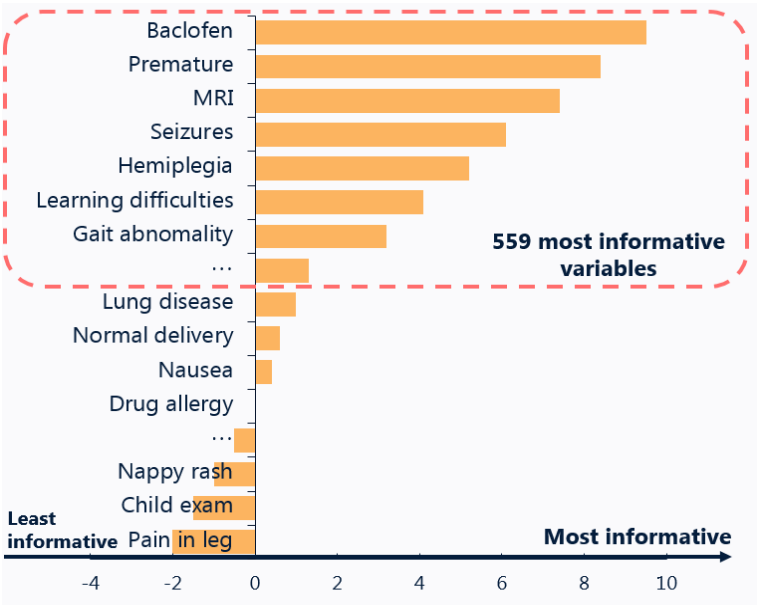
2.2: Predictor selection using Random forests

The random forests method was applied to rank the importance of 2766 predictors with interaction of predictors considered. Random forests is an ensemble machine learning classifier consisting of many local classification tree classifiers.[324] Importantly, the "grouping property" of sub-trees enables the Random forests to adeptly deal with correlation and interaction among variables.[324]

I used the decrease of accuracy quantity to rank the importance of the predictors. The decrease of accuracy quantity gives an indication of the strength of the overall discriminative ability of a particular code for classifying cerebral palsy status. The random forest method drew bootstrap samples from both the cases and non-cases to construct each of the trees and then used the remaining data to estimate the accuracy of the tree. The values of a specific predicator were randomly permuted and the accuracy was calculated repeatedly using the same remaining data. The decrease in accuracy of the predictor is then calculated for all trees and then averaged.[324] Additionally, to deal with the the imbalance in data (cerebral palsy cases constitutes only a very small minority of the data), I under-sampled the majority class (not known cases) by drawing a bootstrap sample with 5 times of the size of the minority class (known cases), to form a more balanced dataset to feed the random forests model. The algorithm implementation and parameter tuning were performed with the packages of 'randomForest' in RStudio version 3.1.2.

Using the balanced dataset, the random forests method calculated a mean decrease in accuracy for each of the 2766 codes. Among these codes, 559 codes with the highest decrease in accuracy were selected as predictors (with mean decrease accuracy ≥1.1 in this case, as illustrated in Figure 6-4). The cut-off for the mean decrease in accuracy was set according to its distribution and the clinical significance of the codes.

Figure 6-4. Variable importance measured by mean decrease in accuracy.



Step 3: Prediction using a logistic regression model

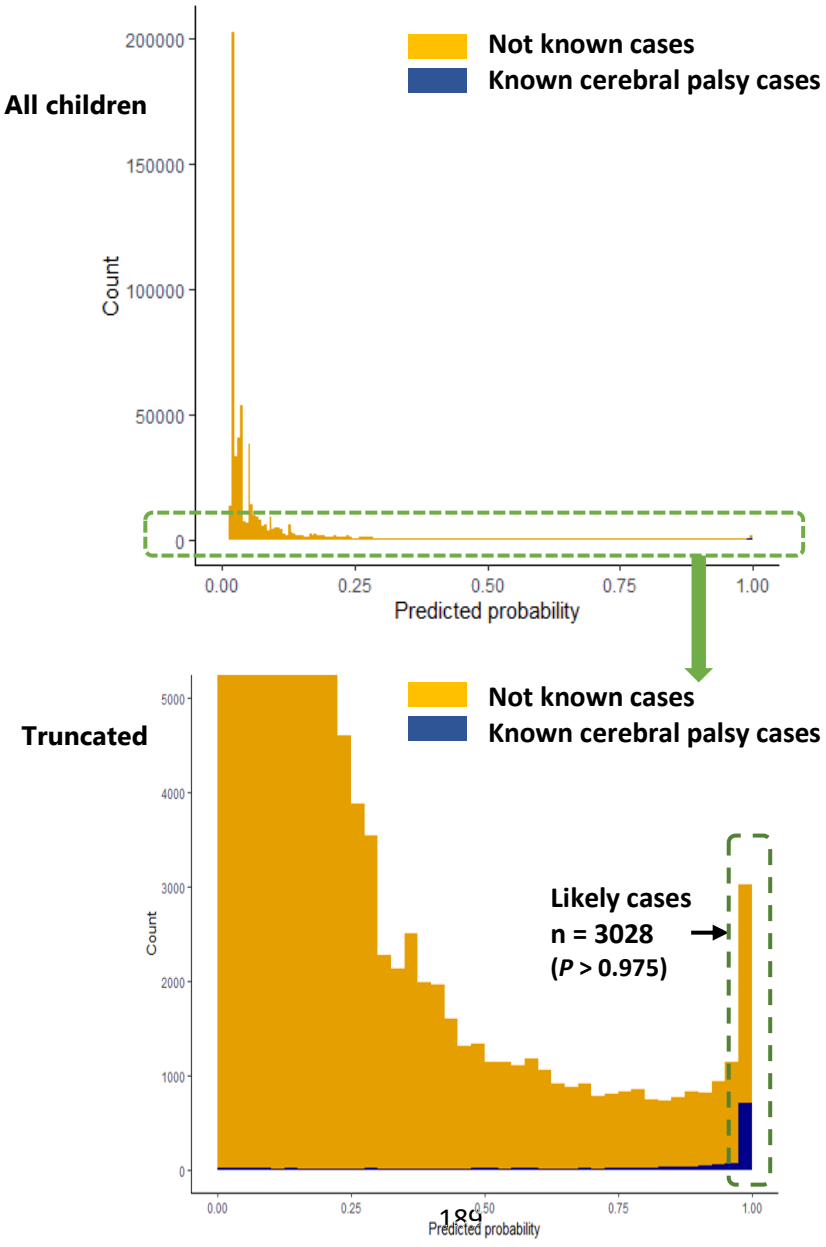
The 559 selected predictors were used to build a logistic prediction model:

$$\text{Logit}\,(P\,(\text{CP}=1)) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots +$$

$$\beta_{559} X_{559}, \text{where } X_1 \text{ was the } i\text{th selected predictor.}$$

The predicted probability for cerebral palsy of each child in CPRD Mother Baby Cohort was then calculated based on the estimated model. As expected, the distribution of predicted probability of known cerebral palsy cases was clustered close to the predicted probability of one (Figure 6-5). Interestingly, the predicted probability of not known cases presented a bimodal distribution: while most not known cases had a lower predicted probability (95% not known cases with predicted probability <0.38), there was also a clustering of predicted probability of cerebral palsy close to one. The cluster of 3028 "not known cases" with a high predicted probability for cerebral palsy diagnosis (p>0.975) were therefore labelled as likely cases.

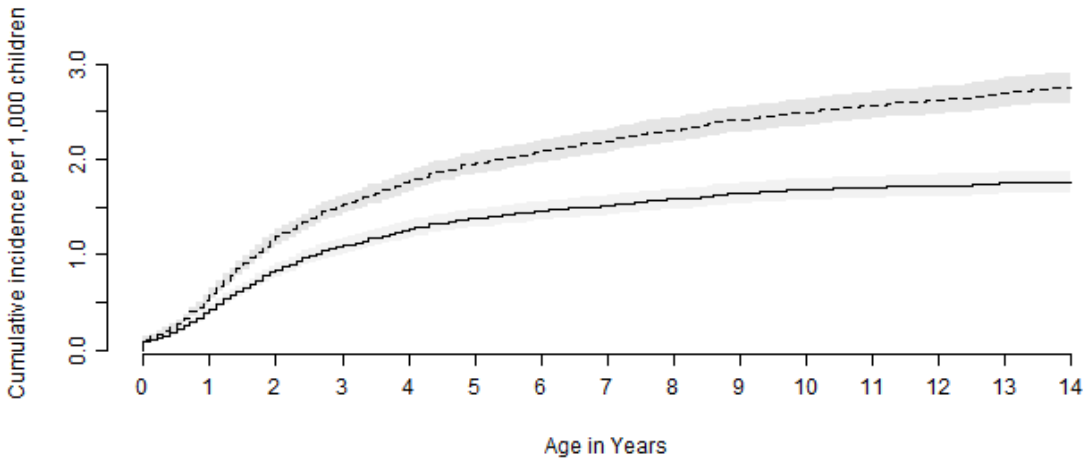
Figure 6-5. Distribution of predicted probability for CP.



An exploration of the medical history of the 3028 likely cases suggested that the likely cases represented a population with some adverse neurological condition (e.g. specific delays in development, neurological referral, gastrostomy feeding, multiple prescription of Hyoscine to control drooling, hip joint X-ray, low vision and seizures), but was not specific enough to ascertain a cerebral palsy diagnosis. Therefore, a code list including rarer but cerebral-palsy-related treatment, symptom or management codes was used to further restrict the likely cases to cerebral palsy cases. The pre-defined code list was derived based on the work of Meeraus et al and incorporated the input of a paediatric neurologist Dr. Finbar O'Callaghan.[56] Examples of the cerebral-palsy-related codes include prescription of muscle relaxer and an antispasmodic agent such as Baclofen, spastic gait, knee joint contracture, and lengthening of tendon Achilles.

672 cases out of the 3028 likely cases were identified as cerebral palsy cases, based on the presence of at least one occurrence of cerebral-palsy-related treatment, symptom or management in their medical history. Together with the 956 known cases identified using diagnostic code, a total of 1628 cerebral palsy cases were identified in the CPRD Mother Baby Cohort (at any age), resulting in an increase in cumulative incidence of cerebral palsy from 1.8 (1.7-1.9), using only diagnostic codes for cerebral palsy, to 2.7 (2.5-2.8) per 1,000 children by age 14, using the random forest method. The cumulative incidence curves by age 14 using the two approaches were shown in Figure 6-6.

Figure 6-6. Kaplan-Meier cumulative incidence risk of CP using the random-forest based identification approach plus diagnosed cases (dashed line), and diagnosed cases alone (solid line).



Reassuringly, the distributions of potential risk factors measured in CP cases using two methods were quite comparable. Also, four out of the six hazard ratios associated with the diagnosed CP cases were also found to be associated with the newly defined CP cases, though with weaker strength (the weaker strength was also observed for the left two risk factors) (Table 6-8).

Table 6-8. Associations between potential risk factors and CP identified using the random forests based prediction (plus diagnostic codes) and diagnostic codes alone.

	Cerebral palsy identified using prediction				Cerebral palsy identified using diagnostic code			
Maternal and pregnancy factors	**Cases (%)**	**Non-cases (%)**	**Std.diff**	**Adj. HR for Cases**	**Cases (%)**	**Non-cases (%)**	**Std.diff**	**Adj. HR for Cases**
Number of pregnancies	1408 (0.2)	727513 (99.8)			947 (0.1)	727974 (99.9)		
Maternal age			0.042				0.063	
13-19	58 (4.1)	28214 (3.9)		0.95 [0.72, 1.26]	34 (3.6)	28238 (3.9)		0.78 [0.55, 1.12]
20-24	215 (15.3)	104150 (14.3)		1.04 [0.88, 1.23]	152 (16.1)	104213 (14.3)		1.05 [0.86, 1.29]
25-29	376 (26.7)	191869 (26.4)		Reference	259 (27.3)	191986 (26.4)		Reference
30-34	430 (30.5)	234887 (32.3)		0.94 [0.82, 1.08]	286 (30.2)	235031 (32.3)		0.92 [0.78, 1.09]
35-50	329 (23.4)	168393 (23.1)		0.98 [0.85, 1.14]	216 (22.8)	168506 (23.1)		0.95 [0.79, 1.74]
Pregnancy year			0.425				0.419	
1990-1994	215 (15.3)	80188 (11.0)		Reference	161 (17.0)	80242 (11.0)		Reference
1995-1999	321 (22.8)	129539 (17.8)		0.93 [0.78, 1.11]	219 (23.1)	129641 (17.8)		0.84 [0.69, 1.04]
2000-2004	390 (27.7)	154294 (21.2)		0.96 [0.81, 1.15]	248 (26.2)	154436 (21.2)		0.78 [0.64, 0.96]
2005-2009	333 (23.7)	174387 (24.0)		0.84 [0.70, 1.00]	213 (22.5)	174507 (24.0)		0.66 [0.53, 0.82]
2010-2016	149 (10.6)	189105 (26.0)		0.55 [0.44, 0.69]	106 (11.2)	189148 (26.0)		0.47 [0.36, 0.61]
Alcohol misuse	69 (4.9)	34234 (4.7)	0.009	1.11 [0.87, 1.42]	49 (5.2)	34254 (4.7)	0.022	1.18 [0.88, 1.58]
Illicit drug use	20 (1.4)	6421 (0.9)	0.050	1.44 [0.92, 2.26]	13 (1.4)	6428 (0.9)	0.046	1.33 [0.76, 2.33]
Tobacco use	421 (29.9)	214339 (29.5)	0.010	0.96 [0.85, 1.08]	285 (30.1)	214475 (29.5)	0.014	0.97 [0.84, 1.12]
Maternal obesity	164 (11.6)	92589 (12.7)	0.033	1.04 [0.88, 1.24]	108 (11.4)	92645 (12.7)	0.041	1.01 [0.82, 1.25]
Hypertension	109 (7.7)	48497 (6.7)	0.042	1.06 [0.87, 1.30]	82 (8.7)	48524 (6.7)	0.075	1.20 [0.95, 1.52]
Diabetes	45 (3.2)	21104 (2.9)	0.017	1.09 [0.80, 1.49]	30 (3.2)	21119 (2.9)	0.016	1.05 [0.72, 1.53]
Anxiety	34 (2.4)	14574 (2.0)	0.028	0.90 [0.63, 1.29]	21 (2.2)	14587 (2.0)	0.015	0.77 [0.49, 1.20]
Depression	153 (10.9)	57414 (7.9)	0.102	1.46 [1.21, 1.74]	111 (11.7)	57456 (7.9)	0.129	1.64 [1.32, 2.03]
Epilepsy	15 (1.1)	4147 (0.6)	0.055	1.51 [0.90, 2.54]	11 (1.2)	4151 (0.6)	0.064	1.64 [0.89, 3.01]
GUTIs during pregnancy	142 (10.1)	61613 (8.5)	0.056	1.18 [0.99, 1.41]	101 (10.7)	61654 (8.5)	0.075	1.24 [1.01, 1.53]
STIs during pregnancy	16 (1.1)	8743 (1.2)	0.006	0.83 [0.51, 1.37]	10 (1.1)	8749 (1.2)	0.014	0.75 [0.40, 1.40]
Chronic condition during pregnancy	215 (15.3)	88914 (12.2)	0.089	1.22 [1.04, 1.44]	148 (15.6)	88981 (12.2)	0.098	1.20 [0.99, 1.46]
Parity >=1	411 (29.2)	238814 (32.8)	0.079	0.89 [0.79, 1.00]	247 (26.1)	238978 (32.8)	0.148	0.78 [0.67, 0.90]

(Continued)

	Cerebral palsy identified using prediction				Cerebral palsy identified using diagnostic code			
Maternal and pregnancy factors	**Cases (%)**	**Non-cases (%)**	**Std.diff**	**Adj. HR for Cases**	**Cases (%)**	**Non-cases (%)**	**Std.diff**	**Adj. HR for Cases**
Multiple births	91 (6.5)	20743 (2.9)	0.172	1.17 [0.94, 1.46]	77 (8.1)	20757 (2.9)	0.233	1.40 [1.09, 1.78]
Sex (Male)	849 (60.3)	373064 (51.3)	0.182	1.42 [1.28, 1.58]	359 (37.9)	354649 (48.7)	0.219	1.53 [1.34, 1.74]
Gestational weeks			0.698				0.804	
23-27	67 (4.8)	3796 (0.5)		12.47 [9.72,	50 (5.3)	3813 (0.5)		14.88 [11.11, 19.92]
28-31	108 (7.7)	6198 (0.9)		11.90 [9.71,	93 (9.8)	6213 (0.9)		16.14 [12.88, 20.21]
32-34	94 (6.7)	11834 (1.6)		5.46 [4.40, 6.79]	65 (6.9)	11863 (1.6)		5.91 [4.54, 7.69]
35-36	185 (13.1)	25086 (3.4)		5.13 [4.36, 6.04]	142 (15.0)	25129 (3.5)		6.17 [5.09, 7.74]
37-38	141 (10.0)	99260 (13.6)		1.15 [0.96, 1.38]	91 (9.6)	99310 (13.6)		1.22 [0.97, 1.52]
>=39	813 (57.7)	581339 (79.9)		Reference	506 (53.4)	581646 (79.9)		Reference

*Std.diff: standardised difference. GUTIs: genitourinary tract infections.HR: hazard ratio

A paediatric neurologist (FC) manually reviewed the full medical history of a 20% randomly selected sample of the 672 identified cases (n=135), and validated 123 cases, resulting in a positive predictive value of 91%.

6.5.3 Discussion

Data-driven schemes, such as the random forest method, have the potential of identifying the most informative predictors in a cost-effective way to reliably identify potential unrecorded cases of cerebral palsy or other complex medical conditions in primary care databases.

Only one previous study applied codes other than diagnostic codes to improve the case identification of cerebral palsy cases in UK's primary care settings.[56] The study developed the code list previously mentioned, including about 430 codes of cerebral-palsy-related symptoms, treatments and managements. Children were identified if they had ≥ one diagnostic codes, or ≥ one code from the symptoms, treatment and management code list. Although the identification rule was simple, after manual review for the full medical history of all he cases identified using the code list, the author excluded 537 out of the 733 cases because of lack of obvious signs for cerebral palsy or identifying alternative explanations of the codes hit. The reason for the inefficiency, as pointed out by the author, was that the code list for symptoms, treatments and managements was not highly specific.

There are several reasons why a complex algorithm improves identification of cerebral palsy in primary care records. Firstly, there was no single, highly specific symptom or treatment for cerebral palsy. Codes related to cerebral-palsy are also used for other conditions. For example, the common prescription for cerebral palsy, Baclofen, could be used for pain of muscle spasm with any cause. Secondly, while a pattern or a combination of traits are necessary to improve the specificity, the heterogeneous phenotypes and rarity of the cerebral palsy related codes make this approach impractical. In other words, the information contained in clinical rule-based cerebral-palsy-related codes might not be adequate to identify cerebral palsy cases.

The strength of the data-driven method in this study, was that it automatically selected the most informative predictors for cerebral palsy diagnosis from a large number of coded data elements. These selected predictors are different from the clinical rule-based cerebral-palsy-related code list in that the method aimed for both high specificity and sensitivity. Data driven predictors identified more common but still relevant predictors, such as "Premature" and "Developmental

delay". These predictors made it possible to identify the pool of likely cases, which could then be readily restricted to more specific cases, without manual review.

The approach could be improved by incorporating timing of the predictors, an iterative approach, and a better resampling and classification algorithm to select more informative codes. Lack of a population-based gold standard for cerebral palsy makes a comprehensive estimation of performance of the approach difficult. Nevertheless, the estimated high positive predicted value implies the identified cerebral palsy cases were appropriate for the analyses of the relative risk in the cohort study.

6.6 Appendix

Appendix 6-1. Associations between any major malformation and potential risk factors.

Maternal and pregnancy factors	Cases (%)	Non-cases (%)	Std.diff	Adj. RR for Outcome
Number of pregnancies	13162 (1.8)	715759 (98.2)		
Maternal age at pregnancy			0.041	
13-19	558 (4.2)	27714 (3.9)		1.09 [1.00, 1.20]
20-24	1951 (14.8)	102414 (14.3)		1.05 [0.99, 1.11]
25-29	3382 (25.7)	188863 (26.4)		Reference
30-34	4089 (31.1)	231228 (32.3)		0.98 [0.93, 1.02]
35-50	3182 (24.2)	165540 (23.1)		1.02 [0.97, 1.07]
Pregnancy start year			0.064	
1990-1994	1343 (10.2)	79060 (11.0)		Reference
1995-1999	2144 (16.3)	127716 (17.8)		0.97 [0.91, 1.04]
2000-2004	2920 (22.2)	151764 (21.2)		1.07 [1.00, 1.14]
2005-2009	3405 (25.9)	171315 (23.9)		1.09 [1.02, 1.16]
2010-2016	3350 (25.5)	185904 (26.0)		0.99 [0.92, 1.05]
Maternal alcohol misuse	659 (5.0)	33644 (4.7)	0.014	1.04 [0.96, 1.12]
Maternal illicit drug use	160 (1.2)	6281 (0.9)	0.033	1.19 [1.02, 1.40]
Recent tobacco use	3936 (29.9)	210824 (29.5)	0.010	0.97 [0.93, 1.01]
Maternal obesity	1892 (14.4)	90861 (12.7)	0.049	1.07 [1.02, 1.13]
Hypertension during pregnancy	1084 (8.2)	47522 (6.6)	0.061	1.12 [1.05, 1.19]
Diabetes during pregnancy	577 (4.4)	20572 (2.9)	0.081	1.31 [1.20, 1.43]
Anxiety during pregnancy	348 (2.6)	14260 (2.0)	0.043	1.17 [1.04, 1.30]
Depression during pregnancy	1162 (8.8)	56405 (7.9)	0.034	1.00 [0.94, 1.07]
Epilepsy during pregnancy	121 (0.9)	4041 (0.6)	0.041	1.39 [1.16, 1.66]
Genitourinary infection during pregnancy	1281 (9.7)	60474 (8.4)	0.045	1.13 [1.07, 1.20]
STIs during pregnancy	139 (1.1)	8620 (1.2)	0.014	0.88 [0.74, 1.03]
Chronic conditions during pregnancy	2038 (15.5)	87091 (12.2)	0.096	1.15 [1.09, 1.21]
Parity >=1	4215 (32.0)	235010 (32.8)	0.017	0.97 [0.93, 1.00]
Multiple births	669 (5.1)	20165 (2.8)	0.117	1.30 [1.20, 1.41]
Sex (Male)	5079 (38.6)	349929 (48.9)	0.209	1.50 [1.45, 1.56]
Gestational weeks			0.316	
23-27	304 (2.3)	3559 (0.5)		4.76 [4.26, 5.31]
28-31	343 (2.6)	5963 (0.8)		3.17 [2.85, 3.52]
32-34	444 (3.4)	11484 (1.6)		2.14 [1.94, 2.35]
35-36	983 (7.5)	24288 (3.4)		2.24 [2.10, 2.40]
37-38	1820 (13.8)	97581 (13.6)		1.11 [1.05, 1.16]
>=39	9268 (70.4)	572884 (80.0)		Reference

*Std.diff: standardised difference. RR: risk ratio.

Appendix 6-2. Associations between nervous system malformation and potential risk factors.

Maternal and pregnancy factors	Cases (%)	Non-cases (%)	Std.diff	Adj. RR for Outcome
Number of pregnancies	687 (0.0)	728234 (100.0)		
Maternal age at pregnancy			0.130	
13-19	37 (5.4)	28235 (3.9)		1.30 [0.91, 1.86]
20-24	118 (17.2)	104247 (14.3)		1.14 [0.91, 1.44]
25-29	190 (27.7)	192055 (26.4)		Reference
30-34	199 (29.0)	235118 (32.3)		0.86 [0.71, 1.05]
35-50	143 (20.8)	168579 (23.1)		0.85 [0.68, 1.06]
Pregnancy start year			0.186	
1990-1994	94 (13.7)	80309 (11.0)		Reference
1995-1999	117 (17.0)	129743 (17.8)		0.76 [0.58, 1.00]
2000-2004	179 (26.1)	154505 (21.2)		0.94 [0.73, 1.22]
2005-2009	163 (23.7)	174557 (24.0)		0.74 [0.57, 0.96]
2010-2016	134 (19.5)	189120 (26.0)		0.55 [0.42, 0.73]
Maternal alcohol misuse	29 (4.2)	34274 (4.7)	0.023	0.91 [0.62, 1.32]
Maternal illicit drug use	11 (1.6)	6430 (0.9)	0.065	1.51 [0.82, 2.78]
Recent tobacco use	208 (30.3)	214552 (29.5)	0.018	0.94 [0.79, 1.11]
Maternal obesity	97 (14.1)	92656 (12.7)	0.041	1.14 [0.91, 1.44]
Hypertension during pregnancy	57 (8.3)	48549 (6.7)	0.062	1.18 [0.89, 1.56]
Diabetes during pregnancy	30 (4.4)	21119 (2.9)	0.078	1.45 [0.99, 2.13]
Anxiety during pregnancy	24 (3.5)	14584 (2.0)	0.091	1.42 [0.92, 2.19]
Depression during pregnancy	75 (10.9)	57492 (7.9)	0.104	1.27 [0.98, 1.65]
Epilepsy during pregnancy	10 (1.5)	4152 (0.6)	0.089	2.07 [1.09, 3.93]
Genitourinary infection during pregnancy	68 (9.9)	61687 (8.5)	0.049	1.10 [0.85, 1.41]
STIs during pregnancy	7 (1.0)	8752 (1.2)	0.017	0.79 [0.38, 1.67]
Chronic conditions during pregnancy	109 (15.9)	89020 (12.2)	0.105	1.17 [0.93, 1.48]
Parity >=1	209 (30.4)	239016 (32.8)	0.052	0.96 [0.81, 1.13]
Multiple births	24 (3.5)	20810 (2.9)	0.036	0.89 [0.59, 1.36]
Sex (Male)	321 (46.7)	354687 (48.7)	0.040	1.07 [0.93, 1.25]
Gestational weeks			0.311	
23-27	15 (2.2)	3848 (0.5)		4.53 [2.71, 7.57]
28-31	16 (2.3)	6290 (0.9)		2.89 [1.76, 4.77]
32-34	30 (4.4)	11898 (1.6)		2.89 [1.99, 4.19]
35-36	49 (7.1)	25222 (3.5)		2.23 [1.65, 3.00]
37-38	85 (12.4)	99316 (13.6)		1.03 [0.82, 1.30]
>=39	492 (71.6)	581660 (79.9)		Reference

*Std.diff: standardised difference. RR: risk ratio.

Appendix 6-3. Associations between cardiovascular malformation and potential risk factors.

Maternal and pregnancy factors	Cases (%)	Non-cases (%)	Std.diff	Adj. RR for Outcome
Number of pregnancies	5156 (0.7)	723765 (99.3)		
Maternal age at pregnancy			0.087	
13-19	218 (4.2)	28054 (3.9)		1.19 [1.03, 1.37]
20-24	731 (14.2)	103634 (14.3)		1.08 [0.98, 1.18]
25-29	1254 (24.3)	190991 (26.4)		Reference
30-34	1583 (30.7)	233734 (32.3)		1.02 [0.95, 1.10]
35-50	1370 (26.6)	167352 (23.1)		1.18 [1.09, 1.28]
Pregnancy start year			0.055	
1990-1994	509 (9.9)	79894 (11.0)		Reference
1995-1999	868 (16.8)	128992 (17.8)		1.03 [0.92, 1.15]
2000-2004	1136 (22.0)	153548 (21.2)		1.08 [0.97, 1.20]
2005-2009	1312 (25.4)	173408 (24.0)		1.06 [0.96, 1.18]
2010-2016	1331 (25.8)	187923 (26.0)		0.97 [0.87, 1.08]
Maternal alcohol misuse	270 (5.2)	34033 (4.7)	0.025	1.09 [0.96, 1.23]
Maternal illicit drug use	66 (1.3)	6375 (0.9)	0.039	1.29 [1.01, 1.65]
Recent tobacco use	1496 (29.0)	213264 (29.5)	0.010	0.94 [0.88, 1.00]
Maternal obesity	779 (15.1)	91974 (12.7)	0.069	1.12 [1.03, 1.21]
Hypertension during pregnancy	420 (8.1)	48186 (6.7)	0.057	1.08 [0.98, 1.20]
Diabetes during pregnancy	284 (5.5)	20865 (2.9)	0.131	1.64 [1.44, 1.85]
Anxiety during pregnancy	131 (2.5)	14477 (2.0)	0.036	1.08 [0.90, 1.29]
Depression during pregnancy	468 (9.1)	57099 (7.9)	0.043	1.03 [0.93, 1.14]
Epilepsy during pregnancy	54 (1.0)	4108 (0.6)	0.054	1.53 [1.17, 2.01]
Genitourinary infection during pregnancy	508 (9.9)	61247 (8.5)	0.048	1.15 [1.05, 1.26]
STIs during pregnancy	55 (1.1)	8704 (1.2)	0.013	0.89 [0.68, 1.16]
Chronic conditions during pregnancy	867 (16.8)	88262 (12.2)	0.131	1.23 [1.13, 1.33]
Parity >=1	1676 (32.5)	237549 (32.8)	0.007	0.97 [0.92, 1.03]
Multiple births	256 (5.0)	20578 (2.8)	0.110	1.33 [1.17, 1.52]
Sex (Male)	2580 (50.0)	352428 (48.7)	0.027	0.95 [0.90, 1.00]
Gestational weeks			0.216	
23-27	43 (0.8)	3820 (0.5)		1.63 [1.21, 2.20]
28-31	101 (2.0)	6205 (0.9)		2.29 [1.88, 2.79]
32-34	174 (3.4)	11754 (1.6)		2.03 [1.74, 2.36]
35-36	325 (6.3)	24946 (3.4)		1.80 [1.61, 2.03]
37-38	754 (14.6)	98647 (13.6)		1.12 [1.03, 1.21]
>=39	3759 (72.9)	578393 (79.9)		Reference

*Std.diff: standardised difference. RR: risk ratio.

Appendix 6-4. Associations between gastrointestinal malformation and potential risk factors.

Maternal and pregnancy factors	Cases (%)	Non-cases (%)	Std.diff	Adj. RR for Outcome
Number of pregnancies	802 (0.1)	728119 (99.9)		
Maternal age at pregnancy			0.085	
13-19	31 (3.9)	28241 (3.9)		1.03 [0.70, 1.51]
20-24	132 (16.5)	104233 (14.3)		1.21 [0.97, 1.51]
25-29	193 (24.1)	192052 (26.4)		Reference
30-34	246 (30.7)	235071 (32.3)		1.04 [0.86, 1.25]
35-50	200 (24.9)	168522 (23.1)		1.12 [0.91, 1.36]
Pregnancy start year			0.146	
1990-1994	64 (8.0)	80339 (11.0)		Reference
1995-1999	119 (14.8)	129741 (17.8)		1.12 [0.82, 1.52]
2000-2004	194 (24.2)	154490 (21.2)		1.44 [1.08, 1.92]
2005-2009	207 (25.8)	174513 (24.0)		1.32 [0.99, 1.76]
2010-2016	218 (27.2)	189036 (26.0)		1.27 [0.95, 1.70]
Maternal alcohol misuse	34 (4.2)	34269 (4.7)	0.023	0.82 [0.58, 1.16]
Maternal illicit drug use	11 (1.4)	6430 (0.9)	0.046	1.20 [0.65, 2.20]
Recent tobacco use	253 (31.5)	214507 (29.5)	0.045	0.99 [0.85, 1.16]
Maternal obesity	124 (15.5)	92629 (12.7)	0.079	1.10 [0.90, 1.34]
Hypertension during pregnancy	73 (9.1)	48533 (6.7)	0.091	1.18 [0.92, 1.52]
Diabetes during pregnancy	32 (4.0)	21117 (2.9)	0.060	1.08 [0.75, 1.56]
Anxiety during pregnancy	26 (3.2)	14582 (2.0)	0.078	1.26 [0.83, 1.90]
Depression during pregnancy	91 (11.3)	57476 (7.9)	0.117	1.25 [0.98, 1.59]
Epilepsy during pregnancy	6 (0.7)	4156 (0.6)	0.022	1.05 [0.47, 2.38]
Genitourinary infection during pregnancy	94 (11.7)	61661 (8.5)	0.108	1.35 [1.09, 1.68]
STIs during pregnancy	11 (1.4)	8748 (1.2)	0.015	1.15 [0.63, 2.09]
Chronic conditions during pregnancy	132 (16.5)	88997 (12.2)	0.121	1.17 [0.95, 1.43]
Parity >=1	264 (32.9)	238961 (32.8)	0.002	0.97 [0.84, 1.13]
Multiple births	30 (3.7)	20804 (2.9)	0.049	0.83 [0.57, 1.21]
Sex (Male)	330 (41.1)	354678 (48.7)	0.153	1.35 [1.17, 1.55]
Gestational weeks			0.369	
23-27	13 (1.6)	3850 (0.5)		3.61 [2.08, 6.25]
28-31	17 (2.1)	6289 (0.9)		2.85 [1.75, 4.62]
32-34	43 (5.4)	11885 (1.6)		3.80 [2.77, 5.21]
35-36	68 (8.5)	25203 (3.5)		2.83 [2.19, 3.66]
37-38	128 (16.0)	99273 (13.6)		1.32 [1.09, 1.61]
>=39	533 (66.5)	581619 (79.9)		Reference

*Std.diff: standardised difference. RR: risk ratio.

Appendix 6-5. Associations between genital tract malformation and potential risk factors.

Maternal and pregnancy factors	Cases (%)	Non-cases (%)	Std.diff	Adj. RR for Outcome
Number of pregnancies	2433 (0.3)	726488 (99.7)		
Maternal age at pregnancy			0.038	
13-19	112 (4.6)	28160 (3.9)		1.18 [0.96, 1.44]
20-24	352 (14.5)	104013 (14.3)		1.00 [0.88, 1.14]
25-29	645 (26.5)	191600 (26.4)		Reference
30-34	767 (31.5)	234550 (32.3)		0.95 [0.86, 1.06]
35-50	557 (22.9)	168165 (23.1)		0.92 [0.82, 1.04]
Pregnancy start year			0.108	
1990-1994	241 (9.9)	80162 (11.0)		Reference
1995-1999	358 (14.7)	129502 (17.8)		0.92 [0.78, 1.08]
2000-2004	547 (22.5)	154137 (21.2)		1.15 [0.99, 1.35]
2005-2009	657 (27.0)	174063 (24.0)		1.21 [1.04, 1.41]
2010-2016	630 (25.9)	188624 (26.0)		1.08 [0.92, 1.25]
Maternal alcohol misuse	109 (4.5)	34194 (4.7)	0.011	0.94 [0.77, 1.14]
Maternal illicit drug use	24 (1.0)	6417 (0.9)	0.011	1.07 [0.71, 1.61]
Recent tobacco use	691 (28.4)	214069 (29.5)	0.023	0.90 [0.82, 0.99]
Maternal obesity	344 (14.1)	92409 (12.7)	0.042	1.05 [0.93, 1.18]
Hypertension during pregnancy	208 (8.5)	48398 (6.7)	0.071	1.20 [1.04, 1.39]
Diabetes during pregnancy	83 (3.4)	21066 (2.9)	0.029	1.02 [0.82, 1.28]
Anxiety during pregnancy	50 (2.1)	14558 (2.0)	0.004	0.96 [0.72, 1.28]
Depression during pregnancy	205 (8.4)	57362 (7.9)	0.019	1.02 [0.87, 1.19]
Epilepsy during pregnancy	19 (0.8)	4143 (0.6)	0.026	1.28 [0.81, 2.03]
Genitourinary infection during pregnancy	222 (9.1)	61533 (8.5)	0.023	1.08 [0.94, 1.24]
STIs during pregnancy	23 (0.9)	8736 (1.2)	0.025	0.81 [0.53, 1.21]
Chronic conditions during pregnancy	344 (14.1)	88785 (12.2)	0.057	1.09 [0.96, 1.23]
Parity >=1	816 (33.5)	238409 (32.8)	0.015	1.04 [0.95, 1.13]
Multiple births	117 (4.8)	20717 (2.9)	0.102	1.44 [1.19, 1.75]
Sex (Male)	112 (4.6)	354896 (48.9)	1.154	19.63 [16.25, 23.73]
Gestational weeks			0.202	
23-27	17 (0.7)	3846 (0.5)		1.38 [0.86, 2.22]
28-31	47 (1.9)	6259 (0.9)		2.16 [1.62, 2.88]
32-34	76 (3.1)	11852 (1.6)		1.85 [1.47, 2.34]
35-36	156 (6.4)	25115 (3.5)		1.80 [1.52, 2.12]
37-38	340 (14.0)	99061 (13.6)		1.05 [0.93, 1.18]
>=39	1797 (73.9)	580355 (79.9)		Reference

*Std.diff: standardised difference. RR: risk ratio.

Appendix 6-6. Associations between urinary malformation and potential risk factors.

Maternal and pregnancy factors	Cases (%)	Non-cases (%)	Std.diff	Adj. RR for Outcome
Number of pregnancies	1143 (0.2)	727778 (99.8)		
Maternal age at pregnancy			0.045	
13-19	43 (3.8)	28229 (3.9)		1.11 [0.80, 1.56]
20-24	174 (15.2)	104191 (14.3)		1.08 [0.78, 1.49]
25-29	310 (27.1)	191935 (26.4)		Reference
30-34	347 (30.4)	234970 (32.3)		0.97 [0.70, 1.33]
35-50	269 (23.5)	168453 (23.1)		1.01 [0.73, 1.41]
Pregnancy start year			0.104	
1990-1994	104 (9.1)	80299 (11.0)		Reference
1995-1999	202 (17.7)	129658 (17.8)		1.19 [0.94, 1.51]
2000-2004	280 (24.5)	154404 (21.2)		1.34 [1.06, 1.69]
2005-2009	285 (24.9)	174435 (24.0)		1.17 [0.93, 1.48]
2010-2016	272 (23.8)	188982 (26.0)		1.02 [0.80, 1.29]
Maternal alcohol misuse	72 (6.3)	34231 (4.7)	0.070	1.38 [1.09, 1.76]
Maternal illicit drug use	8 (0.7)	6433 (0.9)	0.021	0.74 [0.36, 1.48]
Recent tobacco use	340 (29.7)	214420 (29.5)	0.006	0.96 [0.84, 1.09]
Maternal obesity	158 (13.8)	92595 (12.7)	0.032	1.03 [0.86, 1.23]
Hypertension during pregnancy	99 (8.7)	48507 (6.7)	0.075	1.23 [0.99, 1.52]
Diabetes during pregnancy	53 (4.6)	21096 (2.9)	0.091	1.49 [1.12, 1.99]
Anxiety during pregnancy	23 (2.0)	14585 (2.0)	0.001	1.00 [0.65, 1.53]
Depression during pregnancy	84 (7.3)	57483 (7.9)	0.021	0.85 [0.68, 1.08]
Epilepsy during pregnancy	8 (0.7)	4154 (0.6)	0.016	1.13 [0.56, 2.29]
Genitourinary infection during pregnancy	117 (10.2)	61638 (8.5)	0.061	1.22 [1.01, 1.48]
STIs during pregnancy	13 (1.1)	8746 (1.2)	0.006	0.95 [0.55, 1.65]
Chronic conditions during pregnancy	162 (14.2)	88967 (12.2)	0.058	1.11 [0.92, 1.33]
Parity >=1	376 (32.9)	238849 (32.8)	0.002	1.00 [0.88, 1.13]
Multiple births	47 (4.1)	20787 (2.9)	0.069	1.22 [0.90, 1.65]
Sex (Male)	369 (32.3)	354639 (48.7)	0.340	0.50 [0.44, 0.57]
Gestational weeks			0.171	
23-27	10 (0.9)	3853 (0.5)		0.82 [0.36, 1.85]
28-31	14 (1.2)	6292 (0.9)		0.96 [0.47, 1.95]
32-34	31 (2.7)	11897 (1.6)		0.97 [0.50, 1.88]
35-36	66 (5.8)	25205 (3.5)		0.71 [0.38, 1.35]
37-38	183 (16.0)	99218 (13.6)		0.58 [0.31, 1.07]
>=39	839 (73.4)	581313 (79.9)		Reference

*Std.diff: standardised difference. RR: risk ratio.

Appendix 6-7. Associations between cerebral palsy and potential risk factors.

Maternal and pregnancy factors	Cases (%)	Non-cases (%)	Std.diff	Adj. HR for Cases
Number of pregnancies	1408 (0.2)	727513 (99.8)		
Maternal age at pregnancy			0.042	
13-19	58 (4.1)	28214 (3.9)		0.95 [0.72, 1.26]
20-24	215 (15.3)	104150 (14.3)		1.04 [0.88, 1.23]
25-29	376 (26.7)	191869 (26.4)		Reference
30-34	430 (30.5)	234887 (32.3)		0.94 [0.82, 1.08]
35-50	329 (23.4)	168393 (23.1)		0.98 [0.85, 1.14]
Pregnancy start year			0.425	
1990-1994	215 (15.3)	80188 (11.0)		Reference
1995-1999	321 (22.8)	129539 (17.8)		0.93 [0.78, 1.11]
2000-2004	390 (27.7)	154294 (21.2)		0.96 [0.81, 1.15]
2005-2009	333 (23.7)	174387 (24.0)		0.84 [0.70, 1.00]
2010-2016	149 (10.6)	189105 (26.0)		0.55 [0.44, 0.69]
Maternal alcohol misuse	69 (4.9)	34234 (4.7)	0.009	1.11 [0.87, 1.42]
Maternal illicit drug use	20 (1.4)	6421 (0.9)	0.050	1.44 [0.92, 2.26]
Recent tobacco use	421 (29.9)	214339 (29.5)	0.010	0.96 [0.85, 1.08]
Maternal obesity	164 (11.6)	92589 (12.7)	0.033	1.04 [0.88, 1.24]
Hypertension during pregnancy	109 (7.7)	48497 (6.7)	0.042	1.06 [0.87, 1.30]
Diabetes during pregnancy	45 (3.2)	21104 (2.9)	0.017	1.09 [0.80, 1.49]
Anxiety during pregnancy	34 (2.4)	14574 (2.0)	0.028	0.90 [0.63, 1.29]
Depression during pregnancy	153 (10.9)	57414 (7.9)	0.102	1.46 [1.21, 1.74]
Epilepsy during pregnancy	15 (1.1)	4147 (0.6)	0.055	1.51 [0.90, 2.54]
Genitourinary infection during pregnancy	142 (10.1)	61613 (8.5)	0.056	1.18 [0.99, 1.41]
STIs during pregnancy	16 (1.1)	8743 (1.2)	0.006	0.83 [0.51, 1.37]
Chronic condition during pregnancy	215 (15.3)	88914 (12.2)	0.089	1.22 [1.04, 1.44]
Parity >=1	411 (29.2)	238814 (32.8)	0.079	0.89 [0.79, 1.00]
Multiple births	91 (6.5)	20743 (2.9)	0.172	1.17 [0.94, 1.46]
Sex (Male)	849 (60.3)	373064 (51.3)	0.182	1.42 [1.28, 1.58]
Gestational weeks			0.698	
23-27	67 (4.8)	3796 (0.5)		12.47 [9.72, 16.02]
28-31	108 (7.7)	6198 (0.9)		11.90 [9.71, 14.59]
32-34	94 (6.7)	11834 (1.6)		5.46 [4.40, 6.79]
35-36	185 (13.1)	25086 (3.4)		5.13 [4.36, 6.04]
37-38	141 (10.0)	99260 (13.6)		1.15 [0.96, 1.38]
>=39	813 (57.7)	581339 (79.9)		Reference

*Std.diff: standardised difference. RR: risk ratio.

Appendix 6-8. Associations between epilepsy and potential risk factors.

Maternal and pregnancy factors	Cases (%)	Non-cases (%)	Std.diff	Adj. HR for Cases
Number of pregnancies	4682 (0.6)	724239 (99.4)		
Maternal age at pregnancy			0.111	
13-19	222 (4.7)	28050 (3.9)		1.21 [1.05, 1.39]
20-24	778 (16.6)	103587 (14.3)		1.14 [1.04, 1.24]
25-29	1329 (28.4)	190916 (26.4)		Reference
30-34	1406 (30.0)	233911 (32.3)		0.89 [0.83, 0.96]
35-50	947 (20.2)	167775 (23.2)		0.88 [0.81, 0.96]
Pregnancy start year			0.480	
1990-1994	895 (19.1)	79508 (11.0)		Reference
1995-1999	1217 (26.0)	128643 (17.8)		0.90 [0.82, 0.98]
2000-2004	1155 (24.7)	153529 (21.2)		0.83 [0.76, 0.91]
2005-2009	913 (19.5)	173807 (24.0)		0.80 [0.72, 0.89]
2010-2016	502 (10.7)	188752 (26.1)		0.76 [0.67, 0.86]
Maternal alcohol misuse	200 (4.3)	34103 (4.7)	0.021	1.05 [0.91, 1.21]
Maternal illicit drug use	52 (1.1)	6389 (0.9)	0.023	1.34 [1.01, 1.77]
Recent tobacco use	1397 (29.8)	213363 (29.5)	0.008	1.05 [0.98, 1.12]
Maternal obesity	558 (11.9)	92195 (12.7)	0.025	1.23 [1.12, 1.35]
Hypertension during pregnancy	309 (6.6)	48297 (6.7)	0.003	1.08 [0.96, 1.21]
Diabetes during pregnancy	129 (2.8)	21020 (2.9)	0.009	1.18 [0.99, 1.42]
Anxiety during pregnancy	118 (2.5)	14490 (2.0)	0.035	1.15 [0.95, 1.39]
Depression during pregnancy	416 (8.9)	57151 (7.9)	0.036	1.20 [1.08, 1.34]
Epilepsy during pregnancy	82 (1.8)	4080 (0.6)	0.111	2.67 [2.13, 3.35]
Genitourinary infection during pregnancy	513 (11.0)	61242 (8.5)	0.085	1.30 [1.19, 1.43]
STIs during pregnancy	56 (1.2)	8703 (1.2)	0.001	0.85 [0.65, 1.11]
Chronic condition during pregnancy	630 (13.5)	88499 (12.2)	0.037	1.18 [1.08, 1.30]
Parity >=1	1428 (30.5)	237797 (32.8)	0.050	0.99 [0.93, 1.05]
Multiple births	134 (2.9)	20700 (2.9)	<0.001	0.94 [0.79, 1.12]
Sex (Male)	2138 (45.7)	352870 (48.7)	0.061	1.13 [1.06, 1.19]
Gestational weeks			0.143	
23-27	58 (1.2)	3805 (0.5)		2.44 [1.88, 3.16]
28-31	72 (1.5)	6234 (0.9)		1.83 [1.45, 2.31]
32-34	99 (2.1)	11829 (1.6)		1.33 [1.09, 1.63]
35-36	236 (5.0)	25035 (3.5)		1.52 [1.33, 1.73]
37-38	541 (11.6)	98860 (13.7)		1.03 [0.94, 1.13]
>=39	3676 (78.5)	578476 (79.9)		Reference

*Std.diff: standardised difference. RR: risk ratio.

Appendix 6-9. Associations between ADHD and potential risk factors.

Maternal and pregnancy factors	Cases (%)	Non-cases (%)	Std.diff	Adj. HR for Cases
Number of pregnancies	3347 (0.5)	725574 (99.5)		
Maternal age at pregnancy			0.330	
13-19	248 (7.4)	28024 (3.9)		1.89 [1.64, 2.17]
20-24	738 (22.0)	103627 (14.3)		1.45 [1.31, 1.59]
25-29	994 (29.7)	191251 (26.4)		Reference
30-34	841 (25.1)	234476 (32.3)		0.71 [0.65, 0.78]
35-50	526 (15.7)	168196 (23.2)		0.68 [0.61, 0.76]
Pregnancy start year			0.913	
1990-1994	632 (18.9)	79771 (11.0)		Reference
1995-1999	1016 (30.4)	128844 (17.8)		1.06 [0.95, 1.17]
2000-2004	1119 (33.4)	153565 (21.2)		1.23 [1.11, 1.37]
2005-2009	570 (17.0)	174150 (24.0)		1.45 [1.28, 1.64]
2010-2016	10 (0.3)	189244 (26.1)		0.39 [0.21, 0.75]
Maternal alcohol misuse	154 (4.6)	34149 (4.7)	0.005	1.11 [0.94, 1.31]
Maternal illicit drug use	48 (1.4)	6393 (0.9)	0.052	1.57 [1.17, 2.09]
Recent tobacco use	1333 (39.8)	213427 (29.4)	0.220	1.56 [1.45, 1.68]
Maternal obesity	411 (12.3)	92342 (12.7)	0.014	1.54 [1.38, 1.72]
Hypertension during pregnancy	214 (6.4)	48392 (6.7)	0.011	1.09 [0.94, 1.26]
Diabetes during pregnancy	65 (1.9)	21084 (2.9)	0.063	1.07 [0.83, 1.38]
Anxiety during pregnancy	102 (3.0)	14506 (2.0)	0.067	1.21 [0.98, 1.49]
Depression during pregnancy	423 (12.6)	57144 (7.9)	0.157	1.80 [1.61, 2.01]
Epilepsy during pregnancy	29 (0.9)	4133 (0.6)	0.035	1.18 [0.81, 1.71]
Genitourinary infection during pregnancy	401 (12.0)	61354 (8.5)	0.117	1.34 [1.21, 1.49]
STIs during pregnancy	58 (1.7)	8701 (1.2)	0.044	1.19 [0.91, 1.54]
Chronic condition during pregnancy	443 (13.2)	88686 (12.2)	0.030	1.28 [1.15, 1.43]
Parity >=1	1042 (31.1)	238183 (32.8)	0.036	1.08 [1.00, 1.16]
Multiple births	71 (2.1)	20763 (2.9)	0.048	0.80 [0.63, 1.02]
Sex (Male)	515 (15.4)	354493 (48.9)	0.768	5.26 [4.79, 5.77]
Gestational weeks			0.114	
23-27	22 (0.7)	3841 (0.5)		1.19 [0.78, 1.81]
28-31	37 (1.1)	6269 (0.9)		1.19 [0.86, 1.64]
32-34	75 (2.2)	11853 (1.6)		1.28 [1.01, 1.61]
35-36	149 (4.5)	25122 (3.5)		1.24 [1.05, 1.46]
37-38	356 (10.6)	99045 (13.7)		0.97 [0.87, 1.08]
>=39	2708 (80.9)	579444 (79.9)		Reference

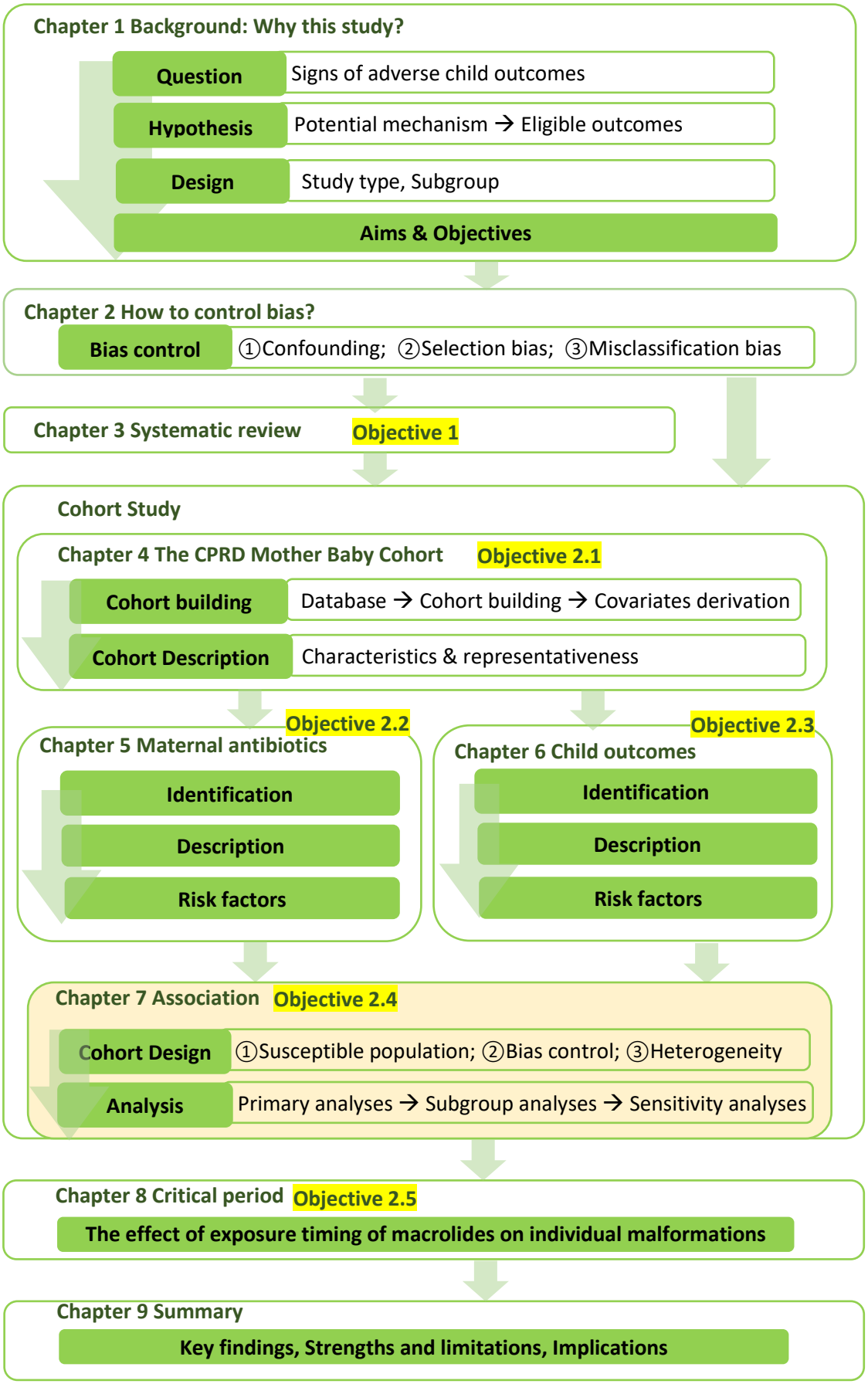
*Std.diff: standardised difference. RR: risk ratio.

Appendix 6-10. Associations between ASD and potential risk factors.

Maternal and pregnancy factors	Cases (%)	Non-cases (%)	Std.diff	Adj. HR for Cases
Number of pregnancies	5719 (0.8)	723202 (99.2)		
Maternal age at pregnancy			0.034	
13-19	205 (3.6)	28067 (3.9)		0.94 [0.81, 1.09]
20-24	838 (14.7)	103527 (14.3)		1.06 [0.98, 1.16]
25-29	1467 (25.7)	190778 (26.4)		Reference
30-34	1820 (31.8)	233497 (32.3)		0.99 [0.92, 1.06]
35-50	1389 (24.3)	167333 (23.1)		1.04 [0.96, 1.12]
Pregnancy start year			0.517	
1990-1994	427 (7.5)	79976 (11.1)		Reference
1995-1999	1320 (23.1)	128540 (17.8)		2.14 [1.92, 2.39]
2000-2004	1866 (32.6)	152818 (21.1)		3.17 [2.84, 3.53]
2005-2009	1594 (27.9)	173126 (23.9)		4.45 [3.97, 4.99]
2010-2016	512 (9.0)	188742 (26.1)		6.20 [5.39, 7.14]
Maternal alcohol misuse	252 (4.4)	34051 (4.7)	0.014	0.93 [0.82, 1.05]
Maternal illicit drug use	60 (1.0)	6381 (0.9)	0.017	1.17 [0.90, 1.52]
Recent tobacco use	1910 (33.4)	212850 (29.4)	0.086	1.15 [1.08, 1.22]
Maternal obesity	890 (15.6)	91863 (12.7)	0.082	1.42 [1.31, 1.53]
Hypertension during pregnancy	490 (8.6)	48116 (6.7)	0.072	1.14 [1.04, 1.26]
Diabetes during pregnancy	189 (3.3)	20960 (2.9)	0.023	1.23 [1.06, 1.43]
Anxiety during pregnancy	171 (3.0)	14437 (2.0)	0.064	1.25 [1.07, 1.47]
Depression during pregnancy	623 (10.9)	56944 (7.9)	0.104	1.36 [1.24, 1.49]
Epilepsy during pregnancy	54 (0.9)	4108 (0.6)	0.043	1.36 [1.03, 1.79]
Genitourinary infection during pregnancy	519 (9.1)	61236 (8.5)	0.021	1.08 [0.99, 1.18]
STIs during pregnancy	84 (1.5)	8675 (1.2)	0.023	1.23 [0.99, 1.53]
Chronic condition during pregnancy	859 (15.0)	88270 (12.2)	0.082	1.24 [1.15, 1.35]
Parity >=1	1824 (31.9)	237401 (32.8)	0.020	0.85 [0.81, 0.90]
Multiple births	160 (2.8)	20674 (2.9)	0.004	0.92 [0.79, 1.08]
Sex (Male)	992 (17.3)	354016 (49.0)	0.713	4.57 [4.27, 4.89]
Gestational weeks			0.056	
23-27	36 (0.6)	3827 (0.5)		1.14 [0.82, 1.58]
28-31	65 (1.1)	6241 (0.9)		1.20 [0.94, 1.54]
32-34	104 (1.8)	11824 (1.6)		1.04 [0.86, 1.27]
35-36	246 (4.3)	25025 (3.5)		1.19 [1.04, 1.35]
37-38	775 (13.6)	98626 (13.6)		1.05 [0.97, 1.14]
>=39	4493 (78.6)	577659 (79.9)		Reference

*Std.diff: standardised difference. RR: risk ratio.

Thesis Structure



Chapter 7 The association between macrolide antibiotic prescribing during pregnancy and adverse child outcomes

7.1 Introduction

In this chapter I used the primary care database to determine the association between macrolide prescribing during pregnancy and adverse child outcomes, based on the previous definition of target population, exposure (macrolides and penicillins), and outcomes (ten adverse child outcomes) from Chapter 4 to 6. The remainder of the introduction describes the design of the cohort analyses. This introduction ends with a description of the objectives specific to this chapter.

7.1.1 Background

In chapter 2, I discussed the general bias structure in evaluating the association between macrolides prescribing during pregnancy and adverse child outcomes, aiming to inform the design for the thesis, i.e. the systematic review (including both RCTs and observational studies) and the cohort study. In this chapter I described the detailed analyses strategy for the cohort study, based on findings from previous chapters.

In Chapter 3 the systematic review showed consistent evidence for an increased risk of miscarriage but less consistent evidence for congenital malformations, cerebral palsy and epilepsy. In Chapter 4, I developed the target population for the cohort study (CPRD Mother Baby Cohort) including all valid mother-baby pairs with or without antibiotics prescribed during pregnancy. The characteristics of the pregnancy cohort were comparable with that of the national statistics except that mothers were older on average. In Chapter 5, I showed that in the CPRD Mother Baby Cohort, among the one-third mothers who were prescribed antibiotics during pregnancy, penicillins were the most frequently prescribed, and macrolides the third most frequently prescribed antibiotic classes. Within these groups, 71% and 84% of the prescriptions were prescribed as a monotherapy, respectively. In the 50% of mothers, where a clinical indication could be matched to the prescription, RTIs accounted for 67% to 75% indications for penicillins and macrolides, respectively. The most significant difference between mothers prescribed macrolides and penicillins was that macrolides were less likely to be prescribed for genitourinary tract infections, and more likely to be prescribed for STIs during pregnancy, than penicillins. In Chapter 6, I identified the ten eligible adverse child outcomes in the CPRD Mother Baby Cohort. Major malformations, cerebral palsy, epilepsy and ASD, were identified using approaches to have high sensitivity and specificity as reported by previous validation studies or as shown by internal validation (cerebral palsy). Although the prevalence

of ADHD identified in the cohort was lower than that reported by surveys, it was comparable with prevalence rates reported by another primary care study, and a high PPV has been reported by previous validation studies (Section 6.4.1).

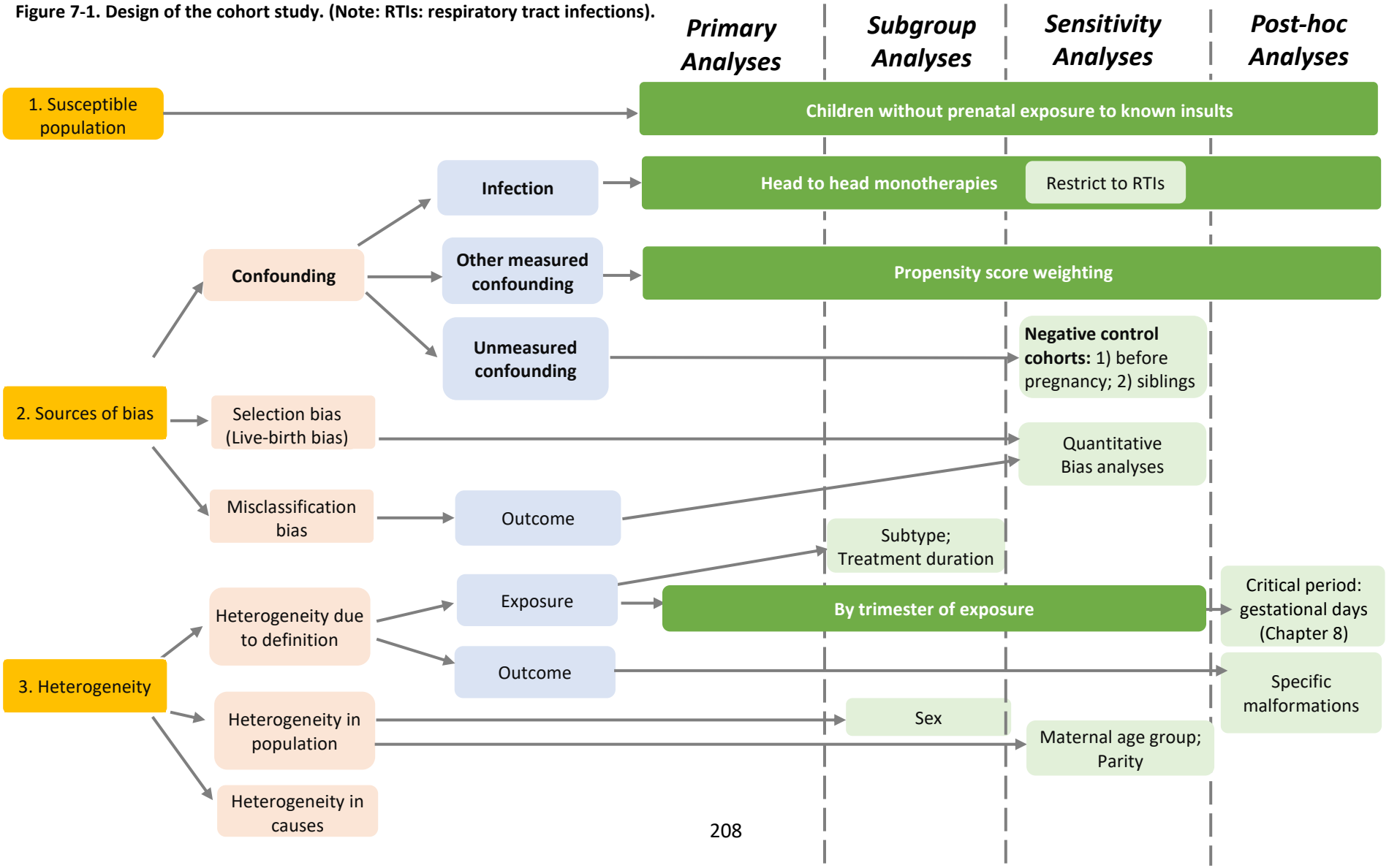
In this chapter, I report the methods and analyses to address the main research question of this thesis: whether prescribing of macrolides during pregnancy is associated with increased risks of adverse child outcomes.

7.1.2 Study design and analyses

7.1.2.1 Susceptible population

To investigate the effect of macrolide prescribing during pregnancy on adverse child outcomes, the study population should be susceptible to the potential insult (i.e. population at risk). I therefore excluded children who had records indicating chromosomal abnormalities or prenatal exposure to known teratogens. Adverse outcomes in these children were most likely to be attributed to these known insults instead of macrolides.

Figure 7-1. Design of the cohort study. (Note: RTIs: respiratory tract infections).



7.1.2.2 Sources of bias

Confounding

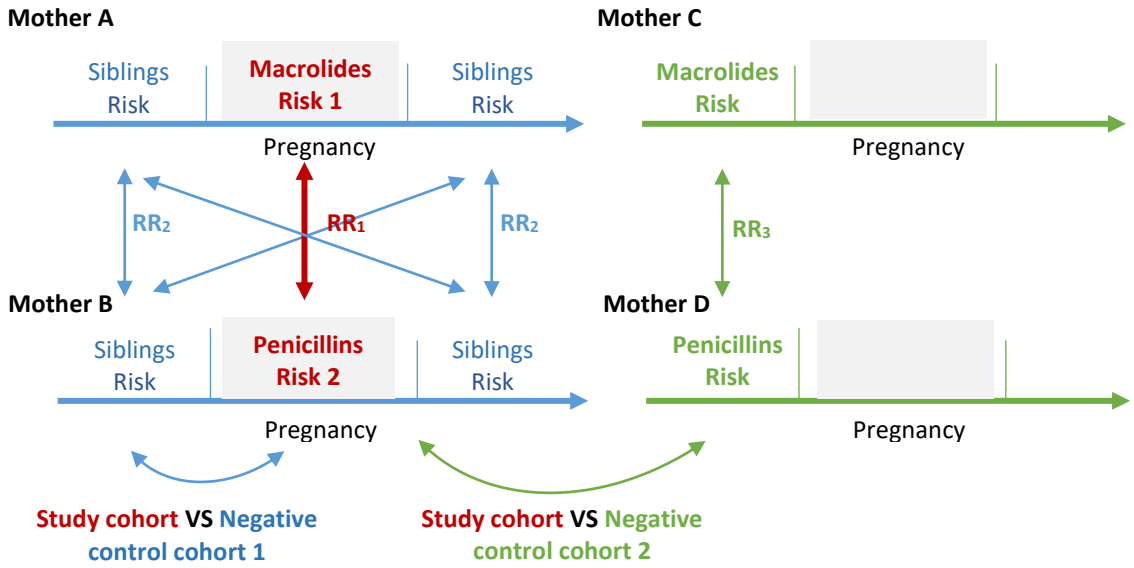
Address the indication bias (due to infection) by head-to-head comparison throughout the cohort study and by a sensitivity analysis restricted to RTIs. The three major sources of bias, confounding bias, selection bias and misclassification bias, have been discussed systematically in Chapter 2. While observational studies are particularly prone to confounding bias, the most influential confounding for drug safety studies is indication bias. I addressed indication bias by using the head-to-head comparison (macrolides versus penicillins) throughout all the cohort analyses (detailed in Section 2.1). The head-to-head design was supported by the previous analyses showing the comparable indication profile for macrolides and penicillins in the CPRD Mother Baby Cohort. Nevertheless, differences in indications between macrolides and penicillins group were still evident (e.g. more genitourinary tract infection in the penicillins group). Therefore, I further controlled indication bias, by conducting a sensitivity analysis in which I restricted the study population to mothers who were prescribed antibiotics for RTIs (reasoning given in Section 2.1).

Adjust for other measured confounders using propensity score weighting throughout the cohort study. I evaluated the associations between measured confounders and macrolide versus penicillin prescribing and ten adverse child outcomes in Chapter 5 and 6, respectively. I adopted a propensity score-based approach to adjust the potential confounding between macrolide and penicillin groups in the cohort study. In theory, propensity scores methods allow for better control of confounding. In practice, I found very little difference between the point estimates of relative measures of risk, using propensity score methods and conventional methods for adjusting for potential confounders. However, I used the propensity score method because it provides a more precise estimation of treatment response,[215] and allows to investigate the association from a perspective of causal inference.

Evaluate unmeasured confounding using two negative control cohorts in sensitivity analyses. Information about genetic predisposition and social-economic status were not available in the CPRD Mother Baby Cohort, but both could be associated with adverse child outcomes. They were thus potential unmeasured confounders. I developed two negative control cohorts to indirectly evaluate the effect of unmeasured confounding. One compared the risks between children whose mothers were prescribed macrolides and children whose mother were penicillins before pregnancy (the cohort in green in Figure 7-2). The other approach compared the risks between siblings of children whose mothers were prescribed macrolides and penicillins during pregnancy (i.e. siblings of children included in the main study cohort, the cohort in blue

in Figure 7-2). As illustrated in Figure 7-2, if an increased risk was observed in the study cohort (RR_1), and null associations were observed in two negative control cohorts (RR_2 and RR_3), this would provide indirect evidence that the increased risk cannot be explained by family-related factors (e.g. genetic factors and social-economic status), or by systematic differences between mothers prescribed macrolides and penicillins during pregnancy.

Figure 7-2. Illustration for two negative control cohorts (Red: study cohort; Blue: negative control cohort--siblings; Green: negative control cohort--prescriptions before pregnancy). RR: risk ratio.



Live-birth bias and outcome misclassification

Evaluate the direction and magnitude of live-birth bias and outcome misclassification using quantitative bias analyses as sensitivity analyses. Outcome measurements derived from administrative databases such as CPRD are not perfect and misclassification bias may exist. CPRD data were collected prospectively as part of routine healthcare. It is therefore reasonable to assume that measurement errors of outcomes were non-differential between macrolides and penicillins groups. This non-differential outcome misclassification is likely to bias the RR estimates towards the null.[234]

As mentioned in Section 2.2, the cohort study included only pregnancies that resulted in live-born children, thus some severe adverse outcomes (e.g. nervous system, cardiovascular and gastrointestinal malformations), that result in fetal deaths, were missed. This depletion of affected fetuses may occur more often among women exposed to macrolides (versus penicillins), as shown in our systematic review [325]. Therefore, the risk ratio of these outcomes

measured only in live births would be subject to selection (live-birth) bias with unknown direction (also known as collider bias).

I addressed these sources of bias by conducting multiple, probabilistic bias analyses to quantify the bias due to outcome misclassification, jointly with the live-birth bias. Specifically, I adjusted the RR (95% CI) for each adverse child outcome using bias parameters stemming from both previous studies and prior, informed opinion. Details are discussed in Section 7.2.5.3..

7.1.1.3 Heterogeneity of the association

Heterogeneity in the definitions of exposures and outcomes

- Evaluate association with antibiotics by trimester of exposure.
- Evaluate malformations by major malformation overall and system-specific malformations.
- Investigate the association with common individual malformations as post-hoc analyses.
- Explore the effect of prescribing timing on the association between macrolides and adverse outcome in Chapter 8.

Apart from confounders that could affect the relative effect estimates, the strength of association also varies according to the definition of outcomes. This is because aetiologies differ for specific malformations within the same system (e.g. Atrial Septal Defects versus the system-specific cardiovascular malformations). A relative effect for a group of malformations related to the same organ system, such as cardiovascular malformations, could be diluted by inclusion of outcomes that are not susceptible to potential adverse effects of macrolide exposure. As was mentioned previously in Section 1.4.3.3, a further source of dilution bias is that specific outcomes could have specific critical period as a short time window during pregnancy. To retain specificity of effect, and avoid dilution bias, an ideal definition for the outcomes would thus be individual outcomes (e.g. individual malformations) with a mechanism known to involve fetal hypoxia, and an ideal definition for exposure would be the corresponding critical period for the individual outcome. However, as was mentioned in Section 6.2.2.1, type II error, incomplete knowledge on mechanisms and the risk of a fishing expedition make this definition unfeasible.

I therefore defined the malformations as 1) major malformation overall and 2) system-specific malformation, according to the explicit classification and coding guidance of EURPCAT, aiming to reach a balance between power and dilution bias. The comparison of macrolides vs penicillins was restricted to prescribing during: 1) the first trimester, 2) second to third

trimester, and 3) any trimester. Additionally, the association for specific malformations were evaluated as a post-hoc analyses, and the effect of prescribing timing on the association were explored in Chapter 8, with the intention of identifying candidate specific outcomes for testing in future aetiological studies.

Perform subgroup analyses according to macrolide subtypes and duration of treatment. As was described in Section 1.5, macrolide subtypes and duration of treatment were evaluated in subgroup analyses to investigate exposure heterogeneity The analyses of duration of treatment may be less informative, as prescribing of macrolides and penicillins is usually 7 days (section 5.3.1.1).

Heterogeneity in population

Perform subgroup analyses by baby gender. Both major malformation and neurodevelopmental disorders are likely to have multiple causes. No causes could be identified for many observed cases (e.g. 65% to 75% for congenital malformation,[107] and 50% for epilepsy[326]). Baby sex is believed to be an important factor involved in known or not known mechanisms, by inducing sex-dependent physiologic and pathologic changes in the placenta and the fetus.[127] For example, baby sex has been reported as an effect modifier for the association of pregnancy outcomes with gestational stress and tobacco smoke with unknown mechanisms.[327,328] I thereby performed subgroup analyses by baby sex.

Evaluate whether maternal age group and parity (nulliparity versus parity ≥ 1) would interact with macrolides for the association with adverse child outcomes, as sensitivity analyses. Compared with the general pregnancy population in UK, the CPRD Mother Baby Cohort seems to include moderately older mothers and more firstborn children (Section 4.5). If these two characteristics would modify the association between macrolides and adverse child outcomes, findings of this study might not readily generalise to a wider population. I therefore evaluated whether these two characteristics would modify the associations, respectively, as sensitivity analyses.

7.1.3 Objectives

The specific objectives of this cohort study were to use primary-care data to:

<u>In the descriptive analyses:</u>

Compare the distribution of covariates in the macrolides and penicillins groups for women in the study cohort and in two negative control cohorts.

In the primary analyses:

Determine whether macrolide (versus penicillin) prescribing during pregnancy was associated with any major malformation, five system-specific malformations recorded in children up to three years of age, and cerebral palsy, epilepsy, ADHD and ASD recorded in children up to fourteen years of age, according to timing of prescribing during the first trimester, second to third trimester and any trimester.

In the subgroup analyses:

Investigate the associations between macrolide (versus penicillin) prescribing during pregnancy and ten adverse child outcomes, according to the timing of prescription (the first trimester, the second to third trimester and any trimester), by 1) macrolides subtype, 2) treatment duration and 3) baby gender.

In the sensitivity analyses:

1. Restrict the association in the main analyses to mothers whose antibiotics were prescribed for RTIs.
2. Analyse using negative control cohort 1 (sibling design): comparing the risks of ten adverse child outcomes between siblings of children whose mothers were prescribed macrolides and penicillins during pregnancy, according to timing of prescribing (the first trimester, the second to third trimester and any trimester).
3. Analyse using negative control cohort 2 (prescription before pregnancy): comparing the risks of ten adverse child outcomes between children whose mothers were prescribed macrolides and penicillins 10-50 weeks before LMP.
4. Conduct quantitative bias analyses for outcome misclassification (for all ten adverse child outcomes) and live-birth bias (for severe adverse outcomes, i.e. nervous system, cardiovascular and gastrointestinal malformations).
5. Evaluate whether maternal age group and birth order modify associations between macrolides and adverse child outcomes, respectively.

In the *post-hoc* analyse:

1. Evaluate the association between macrolide (versus penicillin) prescribing during pregnancy and common specific malformations, according to the timing of prescription (the first trimester, the second to third trimester and any trimester).
2. Explore the effects of exposure timing on associations between macrolides and common specific malformations (Chapter 8).

7.2 Methods

7.2.1 Population

The CPRD Mother Baby Cohort included children registered with the GP within 6 months of birth, whose mother was 14 to 50 year-old and registered with a CPRD practice from at least 50 weeks prior to estimated LMP until delivery. Among the 728,921 live born children included in the CPRD Mother Baby Cohort, 2,647 children were excluded due to chromosomal abnormalities and prenatal exposure to known teratogens. These known teratogens include warfarin, angiotensin-converting enzyme (ACE) inhibitors, antineoplastic agents, isotretinoin, misoprostol, and thalidomide (Figure 7-3). The target population comprised 726,274 children. This cohort was used to identify the study population for analysis: i.e. mothers exposed to macrolide or penicillin prescribing, as described in the next section.

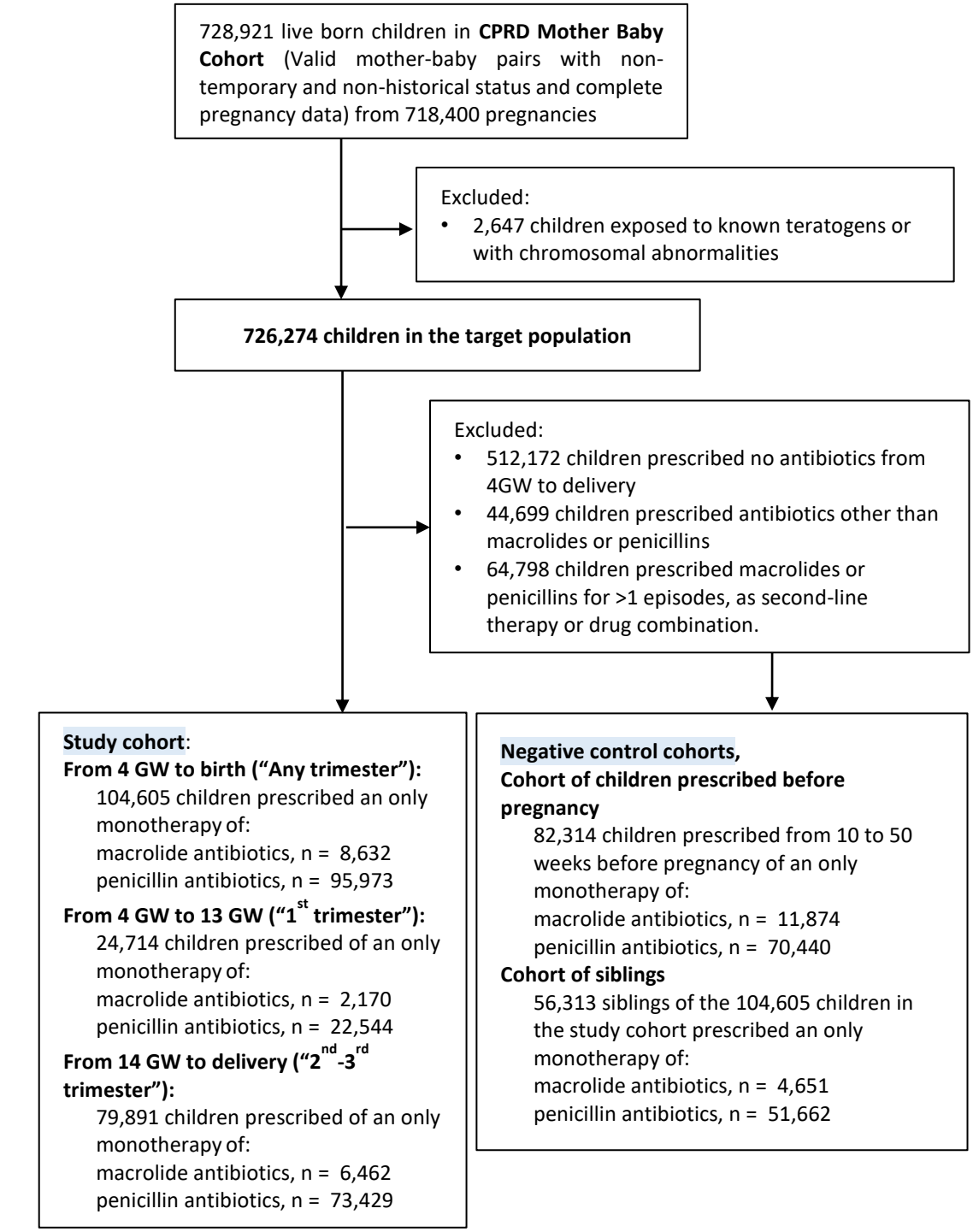
Children were followed from birth to 14 years, death, or end of follow-up (June 2016), whichever came first. The decision to limit follow-up to a maximum of 14 years was supported by the analyses in chapter 6, which showed that most congenital malformations were recorded by age 3. However, neurodevelopmental outcomes were first diagnosed at a later age.

7.2.2 Exposure

The study cohort was restricted to children whose mothers were prescribed one episode of monotherapy of macrolides or penicillins from 4 GW to delivery (hereafter, “any trimester”, n=104,605). The time window started at 4GW to evaluate all prescriptions from 5GW, the start of organogenesis and allow for a typical prescription of one week (as shown in Section 5.3.1.1). I further divided the time window into 4GW to 13GW (“first trimester”, n=44,314), the critical period for most major malformations, and 14GW to birth (“second to third trimester”, n= 79,891) (Figure 7-3). I used a drug code list based on the British National Formulary (chapters 5.1.5 & 5.1.1, as described in Section 5.2.2.1) to identify prescriptions of macrolides and penicillins. The index date of exposure was the date of the first prescription of the monotherapy (Figure 7-3).

Using children in the target population, but not included in the study cohort, I derived two negative control cohorts as described in section 7.1.1.2: (1) children whose mothers were prescribed one macrolide or penicillin monotherapy 10-50 weeks prior to LMP. The 10 weeks gap was to ensure that the prescriptions did not extend into pregnancy (n=82,314); (2) siblings of the children whose mothers were prescribed macrolides or penicillins during pregnancy as included in the study cohort (n=56,313).

Figure 7-3. Study cohort. Both singletons and multiple births were included.



*GW: gestational week.

7.2.3 Outcomes

The ten adverse child outcomes of interest included any major malformation, nervous system malformation, cardiovascular malformation, gastrointestinal malformation, genital malformation and urinary tract malformation, cerebral palsy, epilepsy, ADHD, and ASD. The eligibility criteria for the outcomes, the definition and identification of these outcomes were described in Chapter 6. I evaluated the risks of major malformation recorded in the medical history by age 3 and rates (hazards) of neurodevelopmental disorders by age 14 years. I presented the most frequent read codes of each system-specific malformation in Appendix 7-1.

7.2.4 Covariates

I included the following covariates for calculating the propensity score:

- Maternal characteristics at LMP and chronic risk factors:
    - age at delivery, calendar year at delivery, alcohol misuse, illicit drug use, tobacco use, obesity, hypertension, diabetes, anxiety, depression and epilepsy
- Pregnancy-related variables:
    - Parity, multiple birth, chronic medical treatments, genitourinary tract infections and STIs during pregnancy.

Genitourinary tract infections and STIs could be potential related with preterm labour (and/or congenital malformation). I therefore adjusted for these events as confounders. The definition of the covariates was included in Section 4.4.

7.2.5 Statistical Analyses

7.2.5.1 Descriptive analyses

Before performing the analyses for association described earlier in Section 7.1.2, I first described and compared the covariates of mothers in the macrolides and penicillins groups of the study cohort (by timing of prescribing), and in the two negative control cohorts. I applied the standardized difference (the difference in means in proportion to standard deviation, >0.1 as meaningful imbalances, as described in detail in section 5.2.3.3) to evaluate covariate balance between the macrolides and penicillins groups.

7.2.5.2 Analyses for association

The design for the analyses has been described in Section 7.1.2 and summarised in Section 7.1.3. For malformations, I calculated absolute risks (per 1,000 children) and risk ratios (RR) with 95% confidence intervals (CI), using log-binomial models. The log-binomial model was used to estimate risk ratios. For neurodevelopmental disorders, where the follow-up time was censored,

absolute rates (per 1,000 Person Year) and hazard ratios (HR) with 95% Cis, were estimated using Cox proportional hazard models. Schoenfeld residuals were used to test the proportional hazard assumption.

I used propensity score matching to adjust for potential confounding. Specifically, I adopted the propensity-score-based Fine Stratification approach to address the problem of infrequent exposure: the ratio of number of children in macrolides group versus penicillins group was about 1:11.[323] Exposure propensity scores were estimated as the predicted probability of receiving the macrolides prescription (vs. penicillins), conditioning on the covariates (mentioned in Section 7.2.4), using logistic regression models. The propensity scores of children in the penicillins group were divided into 50 strata based on the distribution of propensity scores of children in the macrolides group.[323] Children in the penicillins group were then weighted in each stratum according to the distribution of the propensity score in the macrolides group. Children in the non-overlapping areas of the propensity-score distributions were trimmed. The weights were then used to estimate adjusted baseline characteristics and adjusted risk ratios or rate ratios and 95% confidence intervals. The clustering of siblings and multiple births within mothers were accounted using robust standard errors. A random effects model with clustering within mother was not considered because more than 94% of mothers had only one child included in the study cohorts by trimester (although 66.5% mothers in the target population had more than one child) (Table 7-1).

Table 7-1. Proportions of mothers with only one or ≥ 2 children included in the study cohorts.

Timing of prescription	Mothers with only one child	Mothers with ≥ 2 children
1st trimester	23338 (97%)	682 (3%)
2nd-3rd trimester	70234 (94%)	4708 (6%)

To evaluate whether maternal age and parity interact with macrolides on associations with adverse child outcomes, I added interaction terms of macrolides × maternal age and macrolides × parity, respectively, into the multivariate logistic regression model (or Cox proportional hazard models) for adverse outcomes, adjusted by other covariates mentioned in section 7.2.4. Adjusted estimates and the standard error of the coefficient were then calculated. I then evaluated the significance of the interactions terms by p values calculated respectively based on Wald tests for the coefficient and the likelihood ratio test between the models with and without the interaction term. Additionally, I performed the Breslow-Day test to evaluate the homogeneity of the crude odds ratios among the subgroups of maternal age and parity.

7.2.5.3 Probabilistic multiple bias analyses

I conducted probabilistic multiple bias analyses to quantify the bias owing to outcome misclassification as well as jointly with live-birth bias (for some severe adverse outcomes, e.g. nervous system, cardiovascular and gastrointestinal malformations) to facilitate interpretation. Multiple bias analyses (which provided bias-adjusted RR estimates using standard 2x2 tables) were described in detail elsewhere.[329] Briefly, frequencies in the tables were adjusted by a set of bias parameters, i.e. sensitivity and specificity for outcome misclassification, and probability of live birth for selection bias. These parameters were randomly sampled from given probability distributions (e.g. 5,000 iterations from triangular distributions in this study). In each iteration, I adjusted for misclassification bias and live-birth bias by sampling and adjusting the frequencies sequentially, incorporated with a random error to obtain the adjusted estimates with 95% limits. The analyses were performed using RStudio version 3.5.1 and R package "episensr".[330] The bias parameters used are included in Appendix 7-2.

7.3 Results

7.3.1 Exposure and covariates

Within the target population (n=726,274), mothers of 31% of children were prescribed at least one antibiotic during pregnancy. Penicillins and macrolides accounted for about 69% and 10% of the prescriptions, respectively, where 65% and 42% were prescribed as the single monotherapy prescribed during pregnancy (Figure 7-3, Figure 7-4)

The study cohort included 104,605 children whose mothers were prescribed a monotherapy macrolides or penicillins from 4 GW to delivery, with 8632 (8.3%) and 95,973 (91.7%) children in macrolides and penicillins groups respectively (Table 7-4). Among these, 2170 (8.8%) were born to mothers prescribed one macrolide monotherapy and 22,544 (91.2%) to mothers prescribed one penicillin monotherapy from 4 to 13 GW (Table 7-2). The children were followed for a median of 5.8 years (interquartile range 2.4-12.1 years) after birth. In negative control cohorts, there were 82,314 children whose mothers were prescribed macrolides or penicillins before conception, and a further 56,313 children identified as siblings of the 104,605 children included in the study cohort (Table 7-5, Table 7-6).

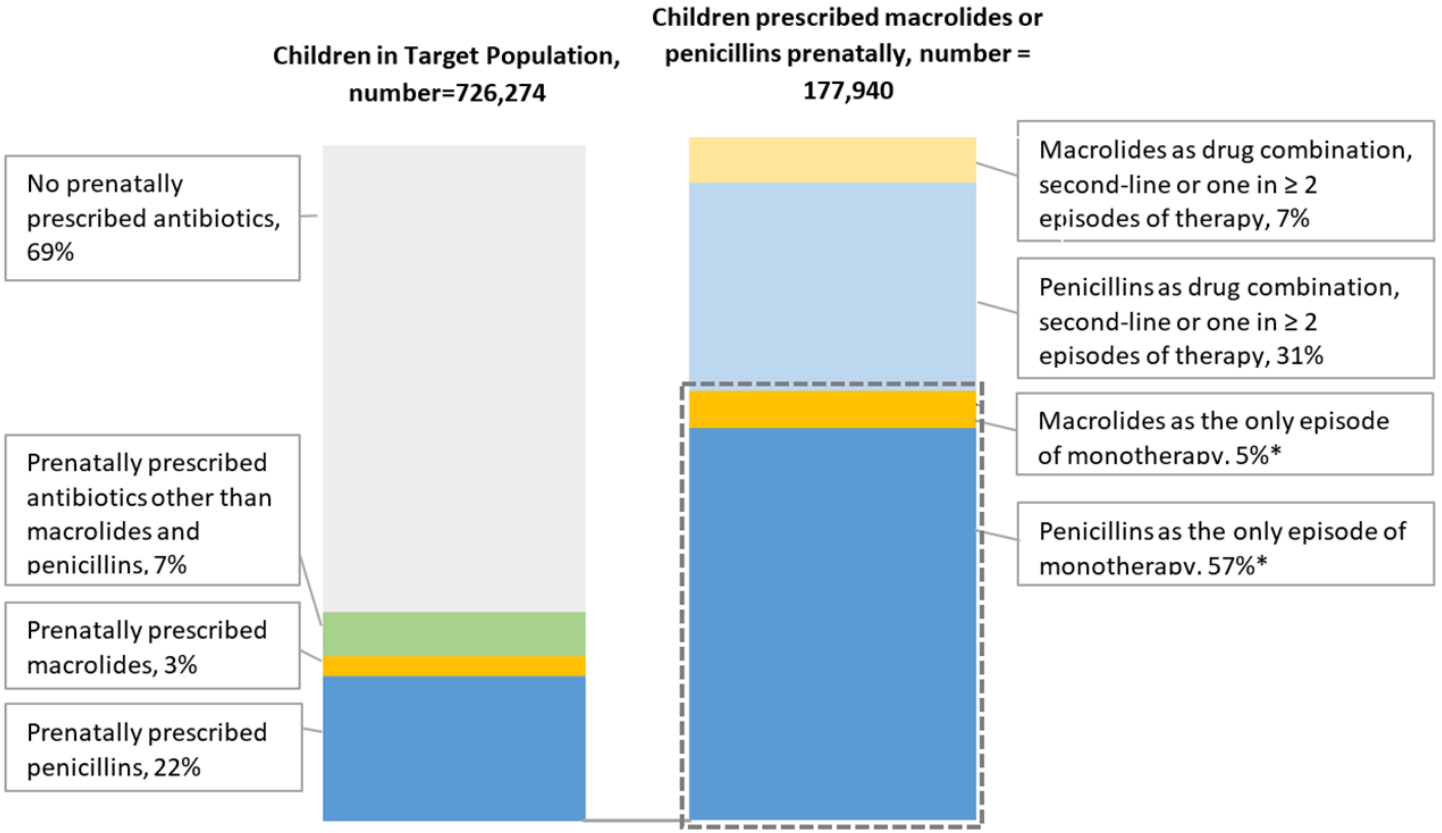
The pattern of the distributions of covariates between the two exposure groups were similar to the distributions with that observed in the CPRD Mother Baby Cohort (including the children with chromosomal abnormalities and prenatal exposure of known teratogens, as described in Chapter 5). Compared with children whose mothers were prescribed penicillins, children whose

mothers were prescribed macrolides were less likely to have records of genitourinary tract infections and more likely to have records of STIs during pregnancy irrespective of trimester of prescriptions. Among mothers prescribed during the first trimester, mothers were younger in the macrolides group as compared with mothers in penicillins group. Other covariates were distributed with no meaningful differences between the macrolides and penicillins groups, regardless of the timing of prescribing during the first trimester, second to third trimesters (Table 7-2, Table 7-3, Table 7-4).

The distributions of covariates were very similar between mothers prescribed 10-50 weeks before pregnancy (negative control cohort 1) and mothers prescribed during pregnancy (the study cohort), except that the macrolides and penicillins groups had comparable prevalence rates of genitourinary tract infections and STIs. Compared with the study cohort, the siblings cohort contained more younger siblings (parity ≥ 1, 56.6% versus 36.1%), with a younger maternal age structure (<= 29y, 51.5% versus 47.6%), and with fewer obesity, hypertension or diabetes (Table 7-5, Table 7-6).

The differences between two exposure groups in each cohort were balanced after propensity score adjustment (Table 7-2 to Table 7-6).

Figure 7-4. Antibiotic prescribing during pregnancy in this study.



*9698 and 101969 children were prenatally prescribed only one episode of monotherapy of macrolides and penicillins, respectively. The antibiotics were issued between 4 gestational week and birth in 8632 and 95973 children, who were included in **the study cohort** (dashed area).

Table 7-2. Crude and adjusted baseline characteristics (N [%]) of children whose mother was prescribed macrolides or penicillins from 4 to 13 gestational week ("the first trimester").

Characteristic	Unadjusted			Propensity-score-adjusted*		
	Macrolides	Penicillins	St.diff	Macrolides	Penicillins	St.diff
Number of children	2170	22544		2170	22509.8	
Maternal baseline characteristic						
Age at delivery			0.108			0.008
13-19	130 (6.0)	986 (4.4)		130 (6.0)	1372.2 (6.1)	
20-24	377 (17.4)	3510 (15.6)		377 (17.4)	3881.9 (17.2)	
25-29	524 (24.1)	6223 (27.6)		524 (24.1)	5437.1 (24.2)	
30-34	664 (30.6)	6892 (30.6)		664 (30.6)	6941.3 (30.8)	
35-50	475 (21.9)	4933 (21.9)		475 (21.9)	4877.3 (21.7)	
Calendar year of delivery			0.115			0.016
1990-1994	170 (7.8)	2225 (9.9)		170 (7.8)	1843.0 (8.2)	
1995-1999	318 (14.7)	3746 (16.6)		318 (14.7)	3323.8 (14.8)	
2000-2004	496 (22.9)	4404 (19.5)		496 (22.9)	5175.9 (23.0)	
2005-2009	568 (26.2)	5594 (24.8)		568 (26.2)	5781.4 (25.7)	
2010-2016	618 (28.5)	6575 (29.2)		618 (28.5)	6385.8 (28.4)	
Alcohol misuse	129 (5.9)	1047 (4.6)	0.058	129 (5.9)	1353.6 (6.0)	0.003
Illicit drug use	31 (1.4)	243 (1.1)	0.032	31 (1.4)	323.6 (1.4)	0.001
Tobacco use	790 (36.4)	7412 (32.9)	0.074	790 (36.4)	8168.1 (36.3)	0.002
Obesity	262 (12.1)	2578 (11.4)	0.020	262 (12.1)	2742.7 (12.2)	0.003
Hypertension	161 (7.4)	1623 (7.2)	0.008	161 (7.4)	1655.9 (7.4)	0.002
Diabetes	68 (3.1)	782 (3.5)	0.019	68 (3.1)	710.3 (3.2)	0.001
Anxiety	74 (3.4)	556 (2.5)	0.056	74 (3.4)	763.1 (3.4)	0.001
Depression	227 (10.5)	2288 (10.1)	0.01	227 (10.5)	2381.0 (10.6)	0.004
Epilepsy	25 (1.2)	155 (0.7)	0.049	25 (1.2)	238.4 (1.1)	0.009
Pregnancy related characteristic						
Parity ≥1	782 (36.0)	8080 (35.8)	0.004	782 (36.0)	8070.8 (35.9)	0.004
Multiple births	52 (2.4)	535 (2.4)	0.002	52 (2.4)	564.5 (2.5)	0.007
Genitourinary tract infection	90 (4.1)	2796 (12.4)	0.303	90 (4.1)	889.2 (4.0)	0.010
Sexually Transmitted Infection	102 (4.7)	301 (1.3)	0.198	102 (4.7)	978.2 (4.3)	0.017
Treatment of chronic medical conditions	422 (19.4)	4066 (18.0)	0.036	422 (19.4)	4388.4 (19.5)	0.001

*A meaningful between-group imbalance was assessed by an absolute standardised difference (St.diff, the difference in means in units of standard deviation) of more than 0.1. Numbers in adjusted penicillins group were non-integer, because they were weighted based on the distribution of the propensity score of the macrolides group.

Table 7-3. Crude and adjusted baseline characteristics (N [%]) of children whose mother was prescribed macrolides or penicillins from 14 gestation weeks to delivery ("the second to third trimester").

Characteristic	Unadjusted			Propensity-score-adjusted*		
	Macrolides	Penicillins	St.diff	Macrolides	Penicillins	St.diff
Number of children	6462	73429		6462	73400	
Maternal baseline characteristic						
Age at delivery			0.08			0.004
13-19	232 (3.6)	2889 (3.9)		232 (3.6)	2631.5 (3.6)	
20-24	825 (12.8)	10560 (14.4)		825 (12.8)	9273.3 (12.6)	
25-29	1562 (24.2)	19105 (26.0)		1562 (24.2)	17779.2 (24.2)	
30-34	2165 (33.5)	23514 (32.0)		2165 (33.5)	24589.0 (33.5)	
35-50	1678 (26.0)	17361 (23.6)		1678 (26.0)	19127.0 (26.1)	
Calendar year of delivery			0.054			0.003
1990-1994	606 (9.4)	7594 (10.3)		606 (9.4)	6907.4 (9.4)	
1995-1999	1067 (16.5)	13023 (17.7)		1067 (16.5)	12111.0 (16.5)	
2000-2004	1344 (20.8)	15025 (20.5)		1344 (20.8)	15234.9 (20.8)	
2005-2009	1688 (26.1)	18005 (24.5)		1688 (26.1)	19248.1 (26.2)	
2010-2016	1757 (27.2)	19782 (26.9)		1757 (27.2)	19898.6 (27.1)	
Alcohol misuse	308 (4.8)	3526 (4.8)	0.002	308 (4.8)	3494.4 (4.8)	<0.001
Illicit drug use	81 (1.3)	739 (1.0)	0.023	81 (1.3)	911.0 (1.2)	0.001
Tobacco use	2136 (33.1)	23351 (31.8)	0.027	2136 (33.1)	24190.8 (33.0)	0.002
Obesity	795 (12.3)	8046 (11.0)	0.042	795 (12.3)	8979.4 (12.2)	0.002
Hypertension	507 (7.8)	5355 (7.3)	0.021	507 (7.8)	5765.8 (7.9)	<0.001
Diabetes	254 (3.9)	2359 (3.2)	0.039	254 (3.9)	2868.7 (3.9)	0.001
Anxiety	187 (2.9)	1820 (2.5)	0.026	187 (2.9)	2118.0 (2.9)	<0.001
Depression	714 (11.0)	6891 (9.4)	0.055	714 (11.0)	8033.0 (10.9)	0.003
Epilepsy	35 (0.5)	474 (0.6)	0.014	35 (0.5)	429.1 (0.6)	0.006
Pregnancy related characteristic						
Parity ≥1	2367 (36.6)	26444 (36.0)	0.013	2367 (36.6)	26964.5 (36.7)	0.002
Multiple births	182 (2.8)	2018 (2.7)	0.004	182 (2.8)	2071.6 (2.8)	<0.001
Genitourinary tract infection	271 (4.2)	8725 (11.9)	0.286	271 (4.2)	3112.9 (4.2)	0.002
Sexually Transmitted Infection	179 (2.8)	936 (1.3)	0.106	179 (2.8)	2079.6 (2.8)	0.004
Treatment of chronic medical conditions	1328 (20.6)	12718 (17.3)	0.083	1328 (20.6)	14976.0 (20.4)	0.004

*A meaningful between-group imbalance was assessed by an absolute standardised difference (Std.diff, the difference in means in units of standard deviation) of more than 0.1. Numbers in adjusted penicillins group were non-integer, because they were weighted based on the distribution of the propensity score of the macrolides group.

Table 7-4. Crude and adjusted baseline characteristics (N [%]) of children whose mother was prescribed macrolides or penicillins from 4 gestation weeks to delivery ("in any trimester").

Characteristic	Unadjusted			Propensity-score-adjusted*		
	Macrolides	Penicillins	St.diff	Macrolides	Penicillins	St.diff
Number of children	8632	95973		8632	95971	
Maternal baseline characteristic						
Age at delivery			0.063			0.003
13-19	362 (4.2)	3875 (4.0)		362 (4.2)	3992.9 (4.2)	
20-24	1202 (13.9)	14070 (14.7)		1202 (13.9)	13291.0 (13.8)	
25-29	2086 (24.2)	25328 (26.4)		2086 (24.2)	23169.9 (24.1)	
30-34	2829 (32.8)	30406 (31.7)		2829 (32.8)	31559.9 (32.9)	
35-50	2153 (24.9)	22294 (23.2)		2153 (24.9)	23957.2 (25.0)	
Calendar year of delivery			0.066			0.003
1990-1994	776 (9.0)	9819 (10.2)		776 (9.0)	8662.3 (9.0)	
1995-1999	1385 (16.0)	16769 (17.5)		1385 (16.0)	15461.5 (16.1)	
2000-2004	1840 (21.3)	19429 (20.2)		1840 (21.3)	20448.7 (21.3)	
2005-2009	2256 (26.1)	23599 (24.6)		2256 (26.1)	25073.7 (26.1)	
2010-2016	2375 (27.5)	26357 (27.5)		2375 (27.5)	26324.9 (27.4)	
Alcohol misuse	437 (5.1)	4573 (4.8)	0.014	437 (5.1)	4823.4 (5.0)	0.002
Illicit drug use	112 (1.3)	982 (1.0)	0.026	112 (1.3)	1193.9 (1.2)	0.005
Tobacco use	2926 (33.9)	30763 (32.1)	0.039	2926 (33.9)	32235.1 (33.6)	0.007
Obesity	1057 (12.2)	10624 (11.1)	0.037	1057 (12.2)	11688.6 (12.2)	0.002
Hypertension	668 (7.7)	6978 (7.3)	0.018	668 (7.7)	7379.0 (7.7)	0.002
Diabetes	322 (3.7)	3141 (3.3)	0.025	322 (3.7)	3551.3 (3.7)	0.002
Anxiety	261 (3.0)	2376 (2.5)	0.034	261 (3.0)	2841.3 (3.0)	0.004
Depression	941 (10.9)	9179 (9.6)	0.044	941 (10.9)	10393.5 (10.8)	0.002
Epilepsy	60 (0.7)	629 (0.7)	0.005	60 (0.7)	666.4 (0.7)	<0.001
Pregnancy related characteristic						
Parity >=1	3149 (36.5)	34524 (36.0)	0.011	3149 (36.5)	35080.6 (36.6)	0.002
Multiple births	234 (2.7)	2553 (2.7)	0.003	234 (2.7)	2594.6 (2.7)	<0.001
Genitourinary tract infection	361 (4.2)	11521 (12.0)	0.29	361 (4.2)	3964.7 (4.1)	0.003
Sexually Transmitted Infection	281 (3.3)	1237 (1.3)	0.132	281 (3.3)	3075.3 (3.2)	0.003
Treatment of chronic medical	1750 (20.3)	16784 (17.5)	0.071	1750 (20.3)	19480.0 (20.3)	0.001

*A meaningful between-group imbalance was assessed by an absolute standardised difference (St.diff, the difference in means in units of standard deviation) of more than 0.1
Numbers in adjusted penicillins group were non-integer, because they were weighted based on the distribution of the propensity score of the macrolides group.

Table 7-5. Crude and adjusted baseline characteristics (N [%]) of children whose mother was prescribed macrolides or penicillins 10 to 50 weeks before pregnancy.

Characteristic	Unadjusted			Propensity-score-adjusted*		
	Macrolides	Penicillins	St.diff	Macrolides	Penicillins	St.diff
Number of children	11874	70440		11874	70425.1	
Maternal baseline characteristic						
Age at delivery			0.028			0.003
13-19	499 (4.2)	3150 (4.5)		499 (4.2)	2975.8 (4.2)	
20-24	1706 (14.4)	10482 (14.9)		1706 (14.4)	10091.3 (14.3)	
25-29	3099 (26.1)	18495 (26.3)		3099 (26.1)	18437.4 (26.2)	
30-34	3760 (31.7)	22346 (31.7)		3760 (31.7)	22240.1 (31.6)	
35-50	2810 (23.7)	15967 (22.7)		2810 (23.7)	16680.6 (23.7)	
Calendar year of delivery			0.038			0.003
1990-1994	1034 (8.7)	6060 (8.6)		1034 (8.7)	6109.9 (8.7)	
1995-1999	1986 (16.7)	12376 (17.6)		1986 (16.7)	11827.1 (16.8)	
2000-2004	2451 (20.6)	14977 (21.3)		2451 (20.6)	14593.5 (20.7)	
2005-2009	3030 (25.5)	18099 (25.7)		3030 (25.5)	17960.3 (25.5)	
2010-2016	3373 (28.4)	18928 (26.9)		3373 (28.4)	19934.4 (28.3)	
Alcohol misuse	607 (5.1)	3248 (4.6)	0.023	607 (5.1)	3584.8 (5.1)	0.001
Illicit drug use	144 (1.2)	695 (1.0)	0.022	144 (1.2)	852.0 (1.2)	<0.001
Tobacco use	3991 (33.6)	22730 (32.3)	0.029	3991 (33.6)	23798.6 (33.8)	0.004
Obesity	1448 (12.2)	8015 (11.4)	0.025	1448 (12.2)	8605.3 (12.2)	0.001
Hypertension	899 (7.6)	5107 (7.3)	0.012	899 (7.6)	5328.1 (7.6)	<0.001
Diabetes	395 (3.3)	2339 (3.3)	<0.001	395 (3.3)	2355.4 (3.3)	0.001
Anxiety	311 (2.6)	1681 (2.4)	0.015	311 (2.6)	1846.0 (2.6)	<0.001
Depression	1180 (9.9)	6601 (9.4)	0.019	1180 (9.9)	7001.3 (9.9)	<0.001
Epilepsy	73 (0.6)	432 (0.6)	<0.001	73 (0.6)	437.7 (0.6)	0.001
Pregnancy related characteristic						
Parity ≥1	4197 (35.3)	26876 (38.2)	0.058	4197 (35.3)	24940.7 (35.4)	0.001
Multiple births	378 (3.2)	1822 (2.6)	0.036	378 (3.2)	2240.8 (3.2)	<0.001
Genitourinary tract infection	1270 (10.7)	6146 (8.7)	0.067	1270 (10.7)	7510.5 (10.7)	0.001
Sexually Transmitted Infection	188 (1.6)	913 (1.3)	0.024	188 (1.6)	1123.6 (1.6)	0.001
Treatment of chronic medical conditions	2175 (18.3)	12110 (17.2)	0.029	2175 (18.3)	12935.9 (18.4)	0.001

*A meaningful between-group imbalance was assessed by an absolute standardised difference (St.diff, the difference in means in units of standard deviation) of more than 0.1. Numbers in adjusted penicillins group were non-integer, because they were weighted based on the distribution of the propensity score of the macrolides group.

Table 7-6. Unadjusted and propensity-score-adjusted baseline characteristics (N [%]) of siblings of children whose mother was prescribed macrolides or penicillins from 4 gestation weeks to delivery ("siblings").

Characteristic	Unadjusted			Propensity-score-adjusted*		
	Macrolides	Penicillins	St.diff	Macrolides	Penicillins	St.diff
Number of children	4651	51662		4651	51579.4	
Maternal baseline characteristic						
Age at delivery			0.053			0.005
13-19	192 (4.1)	2265 (4.4)		192.0 (4.1)	2127.7 (4.1)	
20-24	752 (16.2)	9106 (17.6)		752.0 (16.2)	8405.5 (16.3)	
25-29	1358 (29.2)	15251 (29.5)		1358.0 (29.2)	15102.8 (29.3)	
30-34	1433 (30.8)	15669 (30.3)		1433.0 (30.8)	15857.0 (30.7)	
35-50	916 (19.7)	9371 (18.1)		916.0 (19.7)	10086.5 (19.6)	
Calendar year of delivery			0.054			0.005
1990-1994	436 (9.4)	5057 (9.8)		436.0 (9.4)	4858.9 (9.4)	
1995-1999	860 (18.5)	9925 (19.2)		860.0 (18.5)	9526.9 (18.5)	
2000-2004	1067 (22.9)	12514 (24.2)		1067.0 (22.9)	11917.3 (23.1)	
2005-2009	1227 (26.4)	13346 (25.8)		1227.0 (26.4)	13603.5 (26.4)	
2010-2016	1061 (22.8)	10820 (20.9)		1061.0 (22.8)	11672.8 (22.6)	
Alcohol misuse	222 (4.8)	2316 (4.5)	0.014	222.0 (4.8)	2479.5 (4.8)	0.002
Illicit drug use	63 (1.4)	487 (0.9)	0.039	63.0 (1.4)	673.9 (1.3)	0.004
Tobacco use	1565 (33.6)	16194 (31.3)	0.049	1565.0 (33.6)	17430.2 (33.8)	0.003
Obesity	502 (10.8)	5455 (10.6)	0.008	502.0 (10.8)	5580.5 (10.8)	0.001
Hypertension	332 (7.1)	3416 (6.6)	0.021	332.0 (7.1)	3692.9 (7.2)	0.001
Diabetes	128 (2.8)	1409 (2.7)	0.002	128.0 (2.8)	1420.9 (2.8)	<0.001
Anxiety	135 (2.9)	1144 (2.2)	0.044	135.0 (2.9)	1472.8 (2.9)	0.003
Depression	492 (10.6)	4702 (9.1)	0.05	492.0 (10.6)	5440.6 (10.5)	0.001
Epilepsy	25 (0.5)	306 (0.6)	0.007	25.0 (0.5)	281.2 (0.5)	0.001
Pregnancy related characteristic						
Parity ≥1	2555 (54.9)	29311 (56.7)	0.036	2555.0 (54.9)	28432.0 (55.1)	0.004
Multiple births	84 (1.8)	1006 (1.9)	0.01	84.0 (1.8)	944.4 (1.8)	0.002
Genitourinary tract infection	497 (10.7)	4902 (9.5)	0.04	497.0 (10.7)	5551.7 (10.8)	0.003
Sexually Transmitted Infection	46 (1.0)	687 (1.3)	0.032	46.0 (1.0)	549.3 (1.1)	0.008
Treatment of chronic medical	809 (17.4)	8025 (15.5)	0.05	809.0 (17.4)	8962.5 (17.4)	<0.001

*A meaningful between-group imbalance was assessed by an absolute standardised difference (St.diff, the difference in means in units of standard deviation) of more than 0.1. Numbers in adjusted penicillins group were non-integer, because they were weighted based on the distribution of propensity score of macrolides group.

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7.3.2 Primary analyses

The prevalence of major malformations was 27.7 and 19.5 per 1000 live born in mothers prescribed macrolides during the first trimester and in the second to third trimester, respectively. The rates in the penicillins group were stable from the first to second-to-third trimesters (17.7 and 17.3 per 1000 livebirths, respectively). Compared with penicillins, macrolide prescribed in the first trimester was associated with an increased risk for any malformation (adjusted risk ratio ARR 1.55; 95% CI, 1.19 - 2.03)) and specifically cardiovascular malformations (10.6 versus 6.6 per 1000 livebirths; ARR 1.62 (95% CI, 1.05 - 2.51) (Figure 7-5).

There was no association between macrolide prescribing during the second to third trimester and the risk of any major malformation (ARR 1.13 (95% CI, 0.94 - 1.36)), although a borderline association with gastrointestinal malformations was observed (ARR 1.89 (95% CI, 1.00-3.58)). Macrolide prescribing in any trimester was associated with an increased risk of genital malformations (ARR 1.58 (95% CI, 1.14 - 2.19). Hypospadias accounts for most cases of genital malformations, as shown in our post hoc analyses in Appendix 7-1 (Figure 7-5).

No association has been observed between the four neurodevelopmental disorders and macrolides prescribed in pregnancy (Figure 7-6).

7.3.3 Subgroup analyses

Consistent with the primary results, erythromycin prescribing during the first trimester was associated with an increased risk of any major malformation compared with penicillins (ARR 1.50 (95% CI, 1.13 - 1.99)). The number of malformation events were limited in clarithromycin group, but an increased risk of any major malformation was observed for clarithromycin prescribed during second to third trimester. The analyses for azithromycin were precluded by too few events (Table 7-7). The subgroup analyses of prescribing for less than one week were not informative because 94.7% prescriptions of macrolides lasted for 5 to 7 days (Table 7-8).

It worth noticing that in the analyses by baby gender, the increased risks of any major malformation, cardiovascular malformation and genital malformation were only present in male babies (any major malformation, 1st trimester: 1.73 (95% CI, 1.25-2.38); cardiovascular malformation, 1st trimester: 1.91 (95% CI, 1.06-3.44); genital malformation, 2nd-3rd trimester: 1.66 (95% CI, 1.13-2.44)). The association between first trimester macrolides prescribing and nervous system malformation in male babies was also statistically significant but only based on 5 cases in macrolides group. No increased risks of neurodevelopmental disorders were observed within male babies or female babies (Table 7-9).

To further evaluate whether male versus female gender modifies the potential effect of macrolides on malformations, I performed a post-hoc analyses to test the statistical significance of the gender and gender*antibiotics interaction terms in the association between macrolides (versus penicillins) prescribing during the first trimester and any major malformation, nervous system malformation and cardiovascular malformation (Table 7-10). The post-hoc analyses thereby showed that the interaction term between macrolides and baby gender for these three outcomes were not statistically significant. Even though the subgroup analyse suggested a higher risk among male babies, the estimates were too variable to provide an evidence for this. The overall risk of any malformation was higher in male babies than in female babies, which could be partly explained by the increased risk of hypospadias. Male gender itself does not relates to an increased risk of cardiovascular malformation or nervous system malformation (Table 7-10).

Since no correction for multiple testing was applied, 5 of the 102 subgroup tests (22 tests for macrolides subtypes, 40 tests for duration of treatment and 40 tests for baby gender) would be expected to be statistically significant by chance alone (at α =0.05).

7.3.4 Sensitivity analyses

No association was observed between adverse child outcomes and macrolide prescribing before pregnancy (versus penicillin, Figure 7-5). In the negative control cohorts of siblings, no association was observed between any major malformation, cardiovascular malformations or genital malformations and having siblings who were prenatally prescribed macrolides versus penicillins, although cases were limited for many outcomes by trimester (Table 7-11). Sensitivity analyses restricted to mothers prescribed macrolides or penicillins for RTIs during pregnancy did not alter the findings of the primary analyses, although the statistical power for analyses of many outcomes was limited (Table 7-12).

Given the assumptions of quantitative bias analyses described above, adjustment for the outcome misclassification and live-birth bias resulted in elevated RRs for malformations. The RR increased from 1.62 to 1.78 for cardiovascular malformations, and slightly from 1.55 to 1.58 for any major malformation. RRs for the nervous system and genital malformations increased and became statistically significant with wide 95% limits. The adjustment for outcome misclassification did not alter our findings for neurodevelopmental disorders (Table 7-13)

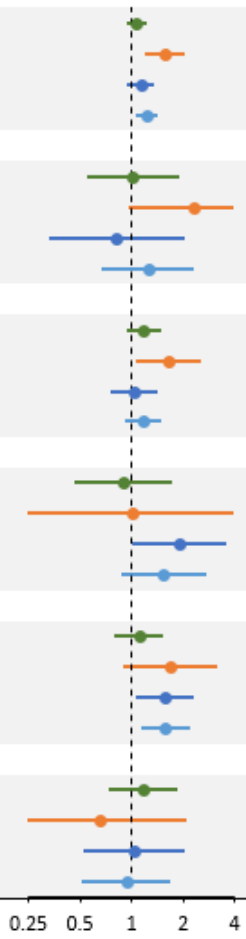
Neither maternal age nor parity modified the association between macrolide prescribing during the first trimester and any major malformations, cardiovascular malformations or genital malformations. (Table 7-14)

7.3.5 *Post-hoc* analyses

Post hoc, exploratory analyses for six common specific malformations revealed no statistically significant associations with macrolide prescribing during the first trimester (with limited power). However, increased risks of hypospadias and craniosynostosis in children of mothers prescribed macrolides during late pregnancy were observed (Table 7-15).

Figure 7-5. The association between major malformations and macrolides (versus penicillins) prescribed before or during pregnancy, by the timing of prescription.

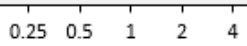
Adverse Outcome	No. of events		Risk per 1,000 live births or Rate per 1,000 person-year		Adj. RR/HR (95% CI)	*P* value
	Macrolides	Penicillins	Macrolide	Penicillins		
Any maior malformation						
Before pregnancy	221	1215	18.61	17.25	1.07 (0.93-1.23)	0.356
1st trimester	60	398	27.65	17.65	1.55 (1.19-2.03)	0.001
2nd -3rd trimester	126	1268	19.50	17.27	1.13 (0.94-1.36)	0.182
Any trimester	186	1666	21.55	17.36	1.23 (1.06-1.43)	0.007
Nervous system malformation						
Before pregnancy	12	68	1.01	0.97	1.02 (0.55-1.88)	0.947
1st trimester	6	27	2.76	1.20	2.29 (0.95-5.55)	0.065
2nd -3rd trimester	5	70	0.77	0.95	0.82 (0.33-2.04)	0.671
Any trimester	11	97	1.27	1.01	1.24 (0.66-2.30)	0.504
Cardiovascular malformation						
Before pregnancy	89	443	7.50	6.29	1.18 (0.94-1.48)	0.159
1st trimester	23	149	10.60	6.61	1.62 (1.05-2.51)	0.030
2nd -3rd trimester	44	477	6.81	6.50	1.03 (0.75-1.40)	0.870
Any trimester	67	626	7.76	6.52	1.17 (0.91-1.50)	0.221
Gastrointestinal malformation						
Before pregnancy	10	65	0.84	0.92	0.89 (0.46-1.72)	0.720
1st trimester	<5	20	-	0.89	1.00 (0.23-4.27)	1.000
2nd -3rd trimester	-	67	-	0.91	1.89 (1.00-3.58)	0.050
Any trimester	13	87	1.51	0.91	1.54 (0.87-2.76)	0.142
Genital malformation						
Before pregnancy	42	225	3.54	3.19	1.10 (0.79-1.53)	0.573
1st trimester	11	68	5.07	3.02	1.68 (0.89-3.16)	0.111
2nd -3rd trimester	30	227	4.64	3.09	1.56 (1.07-2.28)	0.022
Any trimester	41	295	4.75	3.07	1.58 (1.14-2.19)	0.006
Urinary malformation						
Before pregnancy	21	105	1.77	1.49	1.17 (0.73-1.87)	0.513
1st trimester	<5	41	-	1.82	0.65 (0.20-2.07)	0.462
2nd -3rd trimester	-	105	-	1.43	1.03 (0.52-2.03)	0.938
Any trimester	12	146	1.39	1.52	0.93 (0.51-1.67)	0.795



*In accordance with the confidentiality preserving policy of CPRD, I supress the information where the frequency cell contains <5 events (noted as "<5") and where necessary to avoid deduction (noted as "-"). **Before pregnancy**: comparisons of risks (or hazards) between children whose mothers were prescribed macrolides and penicillins during 50-10 weeks before pregnancy; **1st trimester**: comparisons of risks (or hazards) between children whose mothers were prescribed macrolides and penicillins during 4-13 gestational week (GW); **2nd to 3rd trimester**: comparisons of risks (or hazards) between children whose mothers were prescribed macrolides and penicillins from 14 GW to delivery; Any trimester: comparisons of risks (or hazards) between children whose mothers were prescribed macrolides and penicillins from 4 GW to delivery. CI: confidence interval; RR: risk ratio; HR: hazard ratio. ADHD: attention-deficit/hyperactivity disorder; ASD: autism spectrum disorder.

Figure 7-6. The association between neurodevelopmental disorders and macrolides (versus penicillins) prescribed before or during pregnancy, by the timing of prescription (neurodevelopmental disorders).

Adverse Outcome	No. of events		Risk per 1,000 live births or Rate per 1,000 person-year		Adj. RR/HR (95% CI)	*P* value
	Macrolides	Penicillins	Macrolides	Penicillins		
Cerebral palsy						
Before pregnancy	21	136	0.25	0.27	0.92 (0.58-1.46)	0.730
1st trimester	<5	55	-	0.35	0.39 (0.10-1.62)	0.198
2nd -3rd trimester	-	118	-	0.22	1.42 (0.83-2.45)	0.203
Any trimester	17	173	0.28	0.25	1.09 (0.66-1.80)	0.739
Epilepsy						
Before pregnancy	63	433	0.77	0.87	0.86 (0.66-1.12)	0.250
1st trimester	12	160	0.82	1.02	0.79 (0.44-1.42)	0.433
2nd -3rd trimester	36	525	0.80	0.99	0.83 (0.59-1.16)	0.269
Any trimester	48	685	0.80	1.00	0.81 (0.61-1.09)	0.170
ADHD						
Before pregnancy	67	337	0.81	0.67	1.20 (0.92-1.56)	0.178
1st trimester	14	123	0.95	0.78	1.24 (0.71-2.18)	0.445
2nd -3rd trimester	32	392	0.71	0.74	0.97 (0.68-1.40)	0.888
Any trimester	46	515	0.77	0.75	1.05 (0.78-1.42)	0.744
ASD						
Before pregnancy	91	517	1.11	1.04	1.07 (0.85-1.34)	0.564
1st trimester	19	181	1.29	1.15	1.02 (0.63-1.64)	0.937
2nd -3rd trimester	50	595	1.11	1.12	0.98 (0.73-1.31)	0.876
Any trimester	69	776	1.15	1.13	0.99 (0.77-1.27)	0.949



*In accordance with the confidentiality preserving policy of CPRD, I supress the information where the frequency cell contains <5 events (noted as "<5") and where necessary to avoid deduction (noted as "-"). **Before pregnancy**: comparisons of risks (or hazards) between children whose mothers were prescribed macrolides and penicillins during 50-10 weeks before pregnancy; **1st trimester**: comparisons of risks (or hazards) between children whose mothers were prescribed macrolides and penicillins during 4-13 gestational week (GW); **2nd to 3rd trimester**: comparisons of risks (or hazards) between children whose mothers were prescribed macrolides and penicillins from 14 GW to delivery; Any trimester: comparisons of risks (or hazards) between children whose mothers were prescribed macrolides and penicillins from 4 GW to delivery. CI: confidence interval; RR: risk ratio; HR: hazard ratio. ADHD: attention-deficit/hyperactivity disorder; ASD: autism spectrum disorder.

Table 7-7. Subgroup analyses according to macrolides subtypes, on the association between adverse child outcomes and macrolides versus penicillins prescribed during pregnancy.

Adverse Outcomes	No. of events		Risk per 1,000 live births or Rate per 1,000 person-year		Adj. RR/HR (95% CI)	*P* value
	Macr	Penicillin	Macrolides	Penicillin		
Erythromycin						
Any major malformation						
1st trimester	53	398	27.39	17.65	1.50 (1.13-1.99)	0.005
2nd -3rd trimester	112	1268	18.51	17.27	1.07 (0.88-1.29)	0.507
Nervous system malformation						
1st trimester	6	27	3.10	1.20	2.47 (1.03-5.96)	0.044
2nd -3rd trimester	5	70	0.83	0.95	0.84 (0.34-2.08)	0.706
Cardiovascular malformation						
1st trimester	19	149	9.82	6.61	1.48 (0.92-2.37)	0.108
2nd -3rd trimester	41	477	6.77	6.50	1.02 (0.74-1.41)	0.889
Gastrointestinal malformation						
1st trimester	<5	20	-	0.80	0.55 (0.07-4.09)	0.56
2nd -3rd trimester	10	67	1.65	0.91	1.75 (0.90-3.39)	0.099
Genital malformation						
1st trimester	10	68	5.17	3.02	1.62 (0.84-3.14)	0.151
2nd -3rd trimester	26	227	4.30	3.09	1.45 (0.96-2.17)	0.075
Urinary malformation						
1st trimester	<5	41	-	1.82	0.54 (0.13-2.22)	0.392
2nd -3rd trimester	8	105	1.32	1.43	0.96 (0.47-1.97)	0.906
Cerebral palsy						
1st trimester	<5	55	-	0.35	0.21 (0.03-1.56)	0.128
2nd -3rd trimester	15	118	0.35	0.22	1.49 (0.87-2.57)	0.147
Epilepsy						
1st trimester	12	160	0.89	1.02	0.88 (0.49-1.58)	0.663
2nd -3rd trimester	35	525	0.81	0.99	0.84 (0.59-1.18)	0.312
ADHD						
1st trimester	12	123	0.88	0.78	1.12 (0.61-2.04)	0.714
2nd -3rd trimester	31	392	0.72	0.74	0.97 (0.67-1.40)	0.868
ASD						
1st trimester	19	181	1.40	1.15	1.15 (0.71-1.84)	0.575
2nd -3rd trimester	48	595	1.11	1.12	0.99 (0.74-1.33)	0.937
Clarithromycin						
Any major malformation						
1st trimester	6	398	36.81	17.65	1.83 (0.83-4.04)	0.133
2nd -3rd trimester	11	1268	33.23	17.27	2.07 (1.15-3.71)	0.015

*The macrolides group included 7987 (clarithromycin), 494 (clarithromycin) and 151 (azithromycin) children. For clarithromycin, I only analysed any major malformation due to the limited number of events of other adverse child outcomes (there were six events of the four neurodevelopmental disorders in total in children prenatally prescribed clarithromycin). 151 azithromycin were prescribed during the whole pregnancy with <5 events of malformation, which precluded the analyses. ADHD: attention-deficit/hyperactivity disorder; ASD: autism spectrum disorder; CI: confidence interval; RR: risk ratio; HR: hazard ratio.

Table 7-8. Subgroup analyses according to duration of treatment (< 7 days or ≥ 7 days), on the association between adverse child outcomes and macrolides versus penicillins prescribed during pregnancy.

Adverse Outcomes	No. of events		Risk per 1,000 live births or Rate per 1,000 person-year		Adj. RR/HR (95% CI)	*P* value
	Macro-lides	Penici-llins	Macrolides	Penicillins		
Any major malformation						
<7 days, 1st trimester	17	144	37.28	16.58	2.11 (1.27-3.51)	0.004
≥7 days, 1st trimester	33	231	23.98	18.34	1.34 (0.94-1.92)	0.104
<7 days, 2nd -3rd trimester	25	466	15.30	16.46	0.88 (0.59-1.33)	0.553
≥7 days, 2nd -3rd trimester	84	724	20.70	17.81	1.18 (0.94-1.47)	0.154
Nervous system malformation						
<7 days, 1st trimester	<5	11	-	1.27	0 (0-Inf)	0.991
≥7 days, 1st trimester	<5	14	-	1.11	1.96 (0.56-6.82)	0.289
<7 days, 2nd -3rd trimester	<5	26	-	0.92	1.18 (0.28-4.94)	0.820
≥7 days, 2nd -3rd trimester	<5	39	-	0.96	0.54 (0.13-2.24)	0.398
Cardiovascular malformation						
<7 days, 1st trimester	7	59	15.35	6.79	2.39 (1.10-5.23)	0.028
≥7 days, 1st trimester	13	82	9.45	6.51	1.45 (0.81-2.60)	0.207
<7 days, 2nd -3rd trimester	9	167	5.51	5.9	0.87 (0.44-1.69)	0.675
≥7 days, 2nd -3rd trimester	30	271	7.39	6.67	1.10 (0.75-1.60)	0.624
Gastrointestinal malformation						
<7 days, 1st trimester	<5	9	-	1.04	0 (0-Inf)	0.990
≥7 days, 1st trimester	<5	10	-	0.79	0.86 (0.11-6.69)	0.888
<7 days, 2nd -3rd trimester	<5	25	-	0.88	2.36 (0.71-7.87)	0.162
≥7 days, 2nd -3rd trimester	8	37	1.97	0.91	2.02 (0.95-4.32)	0.069
Genital malformation						
<7 days, 1st trimester	<5	25	-	2.88	1.22 (0.29-5.07)	0.787
≥7 days, 1st trimester	6	40	4.36	3.18	0.86 (0.11-6.69)	0.888
<7 days, 2nd -3rd trimester	6	93	3.67	3.28	2.36 (0.71-7.87)	0.162
≥7 days, 2nd -3rd trimester	20	127	4.93	3.12	2.02 (0.95-4.32)	0.069
Urinary malformation						
<7 days, 1st trimester	<5	13	-	1.50	1.55 (0.20-11.85)	0.674
≥7 days, 1st trimester	<5	26	-	2.06	0.76 (0.18-3.20)	0.704
<7 days, 2nd -3rd trimester	<5	44	-	1.55	0.39 (0.05-2.79)	0.345
≥7 days, 2nd -3rd trimester	8	56	1.97	1.38	1.45 (0.69-3.05)	0.322
Cerebral palsy						
<7 days, 1st trimester	<5	20	-	0.28	0 (0-0)	<0.001
≥7 days, 1st trimester	<5	31	-	0.39	0.60 (0.14-2.53)	0.487
<7 days, 2nd -3rd trimester	<5	56	-	0.23	0.31 (0.04-2.25)	0.246
≥7 days, 2nd -3rd trimester	13	57	0.50	0.21	1.98 (1.05-3.73)	0.034
Epilepsy						
<7 days, 1st trimester	<5	71	-	1.01	0.71 (0.22-2.26)	0.559
≥7 days, 1st trimester	5	81	0.57	1.02	0.51 (0.21-1.28)	0.154
<7 days, 2nd -3rd trimester	12	232	0.82	0.98	0.91 (0.51-1.64)	0.762
≥7 days, 2nd -3rd trimester	22	262	0.85	0.99	0.87 (0.56-1.35)	0.543
ADHD						
<7 days, 1st trimester	5	55	1.28	0.78	1.53 (0.61-3.87)	0.367
≥7 days, 1st trimester	8	61	0.91	0.77	1.24 (0.58-2.64)	0.573
<7 days, 2nd -3rd trimester	14	191	0.96	0.80	1.25 (0.73-2.15)	0.418
≥7 days, 2nd -3rd trimester	12	188	0.46	0.71	0.63 (0.35-1.13)	0.122
ASD						
<7 days, 1st trimester	5	74	1.28	1.06	1.43 (0.62-3.30)	0.406
≥7 days, 1st trimester	11	96	1.25	1.21	0.74 (0.38-1.45)	0.385
<7 days, 2nd -3rd trimester	14	257	0.96	1.08	0.93 (0.54-1.59)	0.786
≥7 days, 2nd -3rd trimester	32	299	1.24	1.13	1.07 (0.74-1.54)	0.715

*97772 (93.5%) children in the study cohort were with non-missing duration of treatment. The macrolides group included 456 (<7 days, 1st trimester), 1376 (≥7 days, 1st trimester), 1634 (<7 days, 2nd -3rd trimester) and 4058 (≥7 days, 2nd -3rd trimester) children. The penicillins group included 8683 (<7 days, 1st trimester), 12592 (≥7 days, 1st trimester), 28314 (<7 days, 2nd -3rd trimester) and 40659 (≥7 days, 2nd -3rd trimester) children. Within macrolides prescription during the 1st trimester, 95% prescriptions less than 7 days were of 5-6 days, and 93% prescriptions >= 7 days were of 7 days. Overall, 94.7% macrolides or penicillins prescriptions were of 5 to 7 days. ADHD: attention-deficit/hyperactivity disorder; ASD: autism spectrum disorder; CI: confidence interval; RR: risk ratio; HR: hazard ratio.

Table 7-9. Subgroup analyses according to baby gender, on the association between adverse child outcomes and macrolides versus penicillins prescribed during pregnancy.

Adverse Outcomes	No. of events		Risk per 1,000 live births or Rate per 1,000 person-year		Adj. RR/HR (95% CI)	*P* value
	Mac	Pen	Mac	Pen		
Any major malformation						
Male, 1st trimester	42	238	37.20	20.53	1.73 (1.25-2.38)	<0.001
Female, 1st trimester	18	160	17.29	14.61	1.19 (0.73-1.93)	0.485
Male, 2nd -3rd trimester	78	764	24.49	20.30	1.19 (0.95-1.50)	0.131
Female, 2nd -3rd trimester	48	504	14.65	14.08	1.06 (0.79-1.42)	0.718
Nervous system malformation						
Male, 1st trimester	5	11	4.43	0.95	4.51 (1.58-12.90)	0.005
Female, 1st trimester	<5	16	-	1.46	0.67 (0.09-5.08)	0.701
Male, 2nd -3rd trimester	<5	40	-	1.06	1.23 (0.44-3.44)	0.694
Female, 2nd -3rd trimester	<5	30	-	0.84	0.34 (0.05-2.52)	0.294
Cardiovascular malformation						
Male, 1st trimester	13	69	11.51	5.95	1.91 (1.06-3.44)	0.031
Female, 1st trimester	10	80	9.61	7.31	1.33 (0.69-2.55)	0.399
Male, 2nd -3rd trimester	21	236	6.59	6.27	0.98 (0.63-1.53)	0.934
Female, 2nd -3rd trimester	23	241	7.02	6.73	1.07 (0.70-1.63)	0.770
Gastrointestinal malformation						
Male, 1st trimester	<5	11	-	0.95	0 (0-Inf)	0.990
Female, 1st trimester	<5	9	-	0.82	2.09 (0.46-9.55)	0.339
Male, 2nd -3rd trimester	6	43	1.88	1.14	1.52 (0.65-3.56)	0.331
Female, 2nd -3rd trimester	5	24	1.53	0.67	2.37 (0.90-6.22)	0.080
Genital malformation						
Male, 1st trimester	11	65	9.74	5.61	1.53 (0.81-2.88)	0.186
Female, 1st trimester	<5	<5	-	-	0 (0-Inf)	0.990
Male, 2nd -3rd trimester	29	217	9.11	5.77	1.66 (1.13-2.44)	0.010
Female, 2nd -3rd trimester	<5	10	-	0.28	0.98 (0.13-7.60)	0.986
Urinary malformation						
Male, 1st trimester	<5	32	-	2.76	0.62 (0.15-2.60)	0.518
Female, 1st trimester	<5	9	-	0.82	1.22 (0.15-9.65)	0.850
Male, 2nd -3rd trimester	<5	69	-	1.83	0.53 (0.17-1.70)	0.288
Female, 2nd -3rd trimester	6	36	1.83	1.01	2.05 (0.86-4.91)	0.106
Cerebral palsy						
Male, 1st trimester	<5	31	-	0.38	0.65 (0.15-2.73)	0.555
Female, 1st trimester	<5	24	-	0.31	0 (0-Inf)	<0.001
Male, 2nd -3rd trimester	7	73	0.32	0.27	1.06 (0.48-2.33)	0.892
Female, 2nd -3rd trimester	8	45	0.34	0.17	2.01 (0.94-4.30)	0.071
Epilepsy						
Male, 1st trimester	6	88	0.77	1.09	0.71 (0.31-1.63)	0.418
Female, 1st trimester	6	72	0.87	0.94	0.95 (0.41-2.20)	0.912
Male, 2nd -3rd trimester	18	285	0.82	1.05	0.81 (0.50-1.31)	0.393
Female, 2nd -3rd trimester	18	240	0.77	0.92	0.84 (0.52-1.35)	0.467
ADHD						
Male, 1st trimester	13	112	1.67	1.38	1.28 (0.72-2.30)	0.402
Female, 1st trimester	<5	11	-	0.14	0.99 (0.13-7.74)	0.993
Male, 2nd -3rd trimester	27	327	1.24	1.20	1.03 (0.70-1.53)	0.867
Female, 2nd -3rd trimester	5	65	0.21	0.25	0.93 (0.37-2.33)	0.882
ASD						
Male, 1st trimester	16	156	2.05	1.93	0.96 (0.57-1.62)	0.881
Female, 1st trimester	<5	25	-	0.33	1.34 (0.40-4.46)	0.634
Male, 2nd -3rd trimester	40	511	1.84	1.88	0.96 (0.69-1.32)	0.797
Female, 2nd -3rd trimester	10	84	0.43	0.32	1.33 (0.69-2.58)	0.394

*The macrolides group included 1129 (male, 1st trimester), 1041 (female, 1st trimester), 3185 (male, 2nd -3rd trimester) and 3277 (female, 2nd -3rd trimester) children. The penicillins group included 11595 (male, 1st trimester), 1041 (female, 1st trimester), 37637 (male, 2nd -3rd trimester) and 35798 (female, 2nd -3rd trimester) children. ADHD: attention-deficit/hyperactivity disorder; ASD: autism spectrum disorder; CI: confidence interval; RR: risk ratio; HR: hazard ratio. Mac: macrolides; Pen: penicillins.

Table 7-10. *Post-hoc* analyses: evaluating whether baby sex modified the association between macrolides (versus penicillins) prescribing during the first trimester and major malformations.

Potential effect modifier	Outcomes	Variables	Coefficient[a]	Std.err[a]	P value[a]	Likelihood ratio test[a,b]	Breslow-Day test[c]
Baby sex (ref: female)	Any major malformation	Male gender	0.34	0.10	0.001		
		Macrolides*Gender	0.44	0.30	0.144	0.137	0.144
	Nervous system malformation	Male gender	-0.44	0.39	0.261		
		Macrolides*Gender	1.95	1.16	0.093	0.055	0.062
	Cardiovascular malformation	Male gender	-0.22	0.18	0.178		
		Macrolides*Gender	0.38	0.45	0.400	0.399	0.389

a: Evaluated in model adjusted by 16 covariates previously mentioned in section 7.2.4., with penicillins group as the reference group:

$$\text{Logit}\,(P(\text{Adverse outcome} = 1)) = \beta_0 + \beta_1 \times Macrolides + \beta_2 \times Sex + \beta_3 \times (Macrolides * sex) + \sum_{i=4}^{19} \beta_i \times Covariates_i$$

b: The likelihood ratio tests tested the differences between adjusted models with and without the interaction term.

c: H_0: $OR_{male}=OR_{female}$, using 2×2×2 frequency tables. Ref: reference.

Table 7-11. Sensitivity analyses: comparison of the risks (or hazards) between siblings of children prenatally prescribed macrolides and siblings of children prenatally prescribed penicillins in the study cohort, according to timing of prescribing (the negative control cohort 1: sibling design).

Adverse outcomes	No. of events in siblings of children prescribed		Risk per 1,000 live births or Rate per 1,000 person-year in siblings of children		Adj. RR/HR in siblings (95% CI)	*P* value
	Macrolides	Penicillins	Macrolides	Penicillins		
Any major malformation						
1st trimester	25	210	21.22	18.06	1.18 (0.78-1.78)	0.429
2nd -3rd trimester	65	665	19.50	17.69	1.10 (0.85-1.41)	0.479
Nervous system malformation						
1st trimester	<5	9	-	1.20	0 (0-inf)	0.990
2nd -3rd trimester	6	40	1.80	1.06	1.73 (0.73-4.07)	0.213
Cardiovascular malformation						
1st trimester	7	81	5.94	6.96	0.87 (0.40-1.88)	0.727
2nd -3rd trimester	23	230	6.90	6.12	1.12 (0.73-1.72)	0.598
Gastrointestinal malformation						
1st trimester	<5	20	-	1.72	0.44 (0.06-3.26)	0.422
2nd -3rd trimester	6	33	1.80	0.88	1.88 (0.79-4.46)	0.152
Genital malformation						
1st trimester	6	42	5.09	3.61	1.44 (0.61-3.37)	0.407
2nd -3rd trimester	13	139	3.90	3.70	1.06 (0.60-1.87)	0.844
Urinary malformation						
1st trimester	<5	10	-	0.86	4.08 (1.27-13.07)	0.018
2nd -3rd trimester	5	51	1.50	1.36	1.10 (0.44-2.75)	0.843
Cerebral palsy						
1st trimester	<5	20	-	0.21	0.46 (0.06-3.43)	0.448
2nd -3rd trimester	6	66	0.22	0.21	0.99 (0.42-2.29)	0.973
Epilepsy						
1st trimester	<5	81	0.32	0.84	0.35 (0.11-1.11)	0.075
2nd -3rd trimester	23	276	0.84	0.87	0.96 (0.62-1.47)	0.841
ADHD						
1st trimester	7	76	0.74	0.79	0.91 (0.42-1.98)	0.807
2nd -3rd trimester	22	264	0.80	0.83	0.99 (0.63-1.56)	0.973
ASD						
1st trimester	13	93	1.38	0.96	1.36 (0.76-2.42)	0.297
2nd -3rd trimester	52	369	1.90	1.16	1.59 (1.16-2.17)	0.004

•1178 (macrolides, 1st trimester), 11631 (penicillins, 1st trimester), 3334 (macrolides, 2nd-3rd trimester), and 37592 (penicillins, 2nd-3rd trimester) children were included in the analyses. Higher risks for genital malformation were observed for the both groups in the sibling cohort for unknown reason. ADHD: attention-deficit/hyperactivity disorder; ASD: autism spectrum disorder; CI: confidence interval; RR: risk ratio; HR: hazard ratio.

Table 7-12. Sensitivity analyses on the association between adverse child outcomes and macrolides versus penicillins prescribed during pregnancy: restricting to mothers whose antibiotics were prescribed to respiratory tract infections.

Adverse Outcomes	No. of events		Risk per 1,000 live births or Rate per 1,000 person-year		Adj. RR/HR (95% CI)	*P* value
	Macrolides	Penicillins	Macrolides	Penicillins		
Any major malformation						
1st trimester	30	159	35.42	18.71	1.81 (1.24-2.66)	0.002
2nd -3rd trimester	43	462	16.00	16.52	0.99 (0.73-1.35)	0.944
Nervous system malformation						
1st trimester	<5	7	-	0.82	1.46 (0.18-11.88)	0.723
2nd -3rd trimester	<5	25	-	0.89	0.88 (0.21-3.73)	0.862
Cardiovascular malformation						
1st trimester	11	61	12.99	7.18	1.79 (0.94-3.38)	0.075
2nd -3rd trimester	16	187	5.95	6.69	0.91 (0.55-1.52)	0.723
Gastrointestinal malformation						
1st trimester	<5	7	-	0.82	1.27 (0.16-10.14)	0.823
2nd -3rd trimester	5	25	1.86	0.89	2.19 (0.84-5.75)	0.110
Genital malformation						
1st trimester	9	26	10.63	3.06	3.30 (1.56-6.99)	0.002
2nd -3rd trimester	10	72	3.72	2.57	1.49 (0.77-2.89)	0.235
Urinary malformation						
1st trimester	<5	16	-	1.88	1.24 (0.29-5.37)	0.775
2nd -3rd trimester	5	51	1.12	1.50	0.75 (0.23-2.41)	0.626
Cerebral palsy						
1st trimester	<5	25	-	0.40	0.46 (0.06-3.38)	0.444
2nd -3rd trimester	7	41	0.38	0.20	1.82 (0.81-4.08)	0.146
Epilepsy						
1st trimester	<5	53	-	0.85	0.62 (0.19-1.98)	0.418
2nd -3rd trimester	15	215	0.81	1.05	0.77 (0.45-1.30)	0.332
ADHD						
1st trimester	<5	47	-	0.76	0.70 (0.22-2.24)	0.543
2nd -3rd trimester	15	151	0.81	0.73	1.17 (0.69-1.98)	0.565
ASD						
1st trimester	6	80	1.04	1.29	0.75 (0.33-1.71)	0.491
2nd -3rd trimester	14	195	0.76	0.95	0.78 (0.45-1.34)	0.368

*ADHD: attention-deficit/hyperactivity disorder; ASD: autism spectrum disorder; CI: confidence interval; RR: risk ratio; HR: hazard ratio.

Table 7-13. Sensitivity analyses: results for multiple bias analyses for first trimester macrolide (versus penicillin) prescribing.

Child adverse outcomes	No. of events		Adjusted risk ratio (95% CI)[a]	+ Adjust bias due to outcome misclassification with random error (95% limits)	+ Adjust bias due to live-birth bias with random error (95% limits)
	Macrolides	Penicillins*			
Any major malformation	60	400.7	1.55 (1.21, 2.03)	1.58 (1.22, 2.08)	
Nervous system malformation	6	27.1	2.30 (0.95, 5.55)	5.17 (1.53, 31.24)	5.64 (1.62, 104.15)
Cardiovascular malformation	23	146.9	1.62 (1.05, 2.51)	1.74 (1.11, 2.74)	1.78 (1.12, 2.80)
Gastrointestinal malformation	<5	-	1.00 (0.23, 4.28)	1.04 (0.24, 4.31)	1.00 (0.23, 4.14)
Genital malformation	11	68.1	1.68 (0.89, 3.16)	2.04 (1.03, 3.94)	
Urinary malformation	<5	-	0.65 (0.20, 2.08)	0.49 (0.14, 1.62)	
Cerebral palsy	<5	-	0.39 (0.10, 1.61)	0.27 (0.06, 1.15)	
Epilepsy	12	160.4	0.78 (0.43, 1.39)	0.74 (0.41, 1.30)	
ADHD	14	122.1	1.19 (0.69, 2.06)	1.24 (0.71, 2.16)	
ASD	19	198.9	0.99 (0.62, 1.58)	0.99 (0.60, 1.56)	

*The numbers of event in penicillins group were weighted based on the distribution of propensity score of macrolides group, which were used to calculate the adjusted risk/hazard ratio in the main analyses. Because the risk ratios for cerebral palsy, epilepsy, ADHD and ASD were comparable with the reported hazard ratios, I measured their risk ratios for simplicity. CI: confidence interval; RR: risk ratio; HR: hazard ratio. ADHD: attention-deficit/hyperactivity disorder; ASD: autism spectrum disorder

Table 7-14. Sensitivity analyses: evaluating whether maternal age group and parity modified the association between macrolides (versus penicillins) prescribing during the first trimester and major malformations.

Potential effect modifier	Outcomes	Variables	Coefficient[a]	Std.err[a]	P value[a]	Likelihood ratio test[a,b]	Breslow-Day test[c]
Maternal age group (ref: 25-29)	Any major malformation	Maternal age 35-50	0.05	0.15	0.726		
		Macrolides*age	0.08	0.42	0.850	0.901	0.905
	Cardiovascular malformation	Maternal age 35-50	0.20	0.24	0.418		
		Macrolides*age	0.03	0.61	0.960	0.645	0.650
	Genital malformation[e]	Maternal age 35-50	-0.01	0.37	0.976		
		Macrolides*age	0.29	0.99	0.773	0.626	0.705
Parity (ref: >=1)[d]	Any major malformation	Parity=0	0.04	0.15	0.756		
		Macrolides*Parity	0.25	0.40	0.536	0.478	0.565
	Cardiovascular malformation	Parity=0	-0.17	0.24	0.499		
		Macrolides*Parity	0.26	0.77	0.736	0.774	0.738
	Genital malformation[e]	Parity=0	0.52	0.26	0.046		
		Macrolides*Parity	-0.17	0.66	0.803	0.803	0.816

a: Evaluated in model adjusted by the other 15 covariates previously mentioned in section 7.2.4., with penicillins group as the reference group. E.g. the model evaluating the interaction term of (macrolides × maternal age group):

$$\text{Logit}\left(P(\text{Adverse outcome} = 1)\right) = \beta_0 + \beta_1 \times Macrolides + \beta_2 \times Maternal\ age + \beta_3 \times (Macrolides * maternal\ age) + \sum_{i=3}^{17} \beta_i \times Covariates_i$$

b: The likelihood ratio tests tested the differences of likelihood between adjusted models with and without the interaction term.

c: H_0: $OR_{strata1}=OR_{strata2}$, using 2×2×2 frequency tables. Ref: reference.

d: Analyses were performed within mothers with at least 2 children recorded in the CPRD Mother Baby Cohort, to avoid an influence of baseline difference between mothers with only one child and mothers with more than one children.

e: Analyses were performed within male babies.

Table 7-15. *Post-hoc* analysis: on the association between common specific malformation and macrolides versus penicillins prescribed during pregnancy.

Adverse Outcomes	No. of events		Risk per 1,000 live births or Rate per 1,000 person-year		Adj. RR/HR (95% CI)	P value
	Macro-lides	Penici-llins	Macrolides	Penicillins		
Ventricular septal defect						
1st trimester	13	85	5.99	3.77	1.66 (0.93-2.98)	0.088
2nd -3rd trimester	25	252	3.87	3.43	1.11 (0.73-1.67)	0.626
Hypospadias*						
1st trimester	10	61	8.86	5.26	1.45 (0.75-2.81)	0.268
2nd -3rd trimester	26	206	8.16	5.47	1.56 (1.04-2.35)	0.032
Atrial septal defect						
1st trimester	5	26	2.3	1.15	2.01 (0.77-5.22)	0.154
2nd -3rd trimester	5	89	0.77	1.21	0.59 (0.24-1.44)	0.244
Patent ductus arteriosus						
1st trimester	<5	40	-	1.77	0.84 (0.26-2.74)	0.778
2nd -3rd trimester	12	127	1.86	1.73	1.02 (0.57-1.84)	0.946
Cleft palate/lip						
1st trimester	<5	29	-	1.29	0.75 (0.18-3.14)	0.692
2nd -3rd trimester	11	94	1.7	1.28	1.29 (0.69-2.40)	0.425
Craniosynostosis						
1st trimester	<5	5	-	0.22	4.16 (0.81-21.45)	0.088
2nd -3rd trimester	5	14	0.77	0.19	3.87 (1.40-10.67)	0.009

*Calculated in male babies. In accordance with the confidentiality preserving policy of CPRD, I only analyses outcomes where there were at least 5 cases in 1st trimester or 2nd to 3rd trimester, macrolides group. I suppressed the information where the frequency cell contains <5 events (noted as "<5") and where necessary to avoid deduction. CI: confidence interval; RR: risk ratio; HR: hazard ratio.

7.4 Discussion

7.4.1 Summary

Compared with children of mothers prescribed penicillins during the first trimester of pregnancy, children of mothers prescribed macrolides had increased risks of any major malformation and specifically cardiovascular malformations. These associations were not significant for prescriptions during the second or third trimester. Macrolides prescribed in any trimester were associated with an increased risk of genital malformations compared with penicillins, although not statistically significant for prescribing restricted to the first trimester. More evidence is needed before firm conclusions can be made about greater risks of any major malformation, cardiovascular malformations, and nervous system malformation in male babies. I found no statistically significant associations between macrolide prescriptions and neurodevelopmental disorders. Sensitivity analyses did not alter these findings.

7.4.2 Strengths and weaknesses of study

Strengths of this study include the large, population-based sample of mothers and children registered with primary care in the UK. The key challenge of using observational studies is to disentangle the potential adverse effect of antibiotic prescribing from the effect of infection on the fetus. By comparing mothers prescribed macrolides with those prescribed penicillins, I addressed this potential confounding as the indications for these treatments largely overlapped (as shown in Appendix 7-3). To reduce the risk of confounding due to severe or recurrent infections, I also restricted all analyses to mothers prescribed a single monotherapy of macrolides or penicillins. To minimise treatment benefits of the antibiotics, sensitivity analyses restricting to mothers with RTIs, which are largely caused by virus infections, were performed. I adjusted for measured confounders using propensity score matching, and evaluated effects of unmeasured maternal characteristics using two negative control cohorts: children of mothers prescribed macrolides or penicillins before pregnancy and siblings of children prenatally exposed to macrolides or penicillins in the study cohort.

The increased risks of any major malformation associated with macrolide prescribing in the first trimester, but not later in pregnancy is consistent with the critical period of fetal organogenesis, i.e. 5GW to 13GW for most major malformations. The increased risk of genital malformations (mainly hypospadias) persisted after the first trimester, which is correspondent with evidence from animal studies that genitalia development could be susceptible to insults after early pregnancy.[331-333] Studies in mice suggested that hypospadias can be induced by oestrogenic agents (such as diethylstilbestrol [DES]) not only at prenatal period (E12-E17 of mice,

comparable to week 5 to 17 of human fetuses), but also at neonatal period (comparable to from week 24 onwards in human fetuses) due to impaired growth and tissue fusion events during development.[331-333] Although this mechanism may not directly relate to the effect of macrolides in human, these studies demonstrated that late pregnancy might be not safe from insults by teratogens for hypospadias. This specificity of exposure timing also favours an adverse effect of macrolides rather than effects due to unmeasured systematic differences between groups (e.g. socioeconomic status and genetic factors).

A key limitation of the study is that the study had limited power to investigate treatment exposure during known (narrower) critical periods for specific malformations and neurodevelopmental disorders, which may induce a potential dilution bias towards the null. I grouped prescribing according to trimesters, not known critical periods (e.g. 5GW to 10GW for cardiovascular malformations) to avoid numerous, underpowered comparisons.[274] For same reason, I categorised malformations by organ system instead of specific malformations (e.g. ventricular septum defect). The possible dilution effect of in epidemiological studies due to grouping phenotypes with different inherent susceptibilities has been highlighted by Jenkins et al.[137] I further explored the effect of macrolides prescribing by gestational days for individual malformations in Chapter 8.

Further limitations include analysing antibiotic prescribing as the main exposure, not dispensing or use, because they were not recorded in CPRD. Compliance of erythromycin could be worse than that of penicillin due to its gastrointestinal side effect. But more untreated maternal infection in the macrolide group is unlikely to explain our findings, as sensitivity analysis restricted to RTIs presented similar findings.[136] The potential underestimation of the association due to outcome misclassification and live-birth bias has also been estimated. Although unmeasured confounders still exist, the negative control analyses based on prescribing before conception and among siblings did not change our findings.

For a small proportion of pregnancies, the start date of pregnancy is likely to be overestimated the duration of pregnancy using my algorithm. As mentioned in Section 4.5, the distribution of gestational age is consistent with the UK Office of National Statistics, although about 6%-7% full-term births with "true" gestational age of 37-38 weeks might have been estimated to be 39 weeks or more. [334] The effect of the "estimated longer gestation" for the small proportion of live births equates to move the measurement window by about one or two week earlier (e.g. from gestational week 2-3 instead of 4) and to classify exposure during 12-13 weeks (first trimester)

as 14 weeks (second trimester), which may slightly bias the association for the first trimester towards the null.

7.4.3 Comparison with other studies

A similar magnitude of effect between first trimester erythromycin prescribing (versus all live born) and major cardiovascular malformations was previously reported by a study using the Swedish birth registry.[51] However, most other studies reported no association between the use of macrolides during pregnancy and major malformations (Appendix 7-4). One reason could be a dilution effect due to macrolides prescribing outside the period of organogenesis.[118] This can be especially a concern when a considerable proportion of first-trimester macrolides were prescribed very early in pregnancy (<4GW). In this study, the proportion was over one-third (36%), which is likely to happen before pregnancy has been detected in most cases.[51] Another explanation is limited power, considering five in a total of ten previous studies reported ≤15 malformations in the macrolides group.

7.4.4 Potential mechanisms for the adverse effect of macrolides

The potential mechanisms for the association between adverse child outcomes and prenatal macrolide treatment were discussed following findings reported in the ORACLE II trial and has been discussed in detail in Section 1.4.[24] The arrhythmic effect of macrolides (as I_{kr}-blockers) was hypothesised to be one of the pathways, which has been warned to induce increased risks of cardiovascular events and mortality in high-risk adults.[26,28] In animal studies, the arrhythmic effect of certain I_{kr}-blockers can cause fetal hypoxia and following birth defects.[49,335] There are no animal reproductive toxicology studies with erythromycin available, but experimental studies of clarithromycin and azithromycin have reported increased risk of embryotoxicity (including fetal growth restriction and death) and teratogenicity that are dose dependent.[47,336,337]

The role of sex-dependent factors

Sex-dependent factors may play a role in the underlying mechanisms for the increased risks for malformations. This cohort study observed increased risks of any major malformation, cardiovascular malformation and genital malformation after first-trimester prescribing of macrolides (versus penicillins), which seem to be limited to, or at least more obvious in male babies, although the interaction terms were not statistically significant. This suggested factors related to male gender might be essential to (for example, mediating) the potential underlying mechanism of the adverse effect of fetal exposure of macrolides.

The impact of sex remains unclear in the progression of human disease in utero and in the mechanisms underlying the developmentally programmed responses. The male fetus is at greater risk of death or damage from almost all the obstetric complications.[125] Perinatal brain damage, cerebral palsy, congenital malformations of the genitalia and limbs, premature birth, and stillbirth are more frequent in boys.[126] Males are more likely to develop certain types of cardiovascular malformation, including transposition of the great arteries, aortic stenosis, coarctation of the aorta, single ventricle, and hypoplastic left ventricle syndrome, while females are overrepresented in atrioventricular septal defect, tricuspid atresia, and truncus arteriosus.[338]

Several intriguing mechanistic candidates have been proposed ranging from differences in the amounts of sex hormones (e.g., estrogens, androgens) to recently described sexual dimorphism in the transcriptome of a variety of mammalian tissues.[339] In utero events could then be mediated by the differentiated hormonal levels, epigenetic changes, and other unknown mechanisms. These sex-dependent factors may also be involved in the fetal-hypoxia related pathway. Animal studies in mice observed that male infants were more vulnerable to ischemia/reperfusion injury following prenatal hypoxia, manifesting in both cardiac susceptibility and brain damage.[340,341] The vulnerability of male infants may be associated with lower estrogen levels, as animal studies suggest that estrogen plays an important role in the cardioprotection of global ischemia and reperfusion injury in female hearts.[340,342]

In contrast to previous studies, I observed no increased risk for neurodevelopmental disorders in mother prescribed macrolides during pregnancy.[24,25] This finding of no evidence of an association may be due to the multifactorial aetiology of neurodevelopment disorders. For example, while 30% of cerebral palsy cases are attributable to genetic factors, 30-40% of epilepsy, ADHD, and ASD cases are considered to have non-genetic causes.[97,343-345] Another potential explanation is that the critical period for cerebral palsy could also be restricted (e.g. within third trimester for spastic cerebral palsy). [346] In the ORACLE II trial where increased risks of cerebral palsy were observed in children of mothers in spontaneous preterm labour, erythromycin was prescribed at a median of 31 GW.[24]

7.4.5 How this works informs my thesis

The result of this chapter suggested that prescribing macrolides compared with penicillins during the first trimester of pregnancy was associated with increased risks of any major malformation and specifically cardiovascular malformations. I also found an increased risk of genital malformations associated with macrolide prescribing in any trimester. More evidence is needed before firm conclusions can be made about greater risks in male babies. There was no evidence

about interactions between macrolides and maternal age or parity on the association, therefore the discrepancies between the target population and general population in maternal age structure and parity were unlikely to limit the generalisability of findings of this study.

Indication bias, unmeasured confounding, live-birth bias and outcome misclassification were unlikely to explain the findings. Nevertheless, a dilution bias towards the null may exist due to measuring exposure out of critical period, unspecific definition of exposure timing window and outcomes, as well as grouping phenotypes with different inherent susceptibilities. The next chapter will thus explore the effect of exposure timing on the association between macrolides and individual malformations.

7.5 Chapter appendix

Appendix 7-1. Most frequent five Read codes for each system-specific malformation.

Type	Description	Read code	ICD10 Code	Frequency
Cardiovascular	Ventricular septal defect	P54..00	Q210	382
	Patent ductus arteriosus	P70..00	Q250	189
	Atrial septal defect NOS	P550.00	Q211	116
	Ostium secundum atrial septal defect	P71..00	Q211	35
	Coarctation of aorta	P55..00	Q251	33
Genital	Hypospadias	PC60.00	Q54	293
	Hypospadias, glandular	PC60312	Q540	20
	Hypospadias, penile	PC60000	Q541	14
	Hypospadias, glanular	PC60311	Q540	10
	Hooded penis	PCyy000	Q54	10
Neurological	Microcephalus	P21..00	Q02	44
	Spina bifida	P1...00	Q05	26
	Congenital hydrocephalus	P23..00	Q03	12
	Septo-optic dysplasia	P246.00	Q044	6
	Micrencephaly	P211.00	Q02	6
Eye	Congenital ptosis	P360.00	Q100	46
	Congenital cataract, unspecified	P330.00	Q120	14
	Coloboma of iris	P344200	Q130	14
	Congenital cataract and lens anomalies	P33..00	Q12	12
	Congenital lacrimal passage anomalies	P364.00	Q106	10
Orofacial cleft	Cleft palate	P90..00	Q35	38
	Cleft palate with cleft lip	P92..00	Q37	37
	Repair of cleft palate	7525.12	Q35	34
	Repair of cleft lip operations	7502.11	Q36	34
	Primary repair of cleft palate, unspecified	7525000	Q35	27
Urinary	Congenital hydronephrosis	PD23.00	Q620	30
	Multicystic kidney	PD13.11	Q611-Q614	19
	Congenital absence of kidney	PD02.00	Q600-Q602	15
	Dysplasia of kidney	PD04.00	Q614	14
	Horseshoe kidney	PD38.00	Q631	10
Gastrointestinal	Hirschsprung's disease	PB30.00	Q431	27
	Imperforate anus	PB26.00	Q423	17
	Atresia of oesophagus	PA30.00	Q39	11
	Other anomalies of lip	PA2A.00	Q380	9
	Atresia of duodenum	PB10100	Q410	6
Respiratory	Choanal atresia	P80..00	Q300	7
	Other lung anomalies	P86..00	Q338	6
	Congenital cystic lung	P84..00	Q330	5
	Congenital bronchomalacia	P83yB00	Q322	<5
	Congenital bronchogenic cyst	P843.12	Q330	<5
Ear & face	Ear anomalies with hearing impairment	P40..00	Q169	8
	Eustachian tube anomalies	P423.00	Q164	<5
	Other specified face and neck anomalies	P4y..00	Q188	<5
	Absence of ear NOS	P401011	Q160	<5
	Deafness due to congenital anomaly NEC	P40z.11	Q169	<5
Abdominal wall defects	Gastroschisis	PG71.00	Q793	19
	Exomphalos	PG70.00	Q792	<5

	Abdominal wall anomalies	PG7..00	Q795	<5
Other	Craniosynostosis	PG03.00	Q750	29
	Urticaria pigmentosa	PH32100	Q822	19
	Ichthyosis congenita	PH1..00	Q80	14
	Imperfect fusion of skull	PG06.00	Q750	12
	Scaphocephaly	PG03.11	Q750	8

Appendix 7-2. Summary of Prior Distributions of the Bias Parameters for the Probabilistic Multiple Bias Analyses.

Parameters	Evidence on bias parameters	Distributions of bias parameters
Outcome misclassification		
Sensitivity	**Major malformations**: The CPRD primary care database was considered a more complete source to investigate major malformation compared with national malformation registry, because primary care follow up records for registered patients. In contrast, malformation registry data is based on voluntary reports and active follow-up which is subject to attrition.[166,236,258,262] Based on our data, the prevalence of major malformation and major cardiovascular malformation were 17.0 and 6.3 per 1000 by the age of 3, respectively. These prevalence rates were slightly higher than those reported by the European Surveillance of Congenital Anomalies (EUROCAT) UK estimates (15.3 and 4.3 per 1000). The prevalence of major cardiovascular malformation in our data was also consistent with other reports using CPRD, of 5.1 to 8.3 per 1000 from ages 1 to age 6 in CPRD.[262] Considering there would be a small portion of malformations diagnosed after age 3 years,[258] I hence assume a not perfect but high sensitivity of malformation in our study, e.g. 0.95, with the range from 0.90 to 1.	Triangular (0.90, 0.95, 1)*
	Cerebral palsy: The prevalence is from 2 to 2.5 per 1000 for the whole population in the UK.[301] I observed a prevalence of 1.8 per 1000 live births till age 14 in this study, and thus assumed a sensitivity from 0.70 to 0.90, with a mode of 0.80.	Triangular (0.70, 0.80, 0.90)
	Epilepsy: The prevalence is 7 to 8 per 1000 for the whole population in the UK.[231] I observed a prevalence of 6.2 per 1000 live births till age 14 in this study, and thus assumed a sensitivity from 0.78 to 0.89, with a mode of 0.84.	Triangular (0.78, 0.84, 0.89)
	ADHD: The prevalence estimates vary widely across studies. While the prevalence in screening studies using the Development and Well-Being Assessment (DAWBA) was 36 per 1000 boys and 9 per 1000 girls, studies based on CPRD reported much lower prevalence rates of ADHD ranging from 4.4 to 8.7 per 1000 boys, and 0.5 to 1.2 per 1000 girls.[254,268,347] I observed a prevalence of 7.5 per 1,000 boys and 1.4 per 1,000 girls in this study, comparable to other CPRD studies. The lower prevalence captured in primary care databases is not surprising though, as ADHD is believed to be an underdiagnosed and undertreated condition, with only 43.7%-54.1% children with current ADHD receiving medications in the US and UK.[348,349] I assumed a sensitivity from 0.50 to 0.90, with a mode of 0.70.	Triangular (0.50, 0.70, 0.90)
	ASD: The prevalence is about 10 per 1000 for the whole population in the UK.[233] I observed a prevalence of 7.7 per 1,000 live births till age 14, and thus assumed a sensitivity from 0.77 to 1, with a mode of 0.89.	Triangular (0.77, 0.89, 1)
Specificity	Specificity is not commonly measured for rarer outcomes in CPRD. However, a high specificity for all outcomes was expected in this study, due to both the low prevalence and the high positive predictive value (PPV). The high PPV of diagnosis in CPRD has been addressed by a number of studies. The PPV for major malformations, including cardiovascular malformations and hypospadias, has been reported to be 93% to 96%.[166,236,243] The identification	Triangular (0.997, 0.999, 1)

	criteria I used for neurodevelopmental disorders have also been validated by previous researches in UK's primary care databases.[245,254,255] I thus assume a PPV of 95% for all outcomes in general population. Based on the definition of specificity, $Specificity = 1 - \frac{False\ positive}{True\ negative} = 1 - \frac{N_{Observed\ positive} \times (1 - PPV)}{N_{all} \times (1 - prevalence)}$ $= 1 - \frac{N_{Observed\ positive}}{N_{all}} \times \frac{0.05}{> 0.95} = 1 - (< 0.05) \times (\frac{0.05}{> .95}) > 0.997$ I then assume a specificity for all outcomes from 0.997 to 1, with a mode of 0.999.	
Live-birth bias for the association between first trimester macrolides prescribing and severe malformations (i.e. nervous system malformation, cardiovascular malformation and gastrointestinal malformation)		
Probability of live-birth (selection)	**P (live-birth|(non-malformed, penicillin)):** 0.83. Around 17% pregnancies were terminated with non-clinical indication.[350] I thus assumed that the probability of live birth in penicillins group without malformation was with a mode of 0.83, and a range of 10%.	Triangular (0.78, 0.83, 0.88)
	P (live-birth|(malformed, penicillin)): 0.63, 0.73 and 0.78 for nervous system malformation, cardiovascular malformation and gastrointestinal malformation respectively. Based on estimated risk of termination, stillbirth, and first day neonatal death among cases with specific malformations, I assume 20%, 10% and 5% of cases with nervous system malformation, cardiovascular malformation and gastrointestinal malformation were dead before registration with the general practice.[351] Therefore, the probability of live birth is estimated to be 1-17%-(20%, 10% or 5%)=63%, 73% or 78% for cases with these three malformations, respectively. I estimated a range of 10%.	Nervous system malformation: Triangular (0.58, 0.63, 0.68) Cardiovascular malformation: Triangular (0.68, 0.73, 0.78) Gastrointestinal malformation: Triangular (0.73, 0.78, 0.83)
	P (live-birth|(non-malformed, macrolides)) = P (live birth|(non-malformed, penicillin))-10%=0.73. Based on our previous system review, where the pooled odds ratio for miscarriage between macrolides and penicillins was 1.82, I assumed that first trimester macrolides exposure would decrease the probability of live birth by up to 10% (based on a probability of miscarriage of 12% in penicillin group*82%), compared to penicillins in fetuses with or without malformation.[325]	Triangular (0.68, 0.73, 0.78)
	P (live-birth|(malformed, macrolides)) = P (live birth|(malformed, penicillin))-10%=0.53, 0.63 and 0.68 for nervous system malformation, cardiovascular malformation and gastrointestinal malformation respectively.	Nervous system malformation: Triangular (0.48, 0.53, 0.58) Cardiovascular malformation: Triangular (0.58, 0.63, 0.68) Gastrointestinal malformation: Triangular (0.63, 0.68, 0.73)

*Triangular (min, mode, max): Triangular distribution with minimum value, mode and maximum value. ADHD: attention-deficit/hyperactivity disorder; ASD: autism spectrum disorder.

Appendix 7-3. Number of prescriptions matched or not matched with any indication (infection) and number of any major malformation by each indication.

No. of prescriptions matched or not matched with indication (infection)		Macrolides				Penicillins	No. of any major malformation	
		Total	Erythromycin	Clarithromycin	Azithromycin		Macrolides	Penicillins
Antibiotics matched with any indication		4726 (55%)	4366 (55%)	287 (58%)	73 (48%)	52293 (54%)	94 (1.99%)	915 (1.75%)
Indication	Respiratory tract infection	3534 (75%)	3298 (76%)	224 (78%)	12 (16%)	36462 (70%)	73 (2.07%)	621 (1.70%)
	Skin infection	377 (8%)	363 (8%)	-	<5	3019 (6%)	9 (2.39%)	65 (2.15%)
	Head & Neck infection	306 (6%)	274 (6%)	-	<5	2979 (6%)	6 (1.96%)	51 (1.71%)
	Genitourinary infection	197 (4%)	191 (4%)	<5	<5	9515 (18%)	<5	173 (1.82%)
	Sexual transmitted infection	163 (3%)	107 (2%)	<5	-	63 (0%)	<5	<5
	Gastrointestinal infection	135 (3%)	121 (3%)	-	<5	171 (0%)	<5	5 (2.92%)
	Other infections	14 (0%)	12 (0%)	<5	<5	84 (0%)	<5	<5
Antibiotics unmatched with any indication		3906 (45%)	3621 (45%)	207 (42%)	78 (52%)	43680 (46%)	92 (2.36%)	751 (1.72%)
Total		**8632**	**7987**	**494**	**151**	**95973**	**186**	**1666**

*An indication was defined as an infection episode recorded within 6 days before a macrolide or penicillin prescription. In accordance with the confidentiality preserving policy of CPRD, I suppressed the information where the frequency cell contains <5 events (noted as "<5") and where necessary to avoid deduction.

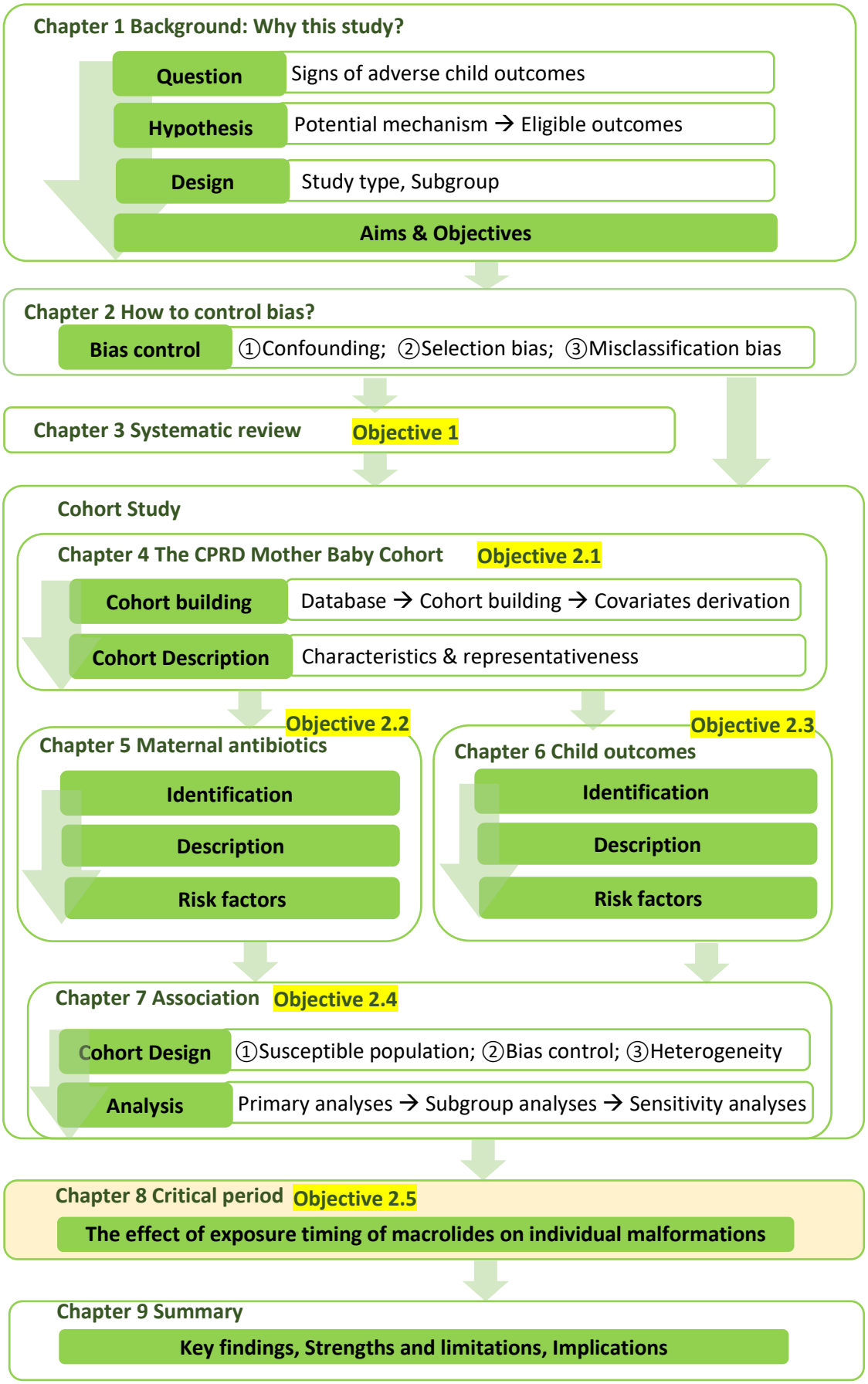
Appendix 7-4. Previously published studies on the association between maternal exposure of macrolides and major congenital malformations or neurodevelopmental disorders.

Studies	Study type	Exposure	Reference group	Outcome	No. of cases/Total in exposure group	RR/OR (95% Confidence interval)	Comments
Einarson, 1990	Prospective cohort	Clarithromycin, 4-14 weeks	Non-teratogenic antibiotics	Major CM	3/157	1.60 (0.26-9.69)	
Czeizel,1999	Paired case-control, Hungarian	Erythromycin, 2-3 month and whole pregnancy	Non-exposure to erythromycin	Isolated CMs	23 cases	1.50 (0.80-2.60) for Cardiovascular CA	
Kallen, 2005	Swedish Medical Birth Register	Erythromycin, 1st trimester	General population and indirectly penicillin V	Cardiovascular CM	31/1844	1.84 (1.29-2.62)	Penicillins Versus general population: 0.99 (0.80-1.23)
Sakar, 2006	Prospective cohort, Canada	Azithromycin, whole pregnancy	Antibiotics, Non-teratogens	Major CM	3/123	Not reported	Under power
Kenyon, 2008	Randomised Clinical Trial	Erythromycin + co-amoxiclav or erythromycin only, 3rd trimester	co-amoxiclav or placebo	Cerebral palsy	18/783 in pPROM, 35/795 in SPL	0.91 (0.48-1.71) in pPROM. 2.28 (1.24-4.21) in SPL	
Kenyon, 2008	Randomised Clinical Trial	Erythromycin + co-amoxiclav or erythromycin only, 3rd trimester	co-amoxiclav or placebo	Epilepsy	18/783 in pPROM, 35/795 in SPL	0.89 (0.59-1.32) in pPROM, 1.18 (0.84-1.66) in SPL	
Cooper, 2009	Tennessee Medicaid	Erythromycin, azithromycin, first 4 lunar months	No antibiotics	Major and system CM	23 major CM/903 in erythromycin group; 23 major CM/559 in azithromycin group	0.86 (0.55-1.34) for erythromycin, major CM; 1.37 (0.85-2.22) for azithromycin, major CM	
Crider, 2009	case-control, Hungarian	Erythromycin, whole pregnancy	No erythromycin	Selected Birth Defects	>300 CM case in total	Anencephaly 2.4 (1.1-5.3) and transverse limb deficiency 2.1(1.0-4.2)	Associations with other outcomes were not significant. Any heart defect 1.0 (0.7-1.3).
Bar-Oz, 2012	Prospective cohort, Czech	Macrolides (Clarithromycin, azithromycin and roxithromycin), 1st trimester	Non-teratogenic exposures	Major and cardiovascular CM	15/441 (Major CM); 7/441 (cardiovascular CM)	1.42 (0.70, 2.88) for macrolides and major CM; 1.91 (0.63, 5.62) for macrolides and cardiovascular CM	
Romoren, 2012	Medical Birth Registry of Norway	Macrolides, 1ST trimester	Penicillin V	Major and cardiovascular CM	69/2549 (Major CM); 25/2549 (cardiovascular CM)	0.96 (0.76,1.22) for major CM; 0.96 (0.65,1.43) for cardiovascular CM	Gestational week 5-8: 1.36 (0.75, 2.47) for cardiovascular CM.
Andersen, 2013	Danish Fertility Database	Clarithromycin, 1ST trimester	No clarithromycin	Major CM	9/253	1.03 (0.53–2.00)	
Bahat, 2013	Retrospective cohort, Israel	Macrolides, 1st and 3rd trimester	No macrolides	Major and cardiovascular CM	Number of cases unreported, 1033 macrolides in total.	1.07 (0.84–1.38) for major CM; 0.95 (0.65–1.40) for cardiovascular CM	
Lin, 2013	Case-control, Slone Epidemiology Center Birth Defects Study	Macrolides and Erythromycin, 1-3 trimester	No erythromycin	Cardiovascular malformation	140 Cardiovascular CM cases in total	0.9 (0.6-1.3) for cardiovascular CM exposed to macrolides during 1st trimester	

Berard, 2015	Prospective cohort, Quebec Pregnancy Cohort	Erythromycin, azithromycin, and clarithromycin, 1st trimester	Unexposed	Major and cardiovascular CM	66/734 erythromycin, 120/914 azithromycin, and 79/686 clarithromycin.	0.96 (0.74–1.24) erythromycin, 1.19 (0.98–1.44) azithromycin and 1.12 (0.99–1.42) clarithromycin	
Meeraus, 2015	Retrospective cohort, UK	Macrolides, whole pregnancy	Penicillins	Cerebral palsy or epilepsy	28/2749	1.78 (1.18-2.69)	
Muanda, 2017	Prospective cohort, Quebec Pregnancy Cohort	Macrolides, 1st trimester	Penicillins	Major and system CM	265/2332 major CM, 35/2332 gastrointestinal CM, 18/2332 genital tract CM	1.13 (0.98–1.31) for major CM, 1.48 (0.99–2.20) for gastrointestinal CM, and 0.93 (0.55–1.56) for genital tract CM	Associations with other outcomes were not significant. High prevalence of major CM, though the author argued this is non-differential between exposure groups.
Damkier, 2019	Danish Medicinal Birth Registry	Erythromycin, azithromycin, gestational day 0 to 90	Penicillins	Major and cardiovascular CM	Major CM: 161/5563 in erythromycin group; 311/5037 in azithromycin group. Cardiac CM: 46/5563 erythromycin; 57/5037 azithromycin	Major CM: 0.94 (0.80-1.11) erythromycin; 1.09 (0.93-1.27) azithromycin Cardiac CM: 0.94 (0.69-1.28) erythromycin; 1.14 (0.86-1.51) azithromycin	

*CM: congenital malformation; pPROM: preterm rupture of the membranes; SPL: spontaneous preterm labour

Thesis Structure



Chapter 8 An exploration of the effect of exposure timing on the association between macrolides and individual malformations

8.1 Background

From the cohort study in Chapter 7, I found associations between maternal prescription of macrolides (vs penicillins) during specific trimesters and increased risks of adverse child outcomes. However, a dilution bias in the estimated associations is possible due to the exposure occurred outside of critical periods and grouping phenotypes with different inherent susceptibilities. As mentioned in section 7.1.1.3, an accurate measurement of risk requires a prior understanding of critical periods of a specific outcome, as well as a large sample size to ensure the statistical power for an individual (and likely rare) phenotype in a narrow time window.

There was limited evidence on critical periods for an individual malformation, although embryological studies have indicated the critical periods for human organ systems in prenatal development (as shown in Figure 1-2). For example, 5 to 14 GW is the period for "organogenesis" and in general the critical period for most major malformation. Specifically, 5 to 9 GW is the critical period for cardiovascular system development and thus most cardiovascular malformation. Also, during the first two weeks the damage caused by a teratogen would most commonly affect all or most cells resulting in the embryo's death. However, a clearly defined critical period for a specific individual malformation is often not known, given the likely multifactorial aetiology and unknown risk factors. For example, patent ductus arteriosus is susceptible not only to rubella infection during the first four GW, but also to insult in later pregnancy or even in neonatal period. Cleft palate, hypospadias and undescended testis are also known to be susceptible to teratogens after the first trimester.

Due to a lack of knowledge about the specific critical period and lack of power for some rare malformations, previous studies on the effect of an exposure in pregnancy commonly applied approaches such as fitting separate regression models for each potential window (i.e. trimester) or fitting a multiple regression that includes all exposure windows. However, considering timing of exposure as a continuous variable may be more informative to investigate the pattern on timing of exposures, avoid a potential dilution bias and facilitate aetiological studies.

This chapter aims to investigate the nonlinear trends of the effect of the timing of the exposure on the association between macrolides and the risks for common individual malformations in children. I measured the timing of prescribing on a continuous scale (not by trimester).

8.2 Methods

I first described the distribution (number of events and the risk) of three most common individual malformations, Ventricular septal defect (VSD), hypospadias and Patent ductus arteriosus (PDA) for children in macrolide and penicillin groups from CPRD Mother Baby Cohort (n=112,376, of which 7771 prescribed between LMP and 4GW and 104,605 prescribed between 4GW and delivery). The cases of VSD, hypospadias and PDA were identified from medical history of each child by age 3 using read code mapping from ICD-10 code Q210, Q250 and Q54, respectively. The exposure dates were defined as from the date of prescription to the last day of this treatment, with a value ranging from 0 to 290 gestational days (GD). Each child may be linked to more than one exposure dates. For prescriptions where the duration was missing (16%), I used 7 days (median) to define the exposure dates.

I modelled the risks of malformations on a continuous scale of exposure timing using nonparametric Poisson regression based on generalized additive mixed models (GAMs).[352] GAM does not require assumptions about the functional form of the exposure-disease relationship except for smoothness, *i.e*. replaces the linear predicator $\sum \beta_p X_p$ by a sum of smooth functions $\sum s_p(X_p)$. GAMs are powerful graphical tools that can provide insights about complex relationships. I did not apply the polynomial models to characterise the curves, as the choice of the shapes are restricted and the estimates may not be as efficient as those from nonparametric models.[352] Additionally, a random effect was adopted to address the correlation among the multiple exposure dates of the children. The regression models were adjusted for 16 covariates previously mentioned (i.e. age at delivery, calendar year at delivery, alcohol misuse, illicit drug use, tobacco use, obesity, hypertension, diabetes, anxiety, depression, epilepsy, parity, multiple birth, chronic medical treatments, genitourinary tract infections and sexually transmitted infections (STIs) during pregnancy).

Suggesting the malformation outcome y_{ij} of the *i* th child (1≤ *i* ≤ 112,376) at the *j* th exposure GD follows a Poisson distribution, the Poisson regression model can be specified as following:

$$log\big(E(y_{ij}|b_i)\big) = \beta_0 + \sum_{p=1}^{16} Covariate_{ip} \times \beta_p + \mathrm{S}_1\big(GD\ for\ Mac_{ij}\big) + \mathrm{S}_2\big(GD\ for\ Pen_{ij}\big) + b_i$$

Where β_0 is the intercept term, β_p is the unknown regression coefficient of the covariate p, $S(\cdot)$ is the unspecified non-parametric functions for GD of macrolides or penicillins exposure estimated using smoothers, and b_i is the random effect for child i, where $b_i \sim N\ (0, \sigma^2)$. The GAM is estimated based on the Iterative Reweighted Least Square procedure, based on thin plate regression splines as used in this study (basis dimension k=10). A 100 (1-α)% pointwise standard error for $S(\cdot)$ was also calculated. The analyses were performed using RStudio version 3.5.1 and R package "mgcv".(17)

8.3 Results

The crude risks of VSD, hypospadias and PDA in children whose mothers were prescribed macrolides or penicillins during the first trimester and the second trimester were given in Table 8-1.

Table 8-1. The association between macrolides (versus penicillins) prescribed during pregnancy and three individual malformations, by trimester of prescription.

Malformations	No. of events		Risk per 1,000 live births or Rate per 1,000 person-year		Adj. RR/HR (95% CI)	P value
	Macro-lides	Penici-llins	Macrolides	Penicillins		
Ventricular septal defect						
1st trimester	13	85	5.99	3.77	1.66 (0.93-2.98)	0.088
2nd -3rd trimester	25	252	3.87	3.43	1.11 (0.73-1.67)	0.626
Hypospadias						
1st trimester	10	61	8.86	5.26	1.45 (0.75-2.81)	0.268
2nd -3rd trimester	26	206	8.16	5.47	1.56 (1.04-2.35)	0.032
Patent ductus arteriosus						
1st trimester	<5	40	-	1.77	0.84 (0.26-2.74)	0.778
2nd -3rd trimester	12	127	1.86	1.73	1.02 (0.57-1.84)	0.946

*Adjusted for age at delivery, calendar year at delivery, alcohol misuse, illicit drug use, tobacco use, obesity, hypertension, diabetes, anxiety, depression, epilepsy, parity, multiple birth, chronic medical treatments, genitourinary tract infections and sexually transmitted infections during pregnancy. Hypospadias were analyses within male babies.

The fitted models were summarised in Table 8-2. The p-value associated with each smooth terms (macrolides and penicillins prescribing) indicated that both smooth terms were significantly different from 0, i.e. non-linear trends exits. Compared with a model not distinguishing S(GD for macrolides) and S(GD for penicillins) (i.e. including one smooth term s(GD for macrolides or penicillins)), the original model (specified above) which distinguished the two antibiotics achieved significantly lower AIC (p<0.001). This suggested a different pattern of the effect of exposure timing between macrolides and penicillins. The k=10 dimension of basis was also checked to be enough to model the complexity.

Table 8-2. Estimation for smooth terms.

	Est.Df	Chisq	*P* value	ΔAIC	ΔDf	P value
Ventricular septal defect						
S(Macrolides)	5.58	22.98	0.001			
S(Penicillins)	8.02	53.45	<0.001			
Model with only one smooth term S(Macrolides or penicillins)				+32.08	-2	<0.001
Hypospadias						
S(Macrolides)	5.40	38.69	<0.001			
S(Penicillins)	7.87	57.91	<0.001			
Model with only one smooth term S(Macrolides or penicillins)				+39.12	-2	<0.001
Patent ductus arteriosus						
S(Macrolides)	4.70	53.58	<0.001			
S(Penicillins)	7.30	82.09	<0.001			
Model with only one smooth term S(Macrolides or penicillins)				+25.64	-2	<0.001

*Est.df: Estimated degrees of freedom. AIC: Akaike information criterion calculated in models with random effect removed.

The effect of exposure timing of macrolides and penicillins on risks of VSD, hypospadias and PDA was estimated using the generalised additive Poisson mixed model as shown in Figure 8-1 and Figure 8-2. While the risk curves for penicillins were relatively stable across exposure timing for all three outcomes, the curves for macrolides had an obvious non-linear pattern although with wider confidence intervals. Compared with penicillins, the risk was significantly higher for macrolides prescribing 1) from late first trimester to second trimester (75-158 GD, about 11-23 weeks) for VSD, and 2) during mid-first trimester and late-second trimester to early third trimester (44-66 and 178-230 GD, about 6-9 and 25-33 weeks) for hypospadias. The risk for PDA was low in both exposure groups with the curves close to each other. A small significant increase of risk was identified during late second trimester to early third trimester (150-230 GD, about 21-33 weeks).

Figure 8-1. Smooths for the risk of malformations according to timing of macrolides or penicillins prescribing (s(GD for Mac) and s(GD for Pen)).

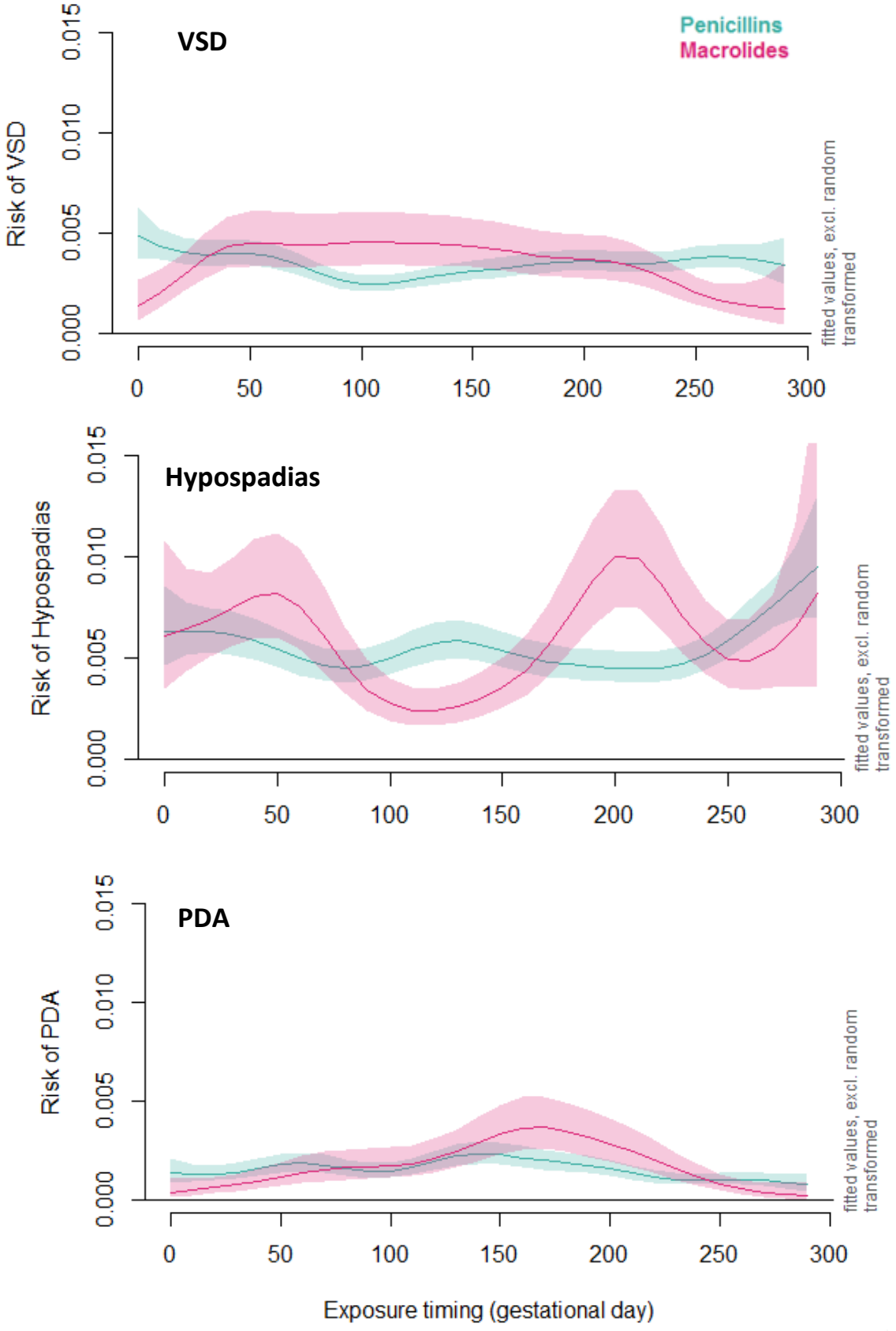
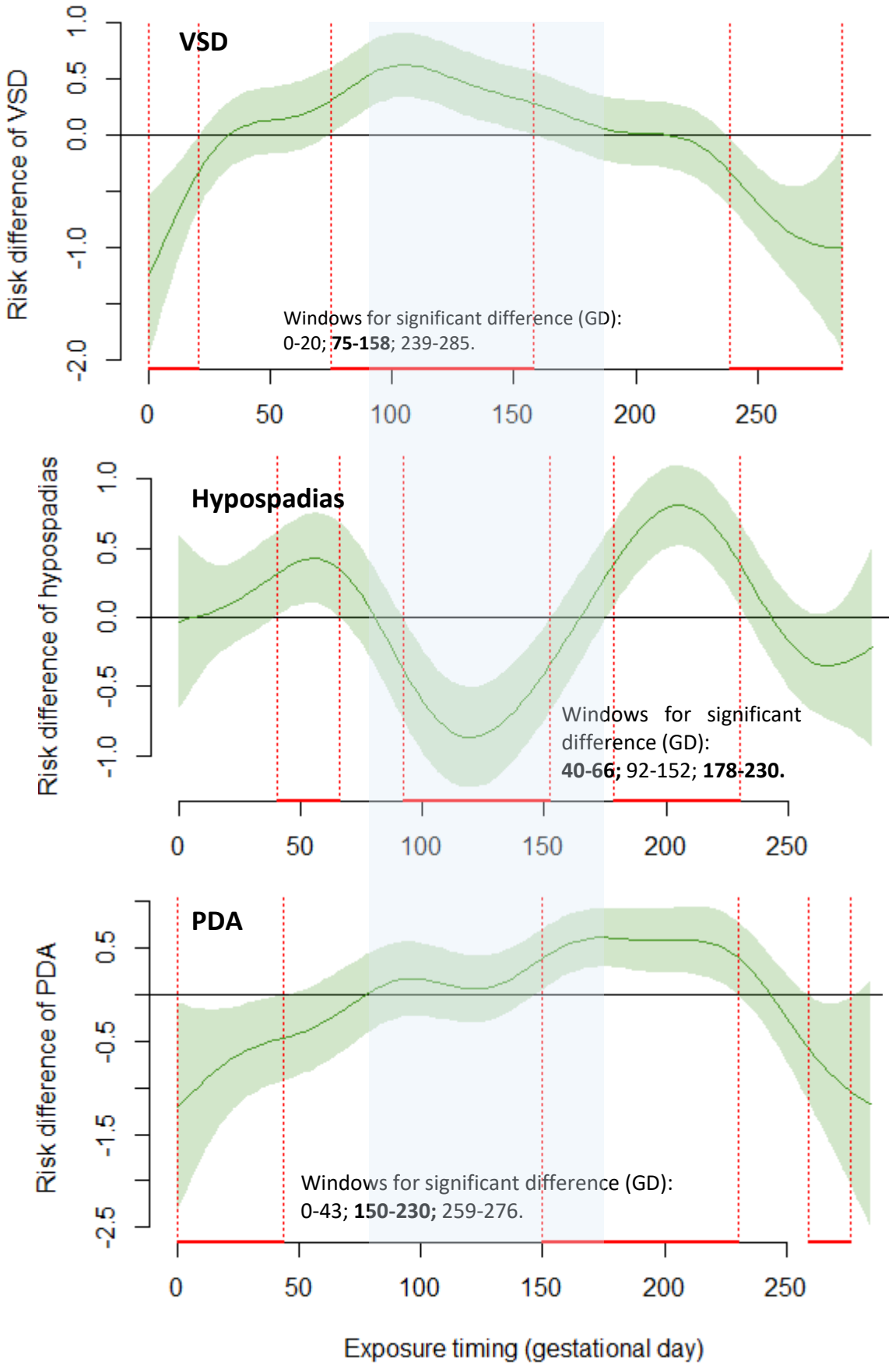


Figure 8-2. Risk difference between S (GD for Mac) and S (GD for Pen))(Note: Transparent area is the second trimester: 14-26 weeks; Red dashed lines: period with significant differences between curves).



8.4 Discussion

Macrolide prescribing during pregnancy presented a time-specific association with two common individual malformations: VSD and hypospadias. Macrolide was associated with a higher risk of VSD for prescribing from late first trimester to second trimester and a higher risk of hypospadias for prescribing in mid-first trimester and from late-second trimester to early third trimester. The risk curves of PDA (which had few events) were in general similar for the two exposure groups.

The increased risk of hypospadias for macrolides prescribing during later pregnancy were consistent with aetiological evidence, while the susceptible period for VSD identified in this study seems to be later than current understanding, probably due to the remaining heterogeneity among subtypes of VSD. Hypospadias could occur during the embryologic development of the urethra between gestational week 6 and 14, which was corresponding to the increased risk observed during early pregnancy in this study. Meanwhile, animal studies suggested that hypospadias could also be induced by oestrogenic agents at neonatal period (comparable to from week 24 onwards in human fetuses) due to impaired growth and tissue fusion events during development.[331-333] For the most common subtype of VSD, perimembranous VSD, embryological studies have shown that its susceptible period is from gestational week 4 to 10, which is the common belief of the critical period for VSD.[274] However, the second common subtype, muscular VSD, has a multifactorial aetiology and are predominantly the result of spontaneous abnormalities in the stage of fetal development (i.e. after embryonic period).[353,354] The susceptible period for muscular VSD was thus suggested to start from 8 gestational week to an unknown point during pregnancy.[57] This study combined the two common subtypes of VSD, which may explain the observed increased risk that persist untill second trimester.

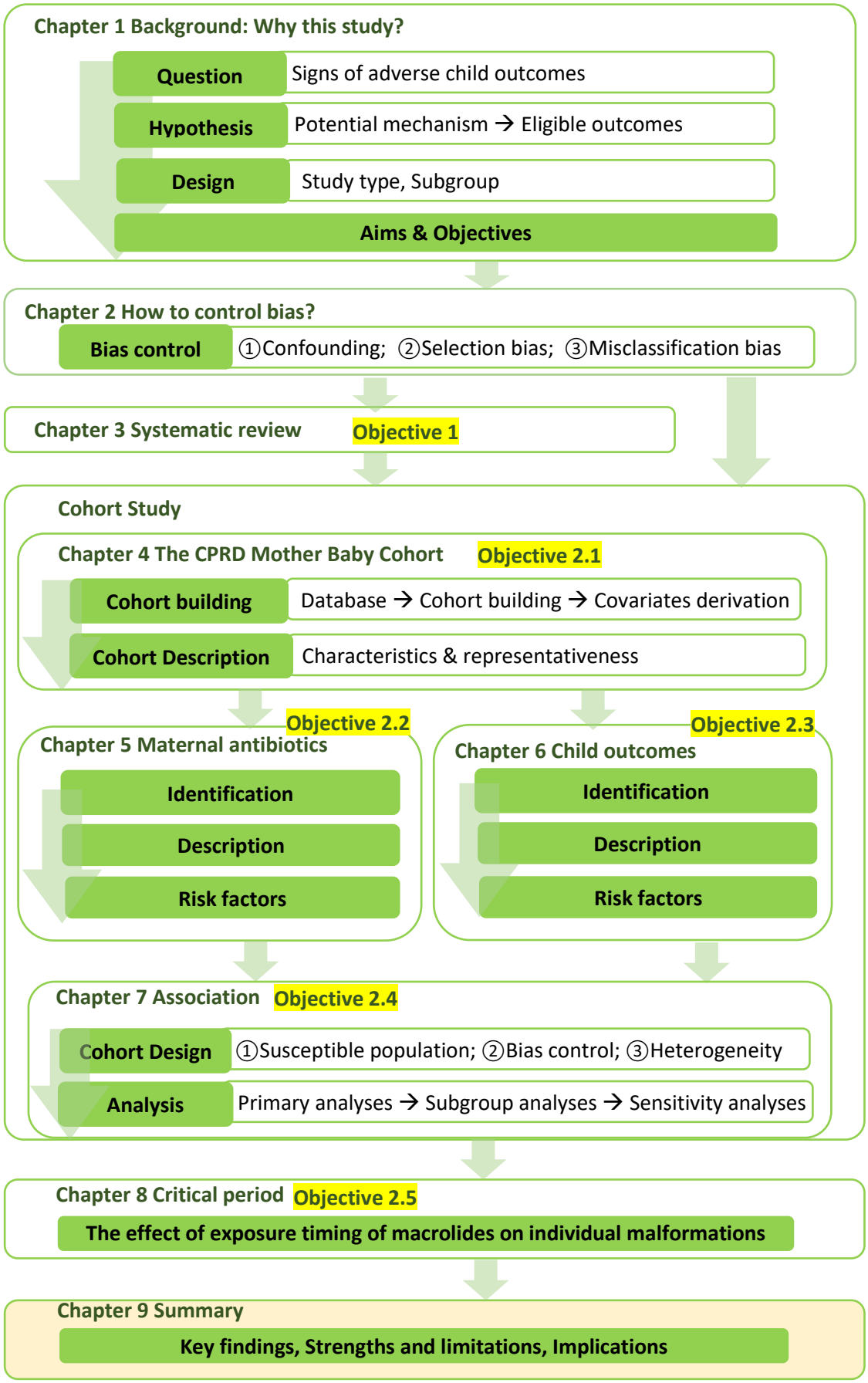
To my knowledge this is the first population-based study which applied a smooth-based model to examine the association between timing of insult and pregnancy outcomes. The flexible nonlinear modelling procedures helps to identify specific outcomes with specific risk patterns in exposure timing as a candidate to test in further aetiological studies. Dilution bias and information loss due to inappropriate cut-off of exposure timing were avoided. Meanwhile, this procedure provides valuable insights about the relationship between the timing of risks and outcomes that cannot be revealed by the use of traditional modelling techniques (e.g. stratification). Given that the critical period for most malformation is still unclear, the study on timing-response relationship could also inform future aetiological studies.

This study has two limitations. Firstly, small number of individual malformation cases in macrolides group may affect the robustness of the model. For example, ≤17 PDA cases in macrolides group might be too sparse to prevent randomness in modelling the timing-response relationship. The pointwise confidence interval cannot fix this as it was based on repeated resampling, and this confidence interval was considered to be too anti-conservative.[355] As a result, the gestational days showing significantly increased risk in macrolides group cannot be explained as the definite dates for a susceptible period for a malformation. I thus explain the finding of the increased risk of PDA observed in macrolides group with caution. Nevertheless, a susceptible period of PDA during late pregnancy was consistent with the aetiological evidence itself. The ductus arteriosus not close until birth, with various environmental factors involved in the process of the closure after birth, such as increased arterial oxygen tension and decreased circulating prostaglandins.[356] The increased risks in the macrolides group during certain periods cannot be explained by differential distributions of timing of prescribing between the two groups, as the distributions have been shown to be similar in Section 5.3.1. Secondly, the error in the estimation of the gestational length and of duration of exposure (rather than duration of prescription as I measured) may affect the results to some extent with unknown direction.

8.5 How this works informs my thesis

The results of this chapter suggest that macrolide prescribing during pregnancy presented a time-specific association with two common individual malformations, VSD and hypospadias. Although confirmation is needed in future larger studies, this study presented differential patterns of risks by timing of prescribing between malformations and between exposure groups, together with a consistency with previous aetiological evidence. These results corroborated my main finding in the Chapter 7 that macrolides prescribed during pregnancy were associated with increased risks of any major malformation and specifically cardiovascular malformations.

Thesis Structure



Chapter 9 Summary of findings, implications and conclusions

9.1 Summary of research

9.1.1 Rationale and thesis aims

Over the last 20 years, concerns have been raised about rare but serious adverse outcomes associated with macrolide use during pregnancy. The large randomised controlled trial (RCT, ORACLE Child Study II) of women with spontaneous preterm labour (SPL) reported an increased risk of cerebral palsy in children whose mothers received erythromycin compared with no erythromycin (3.3% versus 1.7%, Odds Ratio (OR) 1.93, 95% Confidence Interval (CI): 1.21-3.09) [24]. Increased risks of miscarriage, major malformations, and cardiovascular malformation have also been reported in some observational studies [15,22,23,51,147], but not in others [12,357,358]. Animal studies on macrolides observed variable results and the possibility of adverse effects on embryo-foetal development cannot be excluded.[47] Meanwhile, Food and Drug Administration (FDA) has published repeated warnings of an increased risks of cardiovascular event or deaths associated with the used of macrolide antibiotics in high-risk adults.[26,28,29]

Currently there was no consensus about whether macrolides are considered safe in pregnancy or not. While the Swedish national policy advised against the use of erythromycin during early pregnancy in 2005, the British National Formula comments erythromycin as “not known to be harmful”, and suggests to avoid clarithromycin and azithromycin during pregnancy unless potential benefit outweighs risk or if adequate alternatives were not available.[3,51]

Prompt and adequate treatment for infection during pregnancy is vital given the well-established association between maternal infection during pregnancy and adverse birth outcomes, including miscarriage and stillbirth.[54] More evidence is needed on whether there is an association between maternal treatment with macrolide antibiotic during pregnancy and adverse outcomes in children. This PhD study aims to generate further evidence on associations between maternal prescribing of macrolides during pregnancy and adverse child outcomes.

9.1.2 Key findings

The systematic review and meta-analysis found consistent evidence for an association between macrolide antibiotics use during early pregnancy and an increased risk of miscarriage, inconsistent evidence for cerebral palsy and epilepsy, and insufficient evidence for malformations, stillbirth and neonatal death (Chapter 3, Objective 1).

Of 11,186 citations identified, 19 (10 observational, 9 RCTs) studies were included (21 articles including 228,556 participants). Macrolide prescribing during pregnancy was associated with an increased risk of miscarriage (pooled OR_{obs} 1.82, 95% CI 1.57-2.11; I^2=0%), cerebral palsy and/or epilepsy (OR_{obs} 1.78, 1.18-2.69; one study), epilepsy alone (OR_{obs} 2.02, 1.30-3.14, one study; OR_{RCT} 1.03, 0.79- 1.35, two studies), compared with alternative antibiotics. I found no evidence of an adverse effect on malformations, stillbirth, or neonatal death.

To my knowledge, this review is the first to systematically assess the adverse fetal and child outcomes of macrolides use during pregnancy. The increased risk of miscarriage and inconsistent evidence for cerebral palsy and epilepsy suggested that macrolides may have the potential to cause adverse effects when used in pregnancy. Heterogeneity existed among individual studies due to study design (RCT or observational), population of pregnant women studied (with high baseline risk of fetal infection or not), specific types of macrolides, and gestational ages for administering macrolides (potential underestimation of the adverse effect by measuring the exposure outside of critical period). The results of this systematic review and meta-analyses were published in a peer-reviewed journal.[359]

Based on the mother-baby linkage developed from the UK administrative primary care database CPRD, a large Mother Baby Cohort was built as the target population for the cohort study. The gestational length was estimated for each baby. The CPRD Mother Baby Cohort was comparable with the national statistics in most maternal and birth-related characteristics (Chapter 4, Objective 2.1).

To my knowledge, the derived CPRD Mother Baby Cohort, comprising 728,921 children born to 514,139 mothers with complete prenatal follow-up and a median follow-up per child of 6 years, is the largest of its kind using UK primary care data. Besides, the algorithm I developed to estimate the gestational length achieved close agreement with national statistics especially for preterm births, which is an improvement over many other pregnancy cohorts created using routine data. Maternal life style factors, maternal conditions before and during pregnancy, and proportion of multiple births were also comparable with national statistics. Differences with general population exists in that this cohort involves more older mothers at delivery and more firstborn children. The differences did not limit the generalisability of the findings in this study, because no effect modification of the factors for the association between macrolides and adverse child outcomes was observed in the cohort study (Chapter 7).

Penicillins and macrolides were the most and the third commonly prescribed antibiotics class during pregnancy in UK primary care settings. Mothers prescribed macrolides and penicillins

were comparable in most measured maternal and pregnancy-related characteristics. The most significant difference was that macrolides prescribing was less often for genitourinary tract infections and more often for sexually transmitted diseases (STIs) as compared with penicillins group (Chapter 5, objective 2.2).

Among the one-third mothers who were prescribed antibiotics during pregnancy, penicillins and macrolides were the most and the third commonly prescribed antibiotics classes, where 71% and 84% of the prescriptions were prescribed as a monotherapy, respectively. RTIs accounts for about 67% to 75% of matched indications for penicillins and macrolides. Compared with penicillins, macrolides were prescribed less for genitourinary tract infections (5.5% versus 20.3%) and more for STIs (2.6% versus 0.2%). The differences between the cohorts of mothers prescribed macrolides versus penicillins before pregnancy (the negative control cohort) were similar with those observed among mothers prescribed during pregnancy (the study cohort). This implicated the appropriateness of the negative control cohort in terms of indirectly controlling confounding from the systematic difference between macrolides and penicillins prescribing around pregnancy.

The comparisons with external evidence show that the major malformations identified in this study had high sensitivity and specificity, and associations with risk factors were mostly consistent with previous studies. In contrast, neurodevelopment disorders were under-recorded in CPRD, although prevalence rates were still consistent with previous studies using primary care data (Chapter 6, objective 2.3).

Coding of cerebral palsy in UK administrative database is known to be infrequent and non-specific. Previous studies identified cerebral palsy case in ways that were either too specific or too sensitive. This studies proposed a data-driven scheme based on the random forest method which could identify the most informative predictors in a cost-effective way to reliably predict potential unrecorded cases of cerebral palsy, with a potential to generalise to the identification of other complex medical conditions in UK administrative databases.

Compared with children of mothers prescribed penicillins, children of mothers prescribed macrolides during the first trimester of pregnancy (from 4 to 13 gestational week) had increased risks of any major malformation and specifically cardiovascular malformations. These associations were not significant for prescriptions during the second or third trimester. The risk of genital malformations (mostly hypospadias) was increased in children of mothers prescribed macrolides compared with penicillins in any trimester (Chapter 7, objective 2.4).

Subgroup analyses of erythromycin prescribing produce results consistent with any macrolides. More evidence is needed before firm conclusions can be made about greater risks in male babies

for any major malformation, cardiovascular malformations, and nervous system malformation. There was no evidence for interactions between macrolides and maternal age or parity on the association with any major malformation, cardiovascular malformation or genital malformation, therefore the discrepancies between the target population and general population in maternal age structure and parity do not limit the generalisability of findings of this study. Indication bias, unmeasured confounding, live-birth bias and outcome misclassification were unlikely to explain the findings. Nevertheless, a dilution bias towards the null may exist due to grouping phenotypes with different inherent susceptibilities and measuring exposure outside the critical period. A paper based on the results of this analysis are under review in a peer-reviewed journal.

The exploratory study suggested that macrolide was associated with a higher risk of VSD for prescribing from late first trimester to second trimester, and a higher risk of hypospadias for prescribing during mid-first trimester and from late-second trimester to early third trimester (Chapter 8, objective 2.5).

Although confirmation is needed in future larger studies, the study presented differential patterns of risks by timing of prescribing between exposure groups and between specific malformations, together with consistency with previous aetiological evidence. These results corroborated with my main finding in Chapter 7 that macrolide prescribing during early pregnancy was associated with increased risks of cardiovascular malformations and that macrolide prescribing during both early and late pregnancy were associated with increased risk of genital malformations.

9.2 Strength

A major strength of this study was the analysis strategy to systematically address factors that may affect the validity of the estimation of the associations. Firstly, I adopted the head-to-head design to reduce the risk of indication bias, the key concern of observational studies on drug safety, in both the systematic review and the cohort study. Penicillins are particularly suitable comparators for macrolides because the most frequent indication for macrolides' use in pregnancy is as a replacement for suspected penicillin allergy. The definition of exposure restricted to antibiotics monotherapy and a sensitivity analyses restricted to respiratory tract infections further controlled the residual indication bias due to severity of underlying infection and infection types. Secondly, other factors that may affect the estimation of the association were classified and addressed respectively. For example, the potential bias were sorted as three major sources (confounding, selection bias and misclassification bias) and addressed respectively using various designs, including two negative control cohorts (cohorts of sibling and

of prescription before pregnancy), and simulations (multiple bias analyses). Results of these sensitivity analyses showed that unmeasured confounding, restriction of analyses to live-births, and outcome misclassification, were unlikely to explain the findings.

9.3 Limitations and future directions

A key limitation of this study is the limited power that precludes analyses examining specific malformations, thereby inducing a potential dilution bias towards the null and making aetiological interpretation difficult. Specific malformations that belong to the same organ system may share certain aetiological causes, but can often have different, specific, multifactorial aetiological mechanisms. For specific malformations of an organ system, a potential insult induced by macrolides (e.g. fetal hypoxia) may contribute in various degrees to the aetiologies, or may not be involved at all in the aetiology of some specific malformations. For example, while pregestational diabetes has been shown to increase the overall risk of cardiovascular malformation, a much higher risk was observed for patent ductus arteriosus (PDA, with an estimated risk ratio of 57) than for atrioventricular septal defect (with an estimated risk ratio of 11).[137] Therefore the ideal definition for the malformations in this study would be individual malformations for which the aetiological mechanism is known to involve fetal hypoxia.

I grouped malformations by organ system in this study, in order to avoid false-negative findings which is a danger for drug safety studies. This approach increased the risk of dilution bias. Given the limited number of events, defining malformations by specific aetiological mechanisms will end up with few cases in each group of malformations. This classification would thus lead to a major loss in statistical power caused by the sparsity of cases and discarded information. This definition would also make an investigation on patterns by trimester and macrolides subtype nearly implausible even though CPRD is one of the largest datasets for malformation aetiology studies in the world.

For a similar reason, and also due to limited understanding in critical period for specific malformations, I defined antibiotics exposure by trimesters instead of specific gestational days or weeks at the expense of a potential underestimation of effect. In fact, measuring exposure out of the critical period of the malformation could be a common caveat for population-based studies for associations between short-term prenatal exposures and child outcomes, and thus a potential explanation for non-significant findings in some of the previous studies on macrolides. In some studies, women were regarded as exposed if they received a prescription of a macrolide

at any point after their last menstrual period (LMP). As the prescription often lasts for a week or a little more, most women who filled the prescription within the first 3 weeks would not have exposed their infants during organogenesis (from gestational week 5 to gestational week 14 for most malformations), which would then bias the risk ratio estimate towards 1.0.[16,17,19] In a previous study using the Norwegian birth registries, the adjusted OR for the association between cardiovascular malformation and prenatal exposure to erythromycin was notably higher when erythromycin was measured in the most vulnerable period of heart formation (gestational week 5-8, adjusted OR 1.6, 95% CI [0.9, 3.0]), compared with that when erythromycin was measured during the whole first trimester (adjusted OR 1.2, 95% CI [0.8, 1.8]). This is especially a concern when a considerable proportion (over one-third in our study – 36%) of first-trimester macrolides were prescribed very early in pregnancy (<4GW), which is likely to be before pregnancy has been detected in most cases. Similarly, Kallen et al also noticed that only 30% of all women prescribed from LMP to the end of the first trimester were actually prescribed during gestational week 5-9.[51]

Yet an accurate critical period for a specific outcome is often unknown given the limited knowledge from aetiology studies. A remedy suggested in Chapter 8 was to apply the generalised additive model as a flexible tool to model the pattern for the association between macrolides and risks of outcomes according to exposure timing, where prior knowledge of the critical period for outcomes was not needed. Nevertheless, a larger sample size is preferable for a robust inference on the gestational days with significant susceptibility based on this smoothing-based model.

Except for non-specific definition of outcome and exposure timing, two other limitations may also lead to an underestimation of the strength of the associations in the cohort study. Firstly, although the gestational age estimated in CPRD Mother Baby Cohort achieved good comparability with national statistics for preterm births and most term births, about 6-7% of term births with gestational length of 37-38 week were estimated as 40 week. For these children, macrolides or penicillins were measured from about gestational week 2 instead of gestational week 4, therefore diluting the potential association between first-trimester macrolide prescribing and outcomes. Secondly, I measured prescriptions, not use. Due to the gastrointestinal side effect of erythromycin, the compliance of erythromycin could be worse than that of penicillin, resulting a potential underestimation of the effect of macrolides. This underestimation may apply to both the cohort study and the systematic review, where most previous studies used population-based databases of prescription where information on dispensing and/or compliance was not available.

Finally, based on the observational study I cannot conclude that the association observed between prenatal macrolides exposure and major malformations was a causal relationship. However, further trials are unlikely to be conducted.

Given the widespread use of macrolides during pregnancy, there is an urgent need for international collaboration to bring together existing datasets for large-scale analyses of high quality trial and observational cohorts that have accurate measurement of treatment and specific child outcomes. Analyses should pre-specify treatment exposure periods based on gestational days when hazards are likely to impact on organogenesis for specific malformations or on neurodevelopmental outcomes. At least, antibiotics taken before organogenesis (before gestational week 4 or 5) should not be defined as exposure. In larger studies, it is also worth assessing the potential interaction between baby sex and the effect of macrolides, given the differential strength of association between genders observed in this study. Future larger studies enabling these closer investigations on specific outcomes are essential to provide aetiological interpretations for the association between prenatal macrolides exposure and adverse child outcomes.

9.4 Implications for clinical practice and policy

The increased risks of miscarriage found in the systematic review and the increased risks of major malformations found in this cohort study and the study of Kallen et al. provide evidence that macrolide prescribing during pregnancy could be associated with serious pregnancy and child outcomes. If the associations are causal, an additional 5 to 100 miscarriages would occur for every 1000 pregnancies exposed to macrolides instead of penicillins at 6 gestational weeks (with a baseline risk of of 20%) and at 20 gestational weeks (with a baseline risk of 1%), respectively; 4.1 (95% CI: 0.4-9.4) children with cardiovascular malformations and 1.7 (95% CI: 0.4-3.5) children with genital malformations would occur for every 1000 children exposed to macrolides instead of penicillins in the first trimester or in any trimester, respectively.

The MHRA give no direct advices for erythromycin use during pregnancy. The recent Patient information leaflets (PILs) and Summaries of product characteristics (SPCs) issued by the UK MHRA about erythromycin states that “There are no adequate and well-controlled studies in pregnant women. However, observational studies in humans have reported cardiovascular malformations after exposure to medicinal products containing erythromycin during early pregnancy.”[53] The British National Formulary states that erythromycin is “not known to be

harmful".[3] Current National Institute for Health and Care Excellence (NICE) guidance recommends erythromycin as replacements for penicillin allergy for upper respiratory infection in pregnant women.[360] The SPCs and BNF advise to avoid clarithromycin and azithromycin during pregnancy unless the potential benefit outweighs risk or if adequate alternatives are not available.[3,53]

Given the moderate to high absolute risks of potential adverse outcomes of macrolides and the widespread use of macrolides during pregnancy, as well as the cardiotoxicity of macrolides use in adults, a review of the evidence on macrolide safety in pregnancy by the regulatory agency is warranted. Macrolides, including erythromycin, should be prescribed only when there is a clear clinical need and if no alternative is feasible. It is essential that pregnant women receive treatment with an appropriate antibiotic when necessary, as certain infections in pregnancy can cause serious harm both to the mother and the baby. On the other hand, macrolide prescribing without a definite clinical need poses potential risks of adverse child outcomes, and adds to the pressing problem of increasing prevalence of antimicrobial resistance. Mothers better to be sure that they are penicillin-allergic before using macrolides as a replacement for penicillins. In UK 10% people have penicillin allergy recorded in clinical notes – only 1 in 20 are true.[361]

The MHRA should therefore examine the use of macrolides in pregnant women to ensure the best outcome for mother and child. Cost-effective alternatives for macrolides for use in women who are allergic to penicillin also need to be identified. Finally, prescription guidelines and patient information leaflets should report the uncertainty about the safety of macrolides, including erythromycin, and evidence of associations with miscarriage and malformations.

9.5 Concluding remarks

Macrolide prescribing during early pregnancy is associated with increased risks of miscarriage, any major malformation and specifically cardiovascular malformation. Macrolide prescribing during both early and late pregnancy is associated with an increased risk of genital malformation (mainly hypospadias). More evidence is needed before firm conclusions can be made about greater risks in male babies for any major malformation, cardiovascular malformations, and nervous system malformation. The mechanism for the adverse effect is unknown. A short-term fetal hypoxia induced by the arrhythmic effect of macrolides could be a potential pathway, acknowledging the cardiotoxicity of macrolides observed in adults. Considering the moderate-to-high absolute risks of potential adverse outcomes of macrolides and its widespread use during pregnancy, international collaboration is in urgent need to bring together existing

datasets for large-scale analyses of high quality trial and observational cohorts that have accurate measurement of macrolides treatment and specific child outcomes. Analyses should pre-specify treatment exposure periods based on the critical period of specific outcomes. The findings of this study warrant cautious use of macrolides in pregnancy and recommendation of alternative antibiotics where feasible.

Reference

1. Hamilton-Miller JM. Chemistry and biology of the polyene macrolide antibiotics. Bacteriological reviews 1973;37:166-96.
2. Dinos GP. The macrolide antibiotic renaissance. British journal of pharmacology 2017;174:2967-83.
3. Joint Formulary Committee Great Britain. British National Formulary. London: Pharmaceutical Press; 2019.
4. SPIRAMYCIN. The National Institute for Health and Care Excellence (NICE). (Accessed 29 May 2019, at https://bnfc.nice.org.uk/drug/spiramycin.html.)
5. Chlamydia - uncomplicated genital. The National Institute for Health and Care Excellence (NICE). (Accessed 30 May 2019, at https://cks.nice.org.uk/chlamydia-uncomplicated-genital#!scenario.)
6. Miller JM, Martin DH. Treatment of Chlamydia trachomatis Infections in Pregnant Women. Drugs 2000;60:597-605.
7. Gibreel A, Taylor DE. Macrolide resistance in Campylobacter jejuni and Campylobacter coli. Journal of Antimicrobial Chemotherapy 2006;58:243-55.
8. Taylor-Robinson D, Bebear C. Antibiotic susceptibilities of mycoplasmas and treatment of mycoplasmal infections. The Journal of antimicrobial chemotherapy 1997;40:622-30.
9. Petersen I, Gilbert R, Evans S, Ridolfi A, Nazareth I. Oral antibiotic prescribing during pregnancy in primary care: UK population-based study. The Journal of antimicrobial chemotherapy 2010;65:2238-46.
10. Crider KS, Cleves MA, Reefhuis J, Berry RJ, Hobbs CA, Hu DJ. Antibacterial medication use during pregnancy and risk of birth defects: National Birth Defects Prevention Study. Archives of pediatrics & adolescent medicine 2009;163:978-85.
11. Källén BAJ, Otterblad Olausson P. Maternal drug use in early pregnancy and infant cardiovascular defect. Reproductive Toxicology 2003;17:255-61.
12. Romoren M, Lindbaek M, Nordeng H. Pregnancy outcome after gestational exposure to erythromycin - a population-based register study from Norway. British journal of clinical pharmacology 2012;74:1053-62.
13. [Erythromycin should be avoided during early pregnancy]. The Swedish Medical Products Agency, 2006. (Accessed 29 Jan 2019, at https://lakemedelsverket.se/Alla-nyheter/NYHETER---2005/Erytromycin-bor-undvikas-under-tidig-graviditet/.)
14. The Swedish Medical Products Agency. Public Assessment Report Scientific discussion: Erythromycin ELC (SE/H/1486/01/DC). Uppsala: The Swedish Medical Products Agency; 2015.
15. Andersen JT, Petersen M, Jimenez-Solem E, et al. Clarithromycin in early pregnancy and the risk of miscarriage and malformation: a register based nationwide cohort study. PLoS One 2013;8:e53327.
16. Bahat Dinur A, Koren G, Matok I, et al. Fetal safety of macrolides. Antimicrob Agents Chemother 2013;57:3307-11.
17. Bar-Oz B, Weber-Schoendorfer C, Berlin M, et al. The outcomes of pregnancy in women exposed to the new macrolides in the first trimester: A prospective, multicentre, observational study. Drug safety 2012;35:589-98.
18. Berard A, Sheehy O, Zhao JP, Nordeng H. Use of macrolides during pregnancy and the risk of birth defects: a population-based study. Pharmacoepidemiol Drug Saf 2015;24:1241-8.

19. Cooper WO, Hernandez-Diaz S, Arbogast PG, et al. Antibiotics potentially used in response to bioterrorism and the risk of major congenital malformations. Paediatric and perinatal epidemiology 2009;23:18-28.
20. Muanda FT, Sheehy O, Berard A. Use of antibiotics during pregnancy and the risk of major congenital malformations: A population based cohort study. British journal of clinical pharmacology 2017.
21. Damkier P, Bronniche LMS, Korch-Frandsen JFB, Broe A. In utero exposure to antibiotics and risk of congenital malformations: a population-based study. Am J Obstet Gynecol 2019;221:648.e1-.e15.
22. Einarson A, Phillips E, Mawji F, et al. A prospective controlled multicentre study of clarithromycin in pregnancy. Am J Perinatol 1998;15:523-5.
23. Muanda FT, Sheehy O, Berard A. Use of antibiotics during pregnancy and risk of spontaneous abortion. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne 2017;189:E625-e33.
24. Kenyon S, Pike K, Jones DR, et al. Childhood outcomes after prescription of antibiotics to pregnant women with spontaneous preterm labour: 7-year follow-up of the ORACLE II trial. Lancet 2008;372:1319-27.
25. Meeraus WH, Petersen I, Gilbert R. Association between antibiotic prescribing in pregnancy and cerebral palsy or epilepsy in children born at term: a cohort study using the health improvement network. PLoS One 2015;10:e0122034.
26. FDA Drug Safety Communication: Azithromycin (Zithromax or Zmax) and the risk of potentially fatal heart rhythms. U.S. Food and Drug Administration. (Accessed 30 May 2019, at https://www.fda.gov/Drugs/DrugSafety/ucm341822.htm.)
27. Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. Azithromycin and the Risk of Cardiovascular Death. 2012;366:1881-90.
28. U.S. Food and Drug Administration. FDA Drug Safety Communication: FDA review finds additional data supports the potential for increased long-term risks with antibiotic clarithromycin (Biaxin) in patients with heart disease. 2018.
29. The US Food and Drug Administraton. Information for Healthcare Professionals: Clarithromycin (marketed as Biaxin)2005.
30. Jespersen CM, Als-Nielsen B, Damgaard M, et al. Randomised placebo controlled multicentre trial to assess short term clarithromycin for patients with stable coronary heart disease: CLARICOR trial. Bmj 2006;332:22-7.
31. Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. Azithromycin and the Risk of Cardiovascular Death. New England Journal of Medicine 2012;366:1881-90.
32. Schembri S, Williamson PA, Short PM, et al. Cardiovascular events after clarithromycin use in lower respiratory tract infections: analysis of two prospective cohort studies. BMJ : British Medical Journal 2013;346:f1235.
33. Svanstrom H, Pasternak B, Hviid A. Use of clarithromycin and roxithromycin and risk of cardiac death: cohort study. Bmj 2014;349:g4930.
34. Rao GA, Mann JR, Shoaibi A, et al. Azithromycin and levofloxacin use and increased risk of cardiac arrhythmia and death. Ann Fam Med 2014;12:121-7.
35. Winkel P, Hilden J, Hansen JF, et al. Clarithromycin for stable coronary heart disease increases all-cause and cardiovascular mortality and cerebrovascular morbidity over 10years in the CLARICOR randomised, blinded clinical trial. International journal of cardiology 2015;182:459-65.
36. Chou H-W, Wang J-L, Chang C-H, Lai C-L, Lai M-S, Chan KA. Risks of Cardiac Arrhythmia and Mortality Among Patients Using New-Generation Macrolides,

Fluoroquinolones, and β-Lactam/β-Lactamase Inhibitors: A Taiwanese Nationwide Study. Clinical Infectious Diseases 2014;60:566-77.
37. Cheng YJ, Nie XY, Chen XM, et al. The Role of Macrolide Antibiotics in Increasing Cardiovascular Risk. J Am Coll Cardiol 2015;66:2173-84.
38. Guo D, Cai Y, Chai D, Liang B, Bai N, Wang R. The cardiotoxicity of macrolides: a systematic review. Die Pharmazie 2010;65:631-40.
39. Bin Abdulhak AA, Khan AR, Garbati MA, et al. Azithromycin and Risk of Cardiovascular Death: A Meta-Analytic Review of Observational Studies. American journal of therapeutics 2015;22:e122-9.
40. Li X, Wang M, Liu G, Ma J, Li C. Association of macrolides with overall mortality and cardiac death among patients with various infections: A meta-analysis. Eur J Intern Med 2016;28:32-7.
41. Wong AYS, Chan EW, Anand S, Worsley AJ, Wong ICK. Managing Cardiovascular Risk of Macrolides: Systematic Review and Meta-Analysis. Drug safety 2017;40:663-77.
42. Gorelik E, Masarwa R, Perlman A, Rotshild V, Muszkat M, Matok I. Systematic Review, Meta-analysis, and Network Meta-analysis of the Cardiovascular Safety of Macrolides. Antimicrob Agents Chemother 2018;62.
43. Khosropour CM, Capizzi JD, Schafer SD, Kent JB, Dombrowski JC, Golden MR. Lack of Association between Azithromycin and Death from Cardiovascular Causes. New England Journal of Medicine 2014;370:1961-2.
44. Trac MH, McArthur E, Jandoc R, et al. Macrolide antibiotics and the risk of ventricular arrhythmia in older adults. Canadian Medical Association journal 2016;188:E120-E9.
45. Polgreen LA, Riedle BN, Cavanaugh JE, et al. Estimated Cardiac Risk Associated With Macrolides and Fluoroquinolones Decreases Substantially When Adjusting for Patient Characteristics and Comorbidities. J Am Heart Assoc 2018;7.
46. Almalki ZS, Guo JJ. Cardiovascular events and safety outcomes associated with azithromycin therapy: a meta-analysis of randomized controlled trials. American health & drug benefits 2014;7:318-28.
47. Briggs GG, Freeman RK. Drugs in Pregnancy and Lactation. 11 ed. Philadelphia: Wolters Kluwer; 2017.
48. Pfizer Labs. Product information. Zithromax.1994.
49. Nilsson MF, Danielsson C, Skold AC, et al. Improved methodology for identifying the teratogenic potential in early drug development of hERG channel blocking drugs. Reproductive toxicology (Elmsford, NY) 2010;29:156-63.
50. Karabulut AK, Uysal Ii Fau - Acar H, Acar H Fau - Fazliogullari Z, Fazliogullari Z. Investigation of developmental toxicity and teratogenicity of macrolide antibiotics in cultured rat embryos. Anatomia, histologia, embryologia 2008;37:369-75.
51. Kallen B, Danielsson BR. Fetal safety of erythromycin. An update of Swedish data. European journal of clinical pharmacology 2014;70:355-60.
52. Antibiotic used in pregnancy linked to risk of epilepsy and cerebral palsy. The Guardian. (Accessed 29 Aug 2018, at https://www.theguardian.com/science/2015/mar/25/antibiotic-used-in-pregnancy-linked-to-risk-of-epilepsy-and-cerebral-palsy.)
53. Patient information leaflets (PILs) and Summaries of product characteristics (SPCs). The UK Medicines and Healthcare products Regulatory Agency, 2019. (Accessed 1 Dec 2019, at http://www.mhra.gov.uk/spc-pil/index.htm.)
54. Causes of death among stillbirths. JAMA 2011;306:2459-68.

55. Adams Waldorf KM, McAdams RM. Influence of Infection During Pregnancy on Fetal Development. Reproduction (Cambridge, England) 2013;146:R151-R62.
56. Meeraus WH. Adverse Paediatric Outcomes of Antibiotic Treatment in Pregnancy: University College London; 2015.
57. Kanoh S, Rubin BK. Mechanisms of action and clinical application of macrolides as immunomodulatory medications. Clinical microbiology reviews 2010;23:590-615.
58. Grether JK, Nelson KB, Dambrosia JM, Phillips TM. Interferons and cerebral palsy. The Journal of pediatrics 1999;134:324-32.
59. Stanat SJ, Carlton CG, Crumb WJ, Jr., Agrawal KC, Clarkson CW. Characterization of the inhibitory effects of erythromycin and clarithromycin on the HERG potassium channel. Molecular and cellular biochemistry 2003;254:1-7.
60. Walter A. Volberg BJK, Weiguo Su, Jing Lin, Jun Zhou. Blockade of Human Cardiac Potassium Channel Human Ether-a-go-go-Related Gene (HERG) by Macrolide Antibiotics. Journal of Pharmacology and Experimental Therapeutics 2002;302:320-7.
61. Danielsson C, Brask J, Skold AC, et al. Exploration of human, rat, and rabbit embryonic cardiomyocytes suggests K-channel block as a common teratogenic mechanism. Cardiovascular research 2013;97:23-32.
62. Ray WA, Murray KT, Meredith S, Narasimhulu SS, Hall K, Stein CM. Oral Erythromycin and the Risk of Sudden Death from Cardiac Causes. New England Journal of Medicine 2004;351:1089-96.
63. Danielsson BR, SkOld AC, Azarbayjani F. Class III Antiarrhythmics and Phenytoin: Teratogenicity Due to Embryonic Cardiac Dysrhythmia and Reoxygenation Damage. Current pharmaceutical design 2001;7:787-802.
64. Pedersen LH, Henriksen TB, Vestergaard M, Olsen J, Bech BH. Selective serotonin reuptake inhibitors in pregnancy and congenital malformations: population based cohort study. BMJ 2009;339:b3569.
65. Kallen B, Otterblad Olausson P. Antidepressant drugs during pregnancy and infant congenital heart defect. Reproductive toxicology (Elmsford, NY) 2006;21:221-2.
66. Danielsson BR, Johansson A, Danielsson C, Azarbayjani F, Blomgren B, Skold AC. Phenytoin teratogenicity: hypoxia marker and effects on embryonic heart rhythm suggest an hERG-related mechanism. Birth defects research Part A, Clinical and molecular teratology 2005;73:146-53.
67. Bengt R Danielsson A-CS, Alf Johansson, Birgitta Dillner, Bo Blomgren. Teratogenicity by the hERG potassium channel blocking drug almokalant: use of hypoxia marker gives evidence for a hypoxia-related mechanism mediated via embryonic arrhythmia. Toxicology and applied pharmacology 2003;193:168-76.
68. Wellfelt K, Skold AC, Wallin A, Danielsson BR. Teratogenicity of the class III antiarrhythmic drug almokalant. Role of hypoxia and reactive oxygen species. Reproductive toxicology (Elmsford, NY) 1999;13:93-101.
69. Ritchie HE, Ababneh DH, Oakes DJ, Power CA, Webster WS. The teratogenic effect of dofetilide during rat limb development and association with drug-induced bradycardia and hypoxia in the embryo. Birth defects research Part B, Developmental and reproductive toxicology 2013;98:144-53.
70. Brent RL, Franklin JB. Uterine vascular clamping: new procedure for the study of congenital malformations. Science (New York, NY) 1960;132:89-91.
71. Leist KH, Grauwiler J. Fetal pathology in rats following uterine-vessel clamping on day 14 of gestation. Teratology 1974;10:55-67.
72. Fajersztajn L, Veras MM. Hypoxia: From Placental Development to Fetal Programming. Birth defects research 2017;109:1377-85.

73. Gudmundsson S, Tulzer G, Huhta JC, Marsal K. Venous Doppler in the fetus with absent end-diastolic flow in the umbilical artery. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 1996;7:262-7.
74. Meyer K, Lubo Z. Fetal programming of cardiac function and disease. Reprod Sci 2007;14:209-16.
75. Hutter D, Kingdom J, Jaeggi E. Causes and Mechanisms of Intrauterine Hypoxia and Its Impact on the Fetal Cardiovascular System: A Review. International journal of pediatrics 2010;2010.
76. Webster WS, Abela D. The effect of hypoxia in development. Birth Defects Res C Embryo Today 2007;81:215-28.
77. Hutter D, Kingdom J, Jaeggi E. Causes and mechanisms of intrauterine hypoxia and its impact on the fetal cardiovascular system: a review. International journal of pediatrics 2010;2010:401323.
78. Vichinsky EP. Alpha thalassemia major—new mutations, intrauterine management, and outcomes. Hematology 2009;2009:35-41.
79. Blais L, Beauchesne MF. Use of inhaled corticosteroids following discharge from an emergency department for an acute exacerbation of asthma. Thorax 2004;59:943-7.
80. Galinsky R, Lear CA, Dean JM, et al. Complex interactions between hypoxia-ischemia and inflammation in preterm brain injury. Developmental medicine and child neurology 2018;60:126-33.
81. Rees S, Inder T. Fetal and neonatal origins of altered brain development. Early Human Development 2005;81:753-61.
82. Rees S, Breen S, Loeliger M, McCrabb G, Harding R. Hypoxemia near mid-gestation has long-term effects on fetal brain development. Journal of neuropathology and experimental neurology 1999;58:932-45.
83. Mallard EC, Rehn A, Rees S, Tolcos M, Copolov D. Ventriculomegaly and reduced hippocampal volume following intrauterine growth-restriction: implications for the aetiology of schizophrenia. Schizophrenia research 1999;40:11-21.
84. Rees S, Mallard C, Breen S, Stringer M, Cock M, Harding R. Fetal brain injury following prolonged hypoxemia and placental insufficiency: a review. Comparative biochemistry and physiology Part A, Molecular & integrative physiology 1998;119:653-60.
85. Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. Azithromycin and the risk of cardiovascular death. N Engl J Med 2012;366:1881-90.
86. Ingalls TH, Curley FJ, Prindle RA. Experimental production of congenital anomalies; timing and degree of anoxia as factors causing fetal deaths and congenital anomalies in the mouse. N Engl J Med 1952;247:758-68.
87. Franklin JB, Brent RL. The Effect of Uterine Vascular Clamping on the Development of Rat Embryos Three to Fourteen Days Old. Journal of morphology 1964;115:273-90.
88. Poulson E, Robson JM, Sullivan FM. Teratogenic effect of 5-hydroxytryptamine in mice. Science (New York, NY) 1963;141:717-8.
89. Webster WS, Howe AM, Abela D, Oakes DJ. The relationship between cleft lip, maxillary hypoplasia, hypoxia and phenytoin. Current pharmaceutical design 2006;12:1431-48.
90. Skold AC, Wellfelt K, Danielsson BR. Stage-specific skeletal and visceral defects of the I(Kr)-blocker almokalant: further evidence for teratogenicity via a hypoxia-related mechanism. Teratology 2001;64:292-300.

91. Webster WS, Brown-Woodman PD. Cocaine as a cause of congenital malformations of vascular origin: experimental evidence in the rat. Teratology 1990;41:689-97.
92. Webster WS, Brown-Woodman PD, Lipson AH, Ritchie HE. Fetal brain damage in the rat following prenatal exposure to cocaine. Neurotoxicology and teratology 1991;13:621-6.
93. Baud O, Daire JL, Dalmaz Y, et al. Gestational hypoxia induces white matter damage in neonatal rats: a new model of periventricular leukomalacia. Brain pathology (Zurich, Switzerland) 2004;14:1-10.
94. McClain RM, Langhoff L. Teratogenicity of diphenylhydantoin in the New Zealand white rabbit. Teratology 1980;21:371-9.
95. Reddy DV, Adams FH, Baird C. Teratogeneic Effects of Serotonin. The Journal of pediatrics 1963;63:394-7.
96. Ottman R, Annegers JF, Risch N, Hauser WA, Susser M. Relations of Genetic and Environmental Factors in the Etiology of Epilepsy. Annals of neurology 1996;39:442-9.
97. Lim WH. Cerebral palsy: Causes, pathways, and the role of genetic variants. American Journal of Obstetrics and Gynecology 2016;214:670-1.
98. Miscarriage: NICE CKS. The National Institute for Health and Care Excellence, 2018. (Accessed 30 May 2019, at https://cks.nice.org.uk/miscarriage.)
99. National Collaborating Centre for Women's and Children's Health (UK). Ectopic Pregnancy and Miscarriage: Diagnosis and Initial Management in Early Pregnancy of Ectopic Pregnancy and Miscarriage. London2012.
100. Jurkovic D, Overton C, Bender-Atik R. Diagnosis and management of first trimester miscarriage. BMJ : British Medical Journal 2013;346:f3676.
101. BMJ. BMJ Best Practice: Miscarriage. London2018.
102. Flenady V, Wojcieszek AM, Middleton P, et al. Stillbirths: recall to action in high-income countries. The Lancet 2016;387:691-702.
103. Silver RM, Varner MW, Reddy U, et al. Work-up of stillbirth: a review of the evidence. American journal of obstetrics and gynecology 2007;196:433-44.
104. Morrison I, Olsen J. Weight-specific stillbirths and associated causes of death: an analysis of 765 stillbirths. Am J Obstet Gynecol 1985;152:975-80.
105. Maternal NaICORP. MBRRACE-UK Perinatal Mortality Surveillance Report. London2016.
106. Centre for Maternal and Child Enquiries (CMACE). Perinatal Mortality 2009. London2011.
107. Brent RL. Environmental causes of human congenital malformations: the pediatrician's role in dealing with these complex clinical problems caused by a multiplicity of environmental and genetic factors. Pediatrics 2004;113:957-68.
108. Graham HK, Rosenbaum P, Paneth N, et al. Cerebral palsy. Nat Rev Dis Primers 2016;2:15082.
109. Derrick M, Drobyshevsky A, Ji X, Tan S. A model of cerebral palsy from fetal hypoxia-ischemia. Stroke 2007;38:731-5.
110. Bashiri A, Burstein E, Mazor M. Cerebral palsy and fetal inflammatory response syndrome: a review. J Perinat Med 2006;34:5-12.
111. Epilepsy: NICE Clinical knowledge summaries. The National Institute for Health and Care Excellence, 2018. (Accessed 30 May 2019, at https://cks.nice.org.uk/epilepsy.)
112. Attention deficit hyperactivity disorder: NICE CKS. The National Institute for Health and Care Excellence, 2017. (Accessed 30 May 2019, at https://cks.nice.org.uk/attention-deficit-hyperactivity-disorder.)

113. Getahun D, Rhoads GG, Demissie K, et al. In utero exposure to ischemic-hypoxic conditions and attention-deficit/hyperactivity disorder. Pediatrics 2013;131:e53-61.
114. Autism in children: NICE CKS. The National Institute for Health and Care Excellence. (Accessed 30 May 2019, at https://cks.nice.org.uk/autism-in-children.)
115. Samsam M, Ahangari R, Naser SA. Pathophysiology of autism spectrum disorders: revisiting gastrointestinal involvement and immune imbalance. World journal of gastroenterology 2014;20:9942-51.
116. Kolevzon A, Gross R, Reichenberg A. Prenatal and Perinatal Risk Factors for Autism: A Review and Integration of Findings. JAMA pediatrics 2007;161:326-33.
117. Mueller BR, Bale TL. Sex-Specific Programming of Offspring Emotionality after Stress Early in Pregnancy. The Journal of Neuroscience 2008;28:9055-65.
118. Czeizel AE. Specified critical period of different congenital abnormalities: a new approach for human teratological studies. Congenital anomalies 2008;48:103-9.
119. Yarnel J, O'Reilly D. Epidemiology and Disease Prevention: A Global Approach. 2 ed: Oxford University Press; 2013.
120. Khwaja O, Volpe JJ. Pathogenesis of cerebral white matter injury of prematurity. Archives of Disease in Childhood Fetal and Neonatal edition 2008;93:F153-61.
121. McKenna S, Evans G, Committee tCIDSAA. Macrolides: A Canadian Infectious Disease Society position paper. Can J Infect Dis 2001;12:218-31.
122. Witt A, Sommer EM, Cichna M, et al. Placental passage of clarithromycin surpasses other macrolide antibiotics. American Journal of Obstetrics and Gynecology 2003;188:816-9.
123. Federal Drug Comission. FDA/ PhRMA task force to assess QT risk by preclinical markers. Chevy Chase, MD: The Pink Sheet; 1999.
124. Philipson A, Sabath LD, Charles D. Transplacental Passage of Erythromycin and Clindamycin. N Engl J Med 1973;288:1219-21.
125. Mizuno R. The male/female ratio of fetal deaths and births in Japan. Lancet 2000;356:738-9.
126. Kraemer S. The fragile male. BMJ 2000;321:1609-12.
127. O'Driscoll DN, McGovern M, Greene CM, Molloy EJ. Gender disparities in preterm neonatal outcomes. Acta Paediatrica 2018;107:1494-9.
128. Moore KL PT. The developing human: clinically oriented embryology. 6 ed. Philadelphia: W.B. Saunders Company; 1998.
129. Charlton RA, Cunnington MC, de Vries CS, Weil JG. Data resources for investigating drug exposure during pregnancy and associated outcomes: the General Practice Research Database (GPRD) as an alternative to pregnancy registries. Drug safety 2008;31:39-51.
130. Ghosh RE, Crellin E, Beatty S, Donegan K, Myles P, Williams R. How Clinical Practice Research Datalink data are used to support pharmacovigilance. Ther Adv Drug Saf 2019;10:2042098619854010.
131. Guidance and advice list. The National Institute for Health and Care Excellence. (Accessed 30 May 2019, at https://www.nice.org.uk/guidance/published?type=apg.)
132. Lamont HF, Blogg HJ, Lamont RF. Safety of antimicrobial treatment during pregnancy: a current review of resistance, immunomodulation and teratogenicity. Expert opinion on drug safety 2014;13:1569-81.
133. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature reviews Microbiology 2015;13:269-84.

134. Liew Z, Olsen J, Cui X, Ritz B, Arah OA. Bias from conditioning on live birth in pregnancy cohorts: an illustration based on neurodevelopment in children after prenatal exposure to organic pollutants. Int J Epidemiol 2015;44:345-54.
135. Kallen BA, Otterblad Olausson P. Maternal drug use in early pregnancy and infant cardiovascular defect. Reproductive toxicology (Elmsford, NY) 2003;17:255-61.
136. Del Mar C. Antibiotics for acute respiratory tract infections in primary care. BMJ 2016;354:i3482.
137. Jenkins KJ, Correa A, Feinstein JA, et al. Noninherited risk factors and congenital cardiovascular defects: current knowledge: a scientific statement from the American Heart Association Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. Circulation 2007;115:2995-3014.
138. Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ 2011;343:d5928.
139. Sterne JA, Hernan MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ 2016;355:i4919.
140. Sweeting MJS, A.J.; Lambert, P.C. What to add to nothing? Use and avoidance of continuity corrections in meta-analysis of sparse data. Statistics in Medicine 2004;23:1351- 75.
141. Eschenbach DA, Nugent RP, Rao AV, et al. A randomized placebo-controlled trial of erythromycin for the treatment of Ureaplasma urealyticum to prevent premature delivery. The Vaginal Infections and Prematurity Study Group. Am J Obstet Gynecol 1991;164:734-42.
142. Kallen BA, Otterblad Olausson P, Danielsson BR. Is erythromycin therapy teratogenic in humans? Reproductive toxicology (Elmsford, NY) 2005;20:209-14.
143. Kenyon S, Pike K, Jones DR, et al. Childhood outcomes after prescription of antibiotics to pregnant women with preterm rupture of the membranes: 7-year follow-up of the ORACLE I trial. Lancet 2008;372:1310-8.
144. Kenyon SL, Taylor DJ, Tarnow-Mordi W. Broad-spectrum antibiotics for spontaneous preterm labour: the ORACLE II randomised trial. ORACLE Collaborative Group. Lancet 2001;357:989-94.
145. Kenyon SL, Taylor DJ, Tarnow-Mordi W. Broad-spectrum antibiotics for preterm, prelabour rupture of fetal membranes: the ORACLE I randomised trial. ORACLE Collaborative Group. Lancet 2001;357:979-88.
146. Kwak HM, Shin MY, Cha HH, et al. The efficacy of cefazolin plus macrolide (erythromycin or clarithromycin) versus cefazolin alone in neonatal morbidity and placental inflammation for women with preterm premature rupture of membranes. Placenta 2013;34:346-52.
147. Le Nguyen T, Araujo M, Hurault-Delarue C, Lacroix I, Damase-Michel C, Sommet A. Teratogenic risk of macrolides during the first trimester of pregnancy: A study with two complementary approaches within the EFEMERIS database. Fundamental and Clinical Pharmacology 2017;31:25.
148. Martin DH, Eschenbach DA, Cotch MF, et al. Double-Blind Placebo-Controlled Treatment Trial of Chlamydia trachomatis Endocervical Infections in Pregnant Women. Infectious diseases in obstetrics and gynecology 1997;5:10-7.
149. McGregor JA, French JI, Seo K. Antimicrobial therapy in preterm premature rupture of membranes: results of a prospective, double-blind, placebo-controlled trial of erythromycin. Am J Obstet Gynecol 1991;165:632-40.

150. Mercer BM, Moretti ML, Prevost RR, Sibai BM. Erythromycin therapy in preterm premature rupture of the membranes: a prospective, randomized trial of 220 patients. Am J Obstet Gynecol 1992;166:794-802.
151. Tita AT, Szychowski JM, Boggess K, et al. Adjunctive Azithromycin Prophylaxis for Cesarean Delivery. New England journal of medicine2016:1231-41.
152. Ye Y, Tu S, Li H. Clinic intervention study on urogenital mycoplasma infection of pregnant women. [Chinese]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi 2001;22:293-5.
153. Lund M, Pasternak B, Davidsen RB, et al. Use of macrolides in mother and child and risk of infantile hypertrophic pyloric stenosis: nationwide cohort study. Bmj 2014;348:g1908.
154. Anne-Marie Nybo Andersen JW, Peter Christens, Jørn Olsen, Mads Melbye. Maternal age and fetal loss: population based register linkage study. British medical journal 2000;320:1708-12.
155. Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R. How common are the "common" neurologic disorders? Neurology 2007;68:326-37.
156. Mullick S, Watson-Jones D, Beksinska M, Mabey D. Sexually transmitted infections in pregnancy: prevalence, impact on pregnancy outcomes, and approach to treatment in developing countries. Sexually Transmitted Infections 2005;81:294.
157. CLARITHROMYCIN. The National Institute for Health and Care Excellence (NICE). (Accessed 30 May 2019, at https://bnf.nice.org.uk/drug/clarithromycin.html#pregnancy.)
158. PRODUCT MONOGRAPH: rRAN™-CLARITHROMYCIN. Health Canada. (Accessed 30 May 2019, at https://health-products.canada.ca/dpd-bdpp/info.do?lang=en&code=87557.)
159. Clarithromycin. Danish Medicines Agency. (Accessed 28 Sep 2018, at http://produktresume.dk/AppBuilder/search?button=S%C3%B8g&id=&page=0&q=clarithromycin&type=&utf8=%E2%9C%93.)
160. BIAXIN® Filmtab®. The United States Food and Drug Administration. (Accessed 30 May 2019, at https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/050662s044s050,50698s026s030,050775s015s019lbl.pdf.)
161. Births in England and Wales: 2016. Office for National Statistics. at https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/livebirths/bulletins/birthsummarytablesenglandandwales/2016.)
162. Medicines and Healthcare products Regulatory Agency. GPRD Recording Guidelines for Vision Users. London2004.
163. Primary Care Strategy and NHS Contracts Group. 2019/20 General Medical Services (GMS) contract Quality and Outcomes Framework (QOF). 2019.
164. Herrett E, Gallagher AM, Bhaskaran K, et al. Data Resource Profile: Clinical Practice Research Datalink (CPRD). Int J Epidemiol 2015;44:827-36.
165. Herrett E, Thomas SL, Schoonen WM, Smeeth L, Hall AJ. Validation and validity of diagnoses in the General Practice Research Database: a systematic review. British journal of clinical pharmacology 2010;69:4-14.
166. Wurst KE, Ephross SA, Loehr J, Clark DW, Guess HA. The utility of the general practice research database to examine selected congenital heart defects: a validation study. Pharmacoepidemiol Drug Saf 2007;16:867-77.
167. Medicines and Healthcare Products Regulatory Agency. CPRD Mother Baby Link Documentation. London: CPRD; 2017.

168. Devine S, West S, Andrews E, et al. The identification of pregnancies within the general practice research database. Pharmacoepidemiol Drug Saf 2010;19:45-50.
169. Matcho A, Ryan P, Fife D, Gifkins D, Knoll C, Friedman A. Inferring pregnancy episodes and outcomes within a network of observational databases. PLoS One 2018;13:e0192033.
170. Minassian C, Williams R, Meeraus WH, Smeeth L, Campbell OMR, Thomas SL. Methods to generate and validate a Pregnancy Register in the UK Clinical Practice Research Datalink primary care database. Pharmacoepidemiol Drug Saf 2019;28:923-33.
171. Olsen IE, Groveman SA, Lawson ML, Clark RH, Zemel BS. New Intrauterine Growth Curves Based on United States Data. Pediatrics 2010;125:e214-e24.
172. Harris J, Sheiner E. Does an Upper Respiratory Tract Infection During Pregnancy Affect Perinatal Outcomes? A Literature Review. Current infectious disease reports 2013;15:143-7.
173. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassan S. The role of inflammation and infection in preterm birth. Semin Reprod Med 2007;25:21-39.
174. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008;371:75-84.
175. Bell S, Daskalopoulou M, Rapsomaniki E, et al. Association between clinically recorded alcohol consumption and initial presentation of 12 cardiovascular diseases: population based cohort study using linked health records. BMJ 2017;356:j909.
176. Office of National Statistics. Birth statistics: Births and patterns of family building England and Wales2007.
177. Blak BT, Thompson M, Dattani H, Bourke A. Generalisability of The Health Improvement Network (THIN) database: demographics, chronic disease prevalence and mortality rates. Informatics in primary care 2011;19:251-5.
178. Office for National Statistics. Gestation-specific Infant Mortality in England and Wales, 20112013.
179. Smallwood S. New estimates of trends in births by birth order in England and Wales. Population trends 2002:32-48.
180. McIntyre S, Taitz D, Keogh J, Goldsmith S, Badawi N, Blair E. A systematic review of risk factors for cerebral palsy in children born at term in developed countries. Developmental medicine and child neurology 2013;55:499-508.
181. Hewitt C, Gilbody S, Brealey S, et al. Methods to identify postnatal depression in primary care: an integrated evidence synthesis and value of information analysis. Health technology assessment (Winchester, England) 2009;13:1-145, 7-230.
182. National Institute for Health and Care Excellence. Antenatal and postnatal mental health. London2018.
183. Royal College of Obstetricians and Gynaecologists. Pregnancy and complex social factors: A model for service provision for pregnant women with complex social factors. 2010.
184. Syed S, Gilbert R, Wolpert M. Parental alcohol misuse and the impact on children: a rapid evidence review of service presentations and interventions. . London2018.
185. Hernán MA, Jick SS, Logroscino G, Olek MJ, Ascherio A, Jick H. Cigarette smoking and the progression of multiple sclerosis. Brain 2005;128:1461-5.
186. Centre for Maternal and Child Enquiries (CMACE). Maternal obesity in the UK: Findings from a national project. London2010.
187. Royal College of Obstetricians and Gynaecologists. Hypertension in pregnancy: the management of hypertensive disorders during pregnancy. London2011.

188. National Institute for Health and Care Excellence. Diabetes in pregnancy Management of diabetes and its complications from preconception to the postnatal period. London2015.
189. Dennis C-L, Falah-Hassani K, Shiri R. Prevalence of antenatal and postnatal anxiety: Systematic review and meta-analysis. British Journal of Psychiatry 2018;210:315-23.
190. Nath S, Ryan EG, Trevillion K, et al. Prevalence and identification of anxiety disorders in pregnancy: the diagnostic accuracy of the two-item Generalised Anxiety Disorder scale (GAD-2). BMJ open 2018;8:e023766-e.
191. National Institute for Health and Care Excellence. Antenatal and postnatal mental health. London2014.
192. Pearson RM, Carnegie RE, Cree C, et al. Prevalence of Prenatal Depression Symptoms Among 2 Generations of Pregnant Mothers: The Avon Longitudinal Study of Parents and ChildrenPrevalence of Prenatal Depression Symptoms in 2 Generations of Pregnant MothersPrevalence of Prenatal Depression Symptoms in 2 Generations of Pregnant Mothers. JAMA Network Open 2018;1:e180725-e.
193. Royal College of Obstetricians and Gynaecologists. Epilepsy in Pregnancy. London2016.
194. Royal College of Obstetricians and Gynaecologists. Review Urinary tract infection in pregnancy2008.
195. Petrou S, Kupek E, Vause S, Maresh M. Clinical, provider and sociodemographic determinants of the number of antenatal visits in England and Wales. Social Science & Medicine 2001;52:1123-34.
196. Petersen I, Collings SL, McCrea RL, et al. Antiepileptic drugs prescribed in pregnancy and prevalence of major congenital malformations: comparative prevalence studies. Clin Epidemiol 2017;9:95-103.
197. The National Institute for Health and Care Excellence. Antenatal care: routine care for the healthy pregnant woman. London2008.
198. Public Health England. English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR). London2014.
199. Brocklehurst P. Antibiotics for gonorrhoea in pregnancy. The Cochrane database of systematic reviews 2002:Cd000098.
200. Cluver C, Novikova N, Eriksson DO, Bengtsson K, Lingman GK. Interventions for treating genital Chlamydia trachomatis infection in pregnancy. The Cochrane database of systematic reviews 2017;9:Cd010485.
201. Walker GJ. Antibiotics for syphilis diagnosed during pregnancy. The Cochrane database of systematic reviews 2001:Cd001143.
202. Kenyon S, Boulvain M, Neilson JP. Antibiotics for preterm rupture of membranes. The Cochrane database of systematic reviews 2013:Cd001058.
203. Vazquez JC, Abalos E. Treatments for symptomatic urinary tract infections during pregnancy. The Cochrane database of systematic reviews 2011:Cd002256.
204. Smaill FM, Vazquez JC. Antibiotics for asymptomatic bacteriuria in pregnancy. The Cochrane database of systematic reviews 2015:Cd000490.
205. Guinto VT, De Guia B, Festin MR, Dowswell T. Different antibiotic regimens for treating asymptomatic bacteriuria in pregnancy. The Cochrane database of systematic reviews 2010:Cd007855.
206. Ohlsson A, Shah VS. Intrapartum antibiotics for known maternal Group B streptococcal colonization. The Cochrane database of systematic reviews 2014:Cd007467.

207. Sangkomkamhang US, Lumbiganon P, Prasertcharoensuk W, Laopaiboon M. Antenatal lower genital tract infection screening and treatment programs for preventing preterm delivery. The Cochrane database of systematic reviews 2015:Cd006178.
208. Dolk FCK, Pouwels KB, Smith DRM, Robotham JV, Smieszek T. Antibiotics in primary care in England: which antibiotics are prescribed and for which conditions? Journal of Antimicrobial Chemotherapy 2018;73:ii2-ii10.
209. Gulliford M, Latinovic R, Charlton J, Little P, van Staa T, Ashworth M. Selective decrease in consultations and antibiotic prescribing for acute respiratory tract infections in UK primary care up to 2006. Journal of Public Health 2009;31:512-20.
210. Amann U, Egen-Lappe V, Strunz-Lehner C, Hasford J. Antibiotics in pregnancy: analysis of potential risks and determinants in a large German statutory sickness fund population. Pharmacoepidemiol Drug Saf 2006;15:327-37.
211. Pouwels KB, Dolk FCK, Smith DRM, Smieszek T, Robotham JV. Explaining variation in antibiotic prescribing between general practices in the UK. Journal of Antimicrobial Chemotherapy 2018;73:ii27-ii35.
212. Carthy P, Harvey I, Brawn R, Watkins C. A study of factors associated with cost and variation in prescribing among GPs. Family Practice 2000;17:36-41.
213. Shah A, Group CE. CALIBERcodelists user guide2014.
214. Dave S, Petersen I. Creating medical and drug code lists to identify cases in primary care databases. Pharmacoepidemiol Drug Saf 2009;18:704-7.
215. Austin PC. Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Stat Med 2009;28:3083-107.
216. Heikkila AM. Antibiotics in pregnancy--a prospective cohort study on the policy of antibiotic prescription. Annals of medicine 1993;25:467-71.
217. Smith GE, Smith S, Heatlie H, et al. What has happened to antimicrobial usage in primary care in the United Kingdom since the SMAC report? - description of trends in antimicrobial usage using the General Practice Research Database. Journal of public health (Oxford, England) 2004;26:359-64.
218. Dolk FCK, Pouwels KB, Smith DRM, Robotham JV, Smieszek T. Antibiotics in primary care in England: which antibiotics are prescribed and for which conditions? The Journal of antimicrobial chemotherapy 2018;73:ii2-ii10.
219. Daw JR, Mintzes B, Law MR, Hanley GE, Morgan SG. Prescription drug use in pregnancy: a retrospective, population-based study in British Columbia, Canada (2001-2006). Clinical therapeutics 2012;34:239-49.e2.
220. National Institute for Health and Clinical Excellence. Respiratory tract infections – antibiotic prescribing2008.
221. Feijen-de Jong EI, Baarveld F, Jansen DEMC, Ursum J, Reijneveld SA, Schellevis FG. Do pregnant women contact their general practitioner? A register-based comparison of healthcare utilisation of pregnant and non-pregnant women in general practice. BMC family practice 2013;14:10-.
222. NHS England. 2016/17 General Medical Services (GMS) contract Quality and Outcomes Framework (QOF)2016.
223. Palin V, Mölter A, Belmonte M, et al. Antibiotic prescribing for common infections in UK general practice: variability and drivers. Journal of Antimicrobial Chemotherapy 2019;74:2440-50.
224. Hawker JI, Smith S, Smith GE, et al. Trends in antibiotic prescribing in primary care for clinical syndromes subject to national recommendations to reduce antibiotic

resistance, UK 1995-2011: analysis of a large database of primary care consultations. The Journal of antimicrobial chemotherapy 2014;69:3423-30.
225. Gulliford M, Latinovic R, Charlton J, Little P, van Staa T, Ashworth M. Selective decrease in consultations and antibiotic prescribing for acute respiratory tract infections in UK primary care up to 2006. Journal of public health (Oxford, England) 2009;31:512-20.
226. McNulty CAM, Boyle P, Nichols T, Clappison P, Davey P. The public's attitudes to and compliance with antibiotics. Journal of Antimicrobial Chemotherapy 2007;60:i63-i8.
227. Bevan D, White A, Marshall J, Peckham C. Modelling the effect of the introduction of antenatal screening for group B Streptococcus (GBS) carriage in the UK. BMJ open 2019;9:e024324.
228. Ghouri F, Hollywood A, Ryan K. Urinary tract infections and antibiotic use in pregnancy - qualitative analysis of online forum content. BMC Pregnancy and Childbirth 2019;19:289.
229. Bishop CF, Small N, Parslow R, Kelly B. Healthcare use for children with complex needs: using routine health data linked to a multiethnic, ongoing birth cohort. BMJ open 2018;8:e018419.
230. Cerebral palsy: Scenario: Child with confirmed cerebral palsy. The National Institute for Health and Care Excellence. (Accessed 30 May 2019, at https://cks.nice.org.uk/cerebral-palsy#!scenario:1.)
231. The National Institute for Health and Care Excellence. The Epilepsies: The Diagnosis and Management of the Epilepsies in Adults and Children in Primary and Secondary Care: Pharmacological Update of Clinical Guideline 20. In: (UK) NCGC, ed.2012.
232. National Collaborating Centre for Mental Health. Attention deficit hypoeractivity disorder: the NICE guideline on diagnosis and management of ADHD in children, young people and adults. In: Health NCCfM, ed.2016.
233. National Institute for Health and Clinical Excellence. Autism: recognition, referral and diagnosis of children and young people on the autism spectrum. In: National Institute for Health and Clinical Excellence, ed. London2011.
234. Greenland S. Basic methods for sensitivity analysis of biases. Int J Epidemiol 1996;25:1107-16.
235. Jick SS, Kaye JA, Vasilakis-Scaramozza C, et al. Validity of the general practice research database. Pharmacotherapy 2003;23:686-9.
236. Hammad TA, Margulis AV, Ding Y, Strazzeri MM, Epperly H. Determining the predictive value of Read codes to identify congenital cardiac malformations in the UK Clinical Practice Research Datalink. Pharmacoepidemiol Drug Saf 2013;22:1233-8.
237. Nazareth I, King M, Haines A, Rangel L, Myers S. Accuracy of diagnosis of psychosis on general practice computer system. Bmj 1993;307:32-4.
238. Khan NF, Harrison SE, Rose PW. Validity of diagnostic coding within the General Practice Research Database: a systematic review. British Journal of General Practice 2010;60:e128-e36.
239. Charlton RA, McGrogan A, Snowball J, et al. Sensitivity of the UK Clinical Practice Research Datalink to Detect Neurodevelopmental Effects of Medicine Exposure in Utero: Comparative Analysis of an Antiepileptic Drug-Exposed Cohort. Drug safety 2017;40:387-97.
240. The EUROCAT Guide. European Surveillance of Congenital Anomalies. (Accessed 26 May 2019, at http://www.eurocat-network.eu/.)
241. Sewell MD, Rosendahl K, Eastwood DM. Developmental dysplasia of the hip. BMJ 2009;339:b4454.

242. EUROCAT Prevalence Data Tables. European Surveillance of Congenital Anomalies. (Accessed 26 May 2019, at http://www.eurocat-network.eu/.)
243. Charlton MRA, Weil JG, Cunnington MC, de Vries CS. Identifying Major Congenital Malformations in the UK General Practice Research Database (GPRD). Drug safety 2010;33:741-50.
244. Carter B, Verity Bennett C, Bethel J, Jones HM, Wang T, Kemp A. Identifying cerebral palsy from routinely-collected data in England and Wales. Clin Epidemiol 2019;11:457-68.
245. Meeraus WH, Petersen I, Chin RF, Knott F, Gilbert R. Childhood epilepsy recorded in primary care in the UK. Archives of disease in childhood 2013;98:195-202.
246. Holden EW, Grossman E, Nguyen HT, et al. Developing a computer algorithm to identify epilepsy cases in managed care organizations. Disease management : DM 2005;8:1-14.
247. Holden SE, Jenkins-Jones S, Poole CD, Morgan CL, Coghill D, Currie CJ. The prevalence and incidence, resource use and financial costs of treating people with attention deficit/hyperactivity disorder (ADHD) in the United Kingdom (1998 to 2010). Child and Adolescent Psychiatry and Mental Health 2013;7:34.
248. Hagberg KW, Jick SS. Validation of autism spectrum disorder diagnoses recorded in the Clinical Practice Research Datalink, 1990–2014. Clinical Epidemiology 2017;9:475-82.
249. Black C, Kaye JA, Jick H. Relation of childhood gastrointestinal disorders to autism: nested case-control study using data from the UK General Practice Research Database. Bmj 2002;325:419-21.
250. Fombonne E, Heavey L, Smeeth L, et al. Validation of the diagnosis of autism in general practitioner records. BMC public health 2004;4:5.
251. Smeeth L, Cook C, Fombonne E, et al. MMR vaccination and pervasive developmental disorders: a case-control study. Lancet 2004;364:963-9.
252. Taylor B, Jick H, Maclaughlin D. Prevalence and incidence rates of autism in the UK: time trend from 2004-2010 in children aged 8 years. BMJ open 2013;3:e003219.
253. Fan H, Li L, Gilbert R, O'Callaghan F, Wijlaars L. A machine learning approach to identify cases of cerebral palsy using the UK primary care database. The Lancet 2018;392:S33.
254. Holden S, Jenkins-Jones S, Poole C, Morgan C, Coghill D, Currie C. The prevalence and incidence, resource use and financial costs of treating people with attention deficit/hyperactivity disorder (ADHD) in the United Kingdom (1998 to 2010). Child Adolesc Psychiatry Ment Health 2013;7:34.
255. Hagberg KW, Jick SS. Validation of autism spectrum disorder diagnoses recorded in the Clinical Practice Research Datalink, 1990-2014. Clin Epidemiol 2017;9:475-82.
256. British Isles Network of Congenital Anomaly Registers. Congenital Anomaly Statistics 2009: British Isles Network of Congenital Anomaly Registers; 2011.
257. Dolk H. EUROCAT: 25 years of European surveillance of congenital anomalies. Archives of Disease in Childhood Fetal and Neonatal edition 2005;90:F355-8.
258. Sokal R, Fleming KM, Tata LJ. Potential of general practice data for congenital anomaly research: Comparison with registry data in the United Kingdom. Birth defects research Part A, Clinical and molecular teratology 2013;97:546-53.
259. British Isles Network of Congenital Anomaly Registers. Congenital Anomaly Statistics 2012 England and Wales2014.

260. Bishop C, Small N, Mason D, et al. Improving case ascertainment of congenital anomalies: findings from a prospective birth cohort with detailed primary care record linkage. BMJ Paediatrics Open 2017;1:e000171.
261. Hunter S, Heads A, Wyllie J, Robson S. Prenatal diagnosis of congenital heart disease in the northern region of England: benefits of a training programme for obstetric ultrasonographers. Heart 2000;84:294-8.
262. Wurst KE, Ephross SA, Loehr J, Clark DW, Guess HA. Evaluation of the General Practice Research Database congenital heart defects prevalence: comparison to United Kingdom national systems. Birth defects research Part A, Clinical and molecular teratology 2007;79:309-16.
263. Prevalence charts and tables. European Platform on Rare Disease Registration. (Accessed 30 May 2019, at https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-data/prevalence.)
264. Ahmed SF, Dobbie R, Finlayson AR, et al. Prevalence of hypospadias and other genital anomalies among singleton births, 1988–1997, in Scotland. Archives of Disease in Childhood - Fetal and Neonatal Edition 2004;89:F149-F51.
265. Glinianaia SV, Rankin J, Colver A, North of England Collaborative Cerebral Palsy Survey. Cerebral palsy rates by birth weight, gestation and severity in North of England, 1991-2000 singleton births. Archives of disease in childhood 2011;96:180-5.
266. Surveillance of Cerebral Palsy in Europe (SCPE). Prevalence and characteristics of children with cerebral palsy in Europe. Developmental medicine and child neurology 2002;44:633.
267. Kurtz Z, Tookey P, Ross E. Epilepsy in young people: 23 year follow up of the British national child development study. Bmj 1998;316:339-42.
268. Hire AJ, Ashcroft DM, Springate DA, Steinke DT. ADHD in the United Kingdom: Regional and Socioeconomic Variations in Incidence Rates Amongst Children and Adolescents (2004-2013). J Atten Disord 2018;22:134-42.
269. The National Institute for Health and Care Excellence. Attention deficit hyperactivity disorder: diagnosis and management. London2017.
270. Baron-Cohen S, Scott FJ, Allison C, et al. Prevalence of autism-spectrum conditions: UK school-based population study. Br J Psychiatry 2009;194:500-9.
271. Petersen I, McCrea RL, Sammon CJ, et al. Risks and benefits of psychotropic medication in pregnancy: cohort studies based on UK electronic primary care health records. Health technology assessment (Winchester, England) 2016;20:1-176.
272. Charman T, Baird G, Simonoff E, et al. Testing two screening instruments for autism spectrum disorder in UK community child health services. Developmental medicine and child neurology 2016;58:369-75.
273. Ford T, Goodman R, Meltzer H. The British Child and Adolescent Mental Health Survey 1999: the prevalence of DSM-IV disorders. Journal of the American Academy of Child and Adolescent Psychiatry 2003;42:1203-11.
274. Sadler TW. Langman's Medical Embryology. 13 ed. Philadelphia: Lippincott Williams and Wilkins; 2014.
275. Loane M, Dolk H, Morris J, Group aEW. Maternal age-specific risk of non-chromosomal anomalies. BJOG: An International Journal of Obstetrics & Gynaecology 2009;116:1111-9.
276. Schummers L, Hutcheon JA, Hacker MR, et al. Absolute risks of obstetric outcomes by maternal age at first birth: a population-based cohort. Epidemiology 2018;29:379-87.
277. Hollier LM, Leveno KJ, Kelly MA, DD MC, Cunningham FG. Maternal age and malformations in singleton births. Obstet Gynecol 2000;96:701-6.

278. Grewal J, Carmichael SL, Ma C, Lammer EJ, Shaw GM. Maternal periconceptional smoking and alcohol consumption and risk for select congenital anomalies. Birth defects research Part A, Clinical and molecular teratology 2008;82:519-26.
279. van der Zanden LFM, van Rooij IALM, Feitz WFJ, Franke B, Knoers NVAM, Roeleveld N. Aetiology of hypospadias: a systematic review of genes and environment. Human reproduction update 2012;18:260-83.
280. van Gelder MM, Reefhuis J, Caton AR, Werler MM, Druschel CM, Roeleveld N. Maternal periconceptional illicit drug use and the risk of congenital malformations. Epidemiology 2009;20:60-6.
281. Persson M, Cnattingius S, Villamor E, et al. Risk of major congenital malformations in relation to maternal overweight and obesity severity: cohort study of 1.2 million singletons. BMJ 2017;357.
282. Stothard KJ, Tennant PWG, Bell R, Rankin J. Maternal Overweight and Obesity and the Risk of Congenital Anomalies: A Systematic Review and Meta-analysis. JAMA 2009;301:636-50.
283. Akre O, Boyd HA, Ahlgren M, et al. Maternal and gestational risk factors for hypospadias. Environmental health perspectives 2008;116:1071-6.
284. Bateman BT, Huybrechts KF, Fischer MA, et al. Chronic hypertension in pregnancy and the risk of congenital malformations: a cohort study. American Journal of Obstetrics & Gynecology 2015;212:337.e1-.e14.
285. Ramakrishnan A, Lee LJ, Mitchell LE, Agopian AJ. Maternal Hypertension During Pregnancy and the Risk of Congenital Heart Defects in Offspring: A Systematic Review and Meta-analysis. Pediatric cardiology 2015;36:1442-51.
286. Gabbay-Benziv R, Reece EA, Wang F, Yang P. Birth defects in pregestational diabetes: Defect range, glycemic threshold and pathogenesis. World J Diabetes 2015;6:481-8.
287. Simeone RM, Devine OJ, Marcinkevage JA, et al. Diabetes and Congenital Heart Defects: A Systematic Review, Meta-Analysis, and Modeling Project. American Journal of Preventive Medicine 2015;48:195-204.
288. Carmichael SL, Shaw GM, Yang W, Abrams B, Lammer EJ. Maternal stressful life events and risks of birth defects. Epidemiology (Cambridge, Mass) 2007;18:356-61.
289. Nembhard WN, Tang X, Hu Z, MacLeod S, Stowe Z, Webber D. Maternal and infant genetic variants, maternal periconceptional use of selective serotonin reuptake inhibitors, and risk of congenital heart defects in offspring: population based study. Bmj 2017;356:j832.
290. Jentink J, Loane MA, Dolk H, et al. Valproic acid monotherapy in pregnancy and major congenital malformations. N Engl J Med 2010;362:2185-93.
291. Feng Y, Yu D, Chen T, et al. Maternal parity and the risk of congenital heart defects in offspring: a dose-response meta-analysis of epidemiological observational studies. PloS one 2014;9:e108944-e.
292. Akre O, Lipworth L, Cnattingius S, Sparen P, Ekbom A. Risk factor patterns for cryptorchidism and hypospadias. Epidemiology 1999;10:364-9.
293. Glinianaia SV, Rankin J, Wright C. Congenital anomalies in twins: a register-based study. Human Reproduction 2008;23:1306-11.
294. Herskind AM, Almind Pedersen D, Christensen K. Increased prevalence of congenital heart defects in monozygotic and dizygotic twins. Circulation 2013;128:1182-8.
295. Brouwers MM, Feitz WF, Roelofs LA, Kiemeney LA, de Gier RP, Roeleveld N. Risk factors for hypospadias. European journal of pediatrics 2007;166:671-8.

296. Cleves MA, Malik S, Yang S, Carter TC, Hobbs CA. Maternal urinary tract infections and selected cardiovascular malformations. Birth defects research Part A, Clinical and molecular teratology 2008;82:464-73.
297. Howley MM, Feldkamp ML, Papadopoulos EA, Fisher SC, Arnold KE, Browne ML. Maternal genitourinary infections and risk of birth defects in the National Birth Defects Prevention Study. Birth defects research 2018;110:1443-54.
298. Liu S, Joseph KS, Lisonkova S, et al. Association between maternal chronic conditions and congenital heart defects: a population-based cohort study. Circulation 2013;128:583-9.
299. Purisch SE, DeFranco EA, Muglia LJ, Odibo AO, Stamilio DM. Preterm birth in pregnancies complicated by major congenital malformations: a population-based study. American Journal of Obstetrics and Gynecology 2008;199:287.e1-.e8.
300. Tanner K, Sabrine N, Wren C. Cardiovascular Malformations Among Preterm Infants. Pediatrics 2005;116:e833-e8.
301. National Institute for Health and Care Excellence. Cerebral palsy in under 25s: assessment and management. In: National Institute for Health and Care Excellence, ed. London2017.
302. Ackers R, Murray ML, Besag FM, Wong IC. Prioritizing children's medicines for research: a pharmaco-epidemiological study of antiepileptic drugs. British journal of clinical pharmacology 2007;63:689-97.
303. McIntyre S, Taitz D, Keogh J, Goldsmith S, Badawi N, Blair E. A systematic review of risk factors for cerebral palsy in children born at term in developed countries. Developmental Medicine & Child Neurology 2013;55:499-508.
304. O'Callaghan ME, MacLennan AH, Gibson CS, et al. Epidemiologic associations with cerebral palsy. Obstet Gynecol 2011;118:576-82.
305. Cassano PA, Koepsell TD, Farwell JR. Risk of febrile seizures in childhood in relation to prenatal maternal cigarette smoking and alcohol intake. Am J Epidemiol 1990;132:462-73; discussion 74-8.
306. Vahidnia F, Eskenazi B, Jewell N. Maternal smoking, alcohol drinking, and febrile convulsion. Seizure 2008;17:320-6.
307. Forthun I, Wilcox AJ, Strandberg-Larsen K, et al. Maternal Prepregnancy BMI and Risk of Cerebral Palsy in Offspring. Pediatrics 2016;138.
308. Villamor E, Tedroff K, Peterson M, et al. Association Between Maternal Body Mass Index in Early Pregnancy and Incidence of Cerebral Palsy. Jama 2017;317:925-36.
309. Razaz N, Tedroff K, Villamor E, Cnattingius S. Maternal Body Mass Index in Early Pregnancy and Risk of Epilepsy in Offspring. JAMA Neurol 2017;74:668-76.
310. MacLennan AH, Thompson SC, Gecz J. Cerebral palsy: causes, pathways, and the role of genetic variants. American Journal of Obstetrics and Gynecology 2015;213:779-88.
311. Wu CS, Sun Y, Vestergaard M, et al. Preeclampsia and risk for epilepsy in offspring. Pediatrics 2008;122:1072-8.
312. Wu CS, Nohr EA, Bech BH, Vestergaard M, Catov JM, Olsen J. Health of children born to mothers who had preeclampsia: a population-based cohort study. American Journal of Obstetrics & Gynecology 2009;201:269.e1-.e10.
313. Krishnamurthy KB. Epilepsy. Annals of internal medicine 2016;164:ITC17-ITC32.
314. Böhm S, Curran EA, Kenny LC, O'Keeffe GW, Murray D, Khashan AS. The Effect of Hypertensive Disorders of Pregnancy on the Risk of ADHD in the Offspring. Journal of Attention Disorders 2019;23:692-701.

315. Modabbernia A, Velthorst E, Reichenberg A. Environmental risk factors for autism: an evidence-based review of systematic reviews and meta-analyses. Mol Autism 2017;8:13.
316. Banerjee TD, Middleton F, Faraone SV. Environmental risk factors for attention-deficit hyperactivity disorder. Acta Paediatrica 2007;96:1269-74.
317. Andersen CH, Thomsen PH, Nohr EA, Lemcke S. Maternal body mass index before pregnancy as a risk factor for ADHD and autism in children. European Child & Adolescent Psychiatry 2018;27:139-48.
318. Chen Q, Sjolander A, Langstrom N, et al. Maternal pre-pregnancy body mass index and offspring attention deficit hyperactivity disorder: a population-based cohort study using a sibling-comparison design. Int J Epidemiol 2014;43:83-90.
319. Bölte S, Girdler S, Marschik PB. The contribution of environmental exposure to the etiology of autism spectrum disorder. Cell Mol Life Sci 2019;76:1275-97.
320. Ingram Cooke RW. Does neonatal and infant neurodevelopmental morbidity of multiples and singletons differ? Seminars in Fetal and Neonatal Medicine 2010;15:362-6.
321. Zhao L, Li X, Liu G, Han B, Wang J, Jiang X. The association of maternal diabetes with attention deficit and hyperactivity disorder in offspring: a meta-analysis. Neuropsychiatr Dis Treat 2019;15:675-84.
322. Franz AP, Bolat GU, Bolat H, et al. Attention-Deficit/Hyperactivity Disorder and Very Preterm/Very Low Birth Weight: A Meta-analysis. Pediatrics 2018;141.
323. Desai RJ, Rothman KJ, Bateman BT, Hernandez-Diaz S, Huybrechts KF. A Propensity-score-based Fine Stratification Approach for Confounding Adjustment When Exposure Is Infrequent. Epidemiology 2017;28:249-57.
324. Hastie T, Tibshirani R, Friedman J. The Elements of Statistical Learning. 2 ed: Springer; 2009.
325. Fan H, Gilbert R, Li L, Wijlaars L. Associations between use of macrolide antibiotics during pregnancy and adverse child outcomes: a systematic review and meta-analysis Lancet 2018.
326. Neligan A, Hauser WA, Sander JW. The epidemiology of the epilepsies. Handbook of clinical neurology 2012;107:113-33.
327. Barrett ES, Swan SH. Stress and Androgen Activity During Fetal Development. Endocrinology 2015;156:3435-41.
328. Chang L, Cloak CC, Jiang CS, Hoo A, Hernandez AB, Ernst TM. Lower glial metabolite levels in brains of young children with prenatal nicotine exposure. J Neuroimmune Pharmacol 2012;7:243-52.
329. Lash T, Fox M, Fink A. Applying Quantitative Bias Analysis to Epidemiologic Data. 2009.
330. episensr: Basic Sensitivity Analysis of Epidemiological Results. Denis Haine, 2019. (Accessed 30 Sep 2019, at https://CRAN.R-project.org/package=episensr.)
331. Cunha GR, Sinclair A, Risbridger G, Hutson J, Baskin LS. Current understanding of hypospadias: relevance of animal models. Nat Rev Urol 2015;12:271-80.
332. Mahawong P, Sinclair A, Li Y, et al. Comparative effects of neonatal diethylstilbestrol on external genitalia development in adult males of two mouse strains with differential estrogen sensitivity. Differentiation 2014;88:70-83.
333. Govers LC, Phillips TR, Mattiske DM, et al. A critical role for estrogen signaling in penis development. The FASEB Journal 2019;33:10383-92.
334. Office for National Statistics. Gestation-specific Infant Mortality in England and Wales, 2007-2008. In: Office for National Statistics, ed.2014.

335. Danielsson BR, Danielsson C, Nilsson MF. Embryonic cardiac arrhythmia and generation of reactive oxygen species: common teratogenic mechanism for IKr blocking drugs. Reproductive toxicology (Elmsford, NY) 2007;24:42-56.
336. Summary of Product Characteristics: Clarithromycin 500mg film-coated tablets. Medicines & Healthcare Products Regulatory Agency, 2018. (Accessed 30 May 2019, at http://www.mhra.gov.uk/home/groups/spcpil/documents/spcpil/con1551417660825.pdf.)
337. Karabulut AK, Uysal, II, Acar H, Fazliogullari Z. Investigation of developmental toxicity and teratogenicity of macrolide antibiotics in cultured rat embryos. Anatomia, histologia, embryologia 2008;37:369-75.
338. Legato M. Principles of gender specific medicine : gender in the genomic era / edited by Marianne Legato. Third edition. ed: Boston, Massachusetts : Academic Press; 2017.
339. Gilbert JS, Nijland MJ. Sex differences in the developmental origins of hypertension and cardiorenal disease. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 2008;295:R1941-R52.
340. Xue Q, Zhang L. Prenatal hypoxia causes a sex-dependent increase in heart susceptibility to ischemia and reperfusion injury in adult male offspring: role of protein kinase C epsilon. The Journal of pharmacology and experimental therapeutics 2009;330:624-32.
341. Mayoral SR, Omar G, Penn AA. Sex Differences in a Hypoxia Model of Preterm Brain Damage. Pediatric research 2009;66:248-53.
342. Zhai P, Eurell TE, Cotthaus R, Jeffery EH, Bahr JM, Gross DR. Effect of estrogen on global myocardial ischemia-reperfusion injury in female rats. Am J Physiol Heart Circ Physiol 2000;279:H2766-75.
343. Faraone SV, Asherson P, Banaschewski T, et al. Attention-deficit/hyperactivity disorder. Nature Reviews Disease Primers 2015;1:15020.
344. Muhle R, Trentacoste SV, Rapin I. The genetics of autism. Pediatrics 2004;113:e472-86.
345. Hildebrand MS, Dahl H-HM, Damiano JA, Smith RJH, Scheffer IE, Berkovic SF. Recent advances in the molecular genetics of epilepsy. Journal of Medical Genetics 2013;50:271-9.
346. Rumajogee P, Bregman T, Miller SP, Yager JY, Fehlings MG. Rodent Hypoxia-Ischemia Models for Cerebral Palsy Research: A Systematic Review. Frontiers in neurology 2016;7:57-.
347. Ford T, Goodman R, Meltzer H. The British Child and Adolescent Mental Health Survey 1999: The Prevalence of DSM-IV Disorders. Journal of the American Academy of Child & Adolescent Psychiatry 2003;42:1203-11.
348. Danielson ML, Visser SN, Gleason MM, Peacock G, Claussen AH, Blumberg SJ. A National Profile of Attention-Deficit Hyperactivity Disorder Diagnosis and Treatment Among US Children Aged 2 to 5 Years. Journal of developmental and behavioral pediatrics : JDBP 2017;38:455-64.
349. Bushe C, Wilson B, Televantou F, Belger M, Watson L. Understanding the treatment of attention deficit hyperactivity disorder in newly diagnosed adult patients in general practice: a UK database study. Pragmat Obs Res 2015;6:1-12.
350. Mortensen LH, Catalano RA, Bruckner TA. Spontaneous Pregnancy Loss in Denmark Following Economic Downturns. American Journal of Epidemiology 2016;183:701-8.

351. Heinke D. An Evaluation of Competing Risks in Studies of Perinatal Mortality and Birth Defects. Boston: Harvard University; 2018.
352. Hastie T, Tibshirani R. Generalized additive models for medical research. Stat Methods Med Res 1995;4:187-96.
353. Teratogenecity in the setting of cardiac development and maldevelopment. Anderson RH. (Accessed 30 Nov 2019, at https://embryology.med.unsw.edu.au/embryology/index.php/Paper_-_Teratogenecity_in_the_setting_of_cardiac_development_and_maldevelopment.)
354. Muscular Ventricular Septal Defect. Michael D Taylor. (Accessed 30 Nov 2019, at https://emedicine.medscape.com/article/899873-overview#a5.)
355. Simultaneous intervals for smooths revisited. Gavin Simpson, 2016. (Accessed 30 Nov 2019, at https://www.fromthebottomoftheheap.net/2016/12/15/simultaneous-interval-revisited/.)
356. Hermes-DeSantis ER, Clyman RI. Patent ductus arteriosus: pathophysiology and management. Journal of Perinatology 2006;26:S14-S8.
357. Lin KJ, Mitchell AA, Yau WP, Louik C, Hernandez-Diaz S. Safety of macrolides during pregnancy. Am J Obstet Gynecol 2013;208:221.e1-8.
358. Sarkar M, Woodland C, Koren G, Einarson AR. Pregnancy outcome following gestational exposure to azithromycin. BMC Pregnancy Childbirth 2006;6:18.
359. Fan H, Li L, Wijlaars L, Gilbert RE. Associations between use of macrolide antibiotics during pregnancy and adverse child outcomes: A systematic review and meta-analysis. PLoS One 2019;14:e0212212.
360. Public Health England. Management and treatment of common infections: Antibiotic guidance for primary care: For consultation and local adaptation. In: Public Health England, ed.2017.
361. The National Institute for Health and Care Excellence. The risk of MRSA and C difficile in people with documented 'penicillin allergy'. 2018.