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Coláiste na hOllscoile Corcaigh
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School of Medicine
Department of Obstetrics & Gynaecology

OBSTETRIC MODE OF DELIVERY AND CHILD PSYCHOLOGICAL DEVELOPMENT

Thesis presented by

Eileen Ann Curran
BS (Biology/Public Health), MPH (Epidemiology)

Under the supervision of

Dr. Ali S Khashan
Prof. Louise C. Kenny
Prof. Patricia M. Kearney
Prof. John F. Cryan
Prof. Ted Dinan

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Head of Department
Prof. Richard Greene

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Curran EA, O'Neill SM, Cryan JF, Kenny LC, Dinan TG, Khashan AS, Kearney PM (2014). Research review: birth by caesarean section and development of autism spectrum disorder and attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. *J Child Psychol Psychiatry*. 2015; 56 (5): 500-8.

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LIST OF ORAL PRESENTATIONS

Curran EA, Khashan AS, O'Keeffe GW, Kenny LC. Hypertension in pregnancy and autism spectrum disorder in a British cohort. *Society for Social Medicine, Dublin, Ireland.* 2015.

Curran EA, Dalman C, Kearney PM, Kenny L, Cryan JF, Dinan TG, Khashan AS. Obstetric mode of delivery and autism spectrum disorders in Sweden: a sibling design study. *European Congress of Epidemiology (Opening Plenary Session), Maastricht, The Netherlands.* 2015.

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Curran EA, Cryan JF, Kenny LC, Dinan TG, Kearney PM, Khashan AS. Effects of mode of delivery and maternal irritable bowel syndrome on childhood autism spectrum disorder and attention deficit hyperactivity disorder in a British cohort. *IEA World Congress of Epidemiology, Anchorage, AK.* 2014.

LIST OF ABBREVIATIONS

ADHD- Attention-deficit/hyperactivity disorder

aHR- Adjusted hazard ratio

aOR- Adjusted odds ratio

ASD- Autism spectrum disorder

BMI- Body mass index

CI- Confidence interval

CS- Caesarean section

DAG- Directed acyclic graph

DSM- Diagnostic and Statistical Manual of Mental Disorders

HDI- Human Development Index

HR- Hazard ratio

IBS- Irritable bowel syndrome

ICD- International Classification of Disease

ID- Intellectual disability

IOL- Induction of labour

IQ- Intelligence quotient

LGA- Large for gestational age

MCS- Millennium Cohort Study

MeSH- Medical subject headings

NIHR- National Institute for Health Research

NR- Not recorded

OR- Odds ratio

PAR- Population attributable risk

PDD- Pervasive developmental disorder

PDD-NOS- Pervasive developmental disorder-not otherwise specified

PIN- Personal identification number

PPV- Positive predictive value

PRISMA- Preferred Reporting Items for Systematic Reviews and Meta-Analysis

RR- Risk ratio

SDQ- Strengths and Difficulties Questionnaire

SE- Standard error

SGA- Small for gestational age

UK- United Kingdom

VD- Vaginal delivery

WHO- World Health Organization

DECLARATION

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university. The work upon which this thesis is based, was carried out in collaboration with a team of researchers and supervisors who are duly acknowledged in the text of the thesis. The library may lend or copy this thesis upon request.

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THESIS ABSTRACT

Background and aims: Global rates of Caesarean section are high and rising. Birth by Caesarean section has been linked to child development, including psychological development. However results to date have been limited and conflicting. The objective of the current thesis is to investigate the potential impact of birth by Caesarean section on child psychological development, including autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), behavioural difficulties and school performance by systematically reviewing the existing literature and undertaking robust analyses of using two large, population-based cohorts with a range of analytic approaches.

Structure and methods: The published literature to date on birth by Caesarean section and ASD and ADHD was systematically reviewed and meta-analysed (Chapter 2). Data from the UK Millennium Cohort Study (MCS) were analysed to determine the association between mode of delivery and ASD, ADHD and parent-reported behavioural difficulties using logistic regression analysis (Chapter 3). The research questions were further explored using a range of methodological approaches to analyse the Swedish National Registers. Cox regression was used to assess the impact of birth by Caesarean section on ASD in the general cohort, and conditional logistic regression to assess the association in sibling pairs (Chapter 4). Cox regression models were also used to assess the association between birth by Caesarean section and ADHD in the cohort, then stratified Cox regression was used to assess the association amongst siblings (Chapter 5). School performance was assessed using logistic regression ("poor" vs "not poor" school performance) and quantile regression (continuous grade score) (Chapter 6). Finally, the systematic

review and meta-analyses were updated, and included along with a discussion of methods, findings, and strengths and limitations and public health implications of the current thesis as well as recommendations for future research (Chapters 7 and 8).

Results: *Systematic review and meta-analysis:* Children born by Caesarean section were 23% more likely to be diagnosed with ASD after controlling for potential confounders. Only two studies reported adjusted estimates on the association between birth by Caesarean section and ADHD, results were conflicting and limited.

UK MCS: Mode of delivery was not associated with ASD or ADHD in the UK MCS. Birth by elective or emergency Caesarean section was not associated with behavioural difficulties. Induction of labour was associated with behavioural difficulties in unadjusted analysis, but not after adjustment for potential confounders.

Swedish National Registers: In the Swedish National Registers, children born by elective Caesarean section were 15% more likely to be diagnosed with ASD, and children born by emergency Caesarean section were 20% more likely to be diagnosed with ASD. However, amongst sibling pairs there was no association between birth by Caesarean section and ASD. Children born by elective Caesarean section were 15% more likely to be diagnosed with ADHD, and those born by emergency Caesarean section were 16% more likely to be diagnosed with ADHD. In stratified analysis, the association only remained for emergency Caesarean section. There was little evidence of an association between birth by elective Caesarean section and poor school performance. Children born by elective Caesarean section had a 6% increased odds of poor school performance, and children born by emergency Caesarean section had a 12% increased odds of poor school performance. In quantile regression, both elective and emergency Caesarean section were

associated with a 2-5 point decrease (out of a total of 320 points) across the distribution.

Conclusions: This thesis rigorously investigates the association between mode of delivery and psychological development using multiple statistical models, and adjustment for a wide variety of confounders in two large population-based cohorts. While we found an association between birth by Caesarean section and both ASD and ADHD using traditional modelling approaches, the lack of association with the elective Caesarean section in the sibling design studies indicates that this association is most probably due to residual confounding. No association was found between birth by Caesarean section and behavioural difficulties. A small but significant association was found between birth by Caesarean section and school performance. However, given the complexities of the relationship between perinatal risk factors and psychological development, the effect may have been due to residual confounding or confounding by indication and should be interpreted with caution. The overall conclusion is that birth by Caesarean section does not appear to have a causal relationship with the aspects of child psychological development investigated.

CHAPTER 1. INTRODUCTION

1.1 Introduction

1.1.1 Obstetric mode of delivery

The way in which a child is born can be referred to as his or her obstetric mode of delivery. Children can be delivered vaginally or abdominally. or assist delivery through the use of forceps or a vacuum extractor[1]. Abdominal delivery, or Caesarean section, involves a surgical procedure in which the baby is delivered through an incision in the mother's abdomen[2]. This can be planned for medical reasons, such as breech presentation, or psychosocial reasons such as fear of childbirth[3,4]. Additionally, Caesarean section may occur if vaginal delivery has begun but cannot be completed due to complications, such as fetal distress[4].

It is a popular myth that Julius Caesar was the first person to be born by Caesarean section[5], hence the term "Caesarean." This was likely not the case, however, as his mother survived, and at the time Caesarean sections were only performed as an attempt to save the baby if the mother was dying[5]. Though Caesar may not have born through this method, it is true that during his reign, the law was that Caesarean sections be performed in situations where the mother was dead or dying[5]. However, Caesarean sections were performed long before then and have been performed for so long their origin is unclear[5,6]. In fact, references to abdominal birth are even found in Greek mythology[6]. The first Caesarean section where both mother and baby survived was recorded in 1500[6]. Caesarean sections were not performed regularly, however, until the late 19th century, when society as a whole, and consequently medical care, became more centralised. The subsequent advancement of the Caesarean section procedure was a result of medicine increasingly being researched, taught and practiced in a hospital setting[5]. With these

advances, birth by Caesarean section became more common. For example, in 1937, the prevalence rate of birth by Caesarean section at Boston City Hospital over the course of the previous10 years was 3.7%[7].

Indications for Caesarean section have evolved over time. Initially Caesarean sections were performed in cases where the mother was dead or dying and unable to complete labour. As techniques and knowledge improved, Caesarean sections were progressively performed in cases where there were foetal complications[5]. In recent years, psychosocial indications such as fear of childbirth have also become increasingly common[4]. Today, in addition to historically traditional post-labour emergency Caesarean section, the procedure is also planned based on medical indication (such as placenta praevia) or other non-medical reasons, such as maternal request. There are several reasons a non-medically indicated Caesarean section may be performed, including maternal fear of childbirth, cultural influences on the mother or doctor, or maternal or doctor preference[3]. The ethics regarding performing non-medically indicated Caesarean sections based on maternal request has been debated[8,9]. Some believe that medical interventions should not be performed unnecessarily, and performing a Caesarean section because a mother requests it is akin to prescribing antibiotics for influenza because the patient requests them[9]. Others disagree, citing the importance of a woman's right to make informed decisions regarding her own medical care[8,9], and the fact that when women request a Caesarean section it is often because of psychological issues or past trauma[3].

Prevalence of Caesarean section is high and rising globally. A study reporting on over 20 countries in the European Union, as well as Norway, Iceland and Switzerland, reported a median prevalence of Caesarean section in 2010 to be 25.2% (ranging from 14.8% in Iceland to 52.2% in Cyprus)[10]. Prevalence is not only high,

but is rising. For example, in Austria, Ireland and Hungary the rate of Caesarean section more than doubled between 1992 and 2007[11]. Another recent study, which included 21 countries involved in the World Health Organization Global Survey of Maternal and Perinatal Health (2004-2008) and Multi-country Survey of Maternal and Newborn Health (2010-2011), reported a similar increasing trend occurring globally[12]. For instance, Brazil was reported to have a prevalence of 47.0% in the second survey, and increased at an average of 8.5% per year, and Nicaragua reported a prevalence of 44.9% in the second survey, and increased by an average of 9.4% per year[12]. In fact, of the countries included in the study, only Japan did not report an increasing rate of Caesarean section.

Since 1985, the World Health Organization (WHO) has recommended a prevalence of Caesarean section of 10-15%[13]. Recently, the WHO released a statement including data from recent studies including a systematic review of existing literature, as well as analysis on their currently available ecological data from 194 WHO member-countries[13]. The WHO observed that increasing levels of Caesarean section were associated with lower maternal and infant mortality, however this relationship did not hold after adjusting for human development index of the country[14,15]. Following this adjustment, on a population level, increasing prevalence of Caesarean section was associated with lower maternal and infant mortality only up to a prevalence of 10%[13]. For prevalence values from 10-30%, no additional benefit was found with increasing rates of Caesarean section (there was not sufficient data to conclude the effect of prevalence greater than 30%)[15]. On the other hand, another recent study conducted by researchers in Boston on the same countries concluded that increasing prevalence of Caesarean section was inversely related to maternal and infant mortality up to about 20%, even after adjusting for

socio-economic factors (including health expenditure per capita, life expectancy at birth, gross domestic product per capita, total population size, percent urban population, fertility rate, annual number of births and birth rate)[16]. Notably, both studies additionally concluded that the decision of whether or not to proceed with a Caesarean section should be made on a patient-by-patient basis, and not with the goal of achieving a specific prevalence level[15,16].

Regardless of the "ideal" prevalence of Caesarean section, with such high and rising rates, even a small increase in long term risks could have a large impact on society at a global level. In addition to the child psychological development outcomes presented in this thesis, birth by Caesarean section has been reported to be associated with asthma[17], obesity[18], and type 1 diabetes[19]. A better understanding of these potential risks of birth by Caesarean section is becoming increasingly important as Caesarean section is becoming more common.

1.1.2 Caesarean section and psychological development

Two neurodevelopmental disorders which present during childhood are autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD). The definitions of both ASD and ADHD have undergone several changes since their original recognition[20-22]. The history of the definition of these disorders according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) is outlined in **Figures 1-2** and **1-3**. It is worth noting that though DSM is more commonly used in the United States, the definitions of mental disorders according to the WHO's International Classification of Disease (ICD) are generally similar to the current DSM, though the ICD definitions tend to be stricter[23,24]. With regards to autism, prior to DSM-IV the definition of autism was considered to be overly broad,

compared to the ICD[24]. However, changes to the DSM for DSM-IV were specifically created for comparability to the current ICD (ICD-10)[24]. For ADHD, ICD is stricter in that hyperactivity has always been a requirement for diagnosis[20] and impairment must be present in multiple settings[25].

ASD is characterized by impaired social interaction and communication, and obsessive repetitive behaviours[26]. Prevalence of ASD has grown significantly in the last few decades[27]. There are several possible explanations for this, including changes in definitions of ASD, increased awareness, or increased exposure to unknown environmental risk factors. Recent studies have concluded that this rising prevalence can be largely explained by changes in diagnostic practices[27,28], though a significant proportion of the increase cannot be explained in this way, and unknown environmental exposures also likely contribute[27]. Previous results on birth by Caesarean section and ASD have been conflicting, with some reporting an association[29,30] and others reporting none[31,32].

ADHD, characterized by impairment in attention, and increased hyperactivity and impulsivity, affects an estimated 5.59-7.1% of children worldwide[33]. Prevalence of ADHD has also grown in recent years, though similar to ASD, it is possible that this increased prevalence is more related to increased awareness and changes in diagnostic procedures rather than an increase in the disorder itself[34]. Notably, while both disorders are considered to be highly heritable, environmental factors also likely play a role[29,35-37]. Birth by Caesarean section has also been linked to ADHD in some studies, but not others[38,39]. In addition, previous results were limited, and the only study to report separate estimates for elective and emergency Caesarean section only adjusted for year of birth, infant gender and socioeconomic index of the area, and only reported overall Caesarean section in fully adjusted analysis[38].

1.1.3 Caesarean section and behaviour

There are several ways to assess the potential impact of birth by Caesarean section on psychological development. For example, the current thesis includes parent-rated behavioural difficulties and school performance, as well as diagnosis with two neurodevelopmental disorders diagnosed in childhood (i.e. ASD and ADHD). Little research has been done on birth by Caesarean section and behavioural difficulties, and results to date have been conflicting. One study found maternally requested Caesarean section to be associated with lower externalising behavioural problems (such as aggression)[40], while another found it to be associated with increased internalising behavioural problems (such as anxiety or depression) and sleeping problems[41]. Another study found elective Caesarean section to be associated with a delay in personal social skills, but not behavioural difficulties[42].

School performance is associated with behavioural difficulties[43], as well as psychological outcomes including insomnia[44,45] and depression[46]. In that way, assessing school performance could be a good way to "catch" any potential behavioural issues, as well as potential cognitive effects. Some perinatal risk factors, such as being small for gestational age[47], have been associated with school performance. To our knowledge, the association between birth by Caesarean section and school performance has not been investigated previously, but, pre-labour emergency Caesarean section has been reported to be associated with increased special education requirements (though notably the same study found no association with elective Caesarean section or emergency post-labour Caesarean section)[48].

1.1.4 Biological mechanisms

There are several proposed biological mechanisms regarding a potential association between birth by Caesarean section and child development, including "early term" birth[48], and changes in microbiota and stress response[49-51] (**Figure 1-1**) (figures and tables are located at the end of each chapter). With regards to these biological mechanisms, Caesarean sections can be divided into two relevant types: elective (i.e. pre-labour) and emergency (i.e. post-labour) Caesarean section. Elective Caesarean section is scheduled prior to the onset of labour, either by maternal request, or medical reasons such as pre-eclampsia, narrow pelvis, or breech presentation[4]. Emergency Caesarean section, on the other hand, is unplanned and begins after the onset of labour, generally due to a complication during labour such as prolonged labour or foetal compromise[4]. Notably, theories regarding the association between birth by Caesarean section and psychological development are more related to elective, or pre-labour, Caesarean section.

Though 40 weeks gestation is considered "full term," babies are defined as "term" if they are born any time at or after 37 weeks gestation[52]. It is possible that babies born in this window, after 37 weeks but before 40 weeks, i.e. "early term", miss out on some development we don't fully understand. Indeed, a recent study found this "early term" birth to be associated with special education requirements[48]. Caesarean section procedures are generally scheduled for 38 or 39 weeks gestation[52] in order to avoid spontaneous labour at 40 weeks. Therefore, it is possible that babies who are born through scheduled Caesarean section are not exposed to some developmental factor that occurs in these last week or two, and so develop somewhat differently to babies born vaginally at full term.

Caesarean section may also affect child development through changes in the bacterial colonisation of the infant, or the infant's "microbiota"[50,51]. In animal models, changes in microbiota have been associated with changes in behaviour and neurodevelopment[53-55]. Children who are born vaginally are exposed to their mother's microbiota through the birth canal, and thus have a similar microbiota make-up to their mother[51]. However, when a baby is born through by elective Caesarean section, he or she is instead exposed to the bacteria on their mother's skin and in the hospital[50,51,56,57]. Children who are born through this method have been shown to have altered microbiota until at least age 7[56]. This theory is particularly compelling for the development of ASD, which is itself associated with gut disorders and changes in microbiota[55,58].

Another theory is related to stress-response. This theory posits that the act of being born, and the stress that is experienced by an infant when born vaginally, as well as exposure to the elevated stress hormones of the mother, primes the infant's HPA-axis and helps to determine their stress response in the future[49,50]. It is possible that since children who are born through elective Caesarean section do not experience the same stress as children who are born vaginally, their HPA axis is not primed in the same manner, thus altering their behavioural development. Altered microbiota and stress response have both also been suggested as mechanisms for the association between birth by Caesarean section and immune-related outcomes[50,59].

There are several reasons these explanations apply more to elective Caesarean section than emergency Caesarean section. As emergency Caesarean section begins after labour has started, the gestational age is already determined (i.e. emergency Caesarean section does not cause early term birth as labour has already begun). Additionally, in emergency Caesarean section, the baby is likely exposed to

the mother's microbiota and elevated stress hormones during labour prior to the start of the Caesarean section[49]. On the other hand, as elective Caesarean section begins prior to the onset of labour, early term birth is planned and as the membranes have not ruptured, the baby is not exposed to the mother's microbiota or elevated stress hormones.

1.1.5 Public health implications

Over 1 in 4 children in Europe alone, and as many as 1 in 2 in certain countries[10,12] are born through Caesarean section. Consequently, it is vital to understand how previous results and theories on the potential effect of birth by Caesarean section on child psychological development may translate into human populations. With such a high prevalence, even a small increase in absolute risk in a long-term developmental outcome in children could lead to a large population attributable risk. Previous work has linked birth by Caesarean section to asthma[17], obesity[17], and type 1 diabetes[19], all of which are significant public health issues. A better understanding of such potential long-term effects of Caesarean section could help inform parents, clinicians and policy makers in the future.

Neurodevelopmental disorders such as autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) can have a large impact on those diagnosed, not only through direct effects, but also through common co-morbidities including intellectual disability[60], schizophrenia[61], addiction[62] and depression[62] (among others). In addition, diagnosis with ASD or ADHD may affect inter-personal and familial relationships[63-65] and academic achievement[66,67]. They are also economically costly for society as a whole, due to required medical services,

caretaking and educational services, and missed work and lower productivity in adulthood[68-71].

Pre- and perinatal risk factors have previously been associated with ASD, ADHD and school performance. For example, previous associations have been reported with maternal acetaminophen use[72], hospitalisation during pregnancy[73], and gestational weight gain[74], or pre-term birth[75,76], small for gestational age[38,76,77] and Apgar score[78]. Examining potential perinatal risk factors such as birth by Caesarean section could result in a better understanding of the causes of these disorders.

1.2 Causal inference

In observational research, it is impossible to make a definitive causal claim with any association. Instead, various methods such as statistical adjustment are used to account for factors outside of a direct causal relationship which could be leading to a measured association. By accounting for these external factors, observational epidemiological research can provide evidence for a potential causal relationship. There are three potential alternative explanations for a detected non-causal association: chance, confounding and bias.

1.2.1 Chance

Random error occurs in every study, and leads to a measured association that is different than the true association in a population. Avoiding reporting an association by chance is the basis for many statistical methods. The probability of achieving a result at least as extreme as the one detected can be used to create confidence intervals around the measured association[79]. By convention, 95% confidence is considered sufficient[79]. The way to interpret 95% confidence intervals

is that if the same study were to be conducted many times, then 95% of the time the true value of the association of interest in the population would fall within the intervals calculated. Therefore, if the 95% confidence interval does not include a null association, it is said to be "statistically significant," and the association is deemed likely different from the null in the underlying population.

1.2.2 Confounding

Confounding is a mixing of effects between the exposure of interest and another variable[80]. Confounding results when a particular factor is associated with both the exposure and outcome of interest, resulting in a change in the measured association. For example, maternal psychiatric history is associated with both birth by Caesarean section[81] and child psychological development[82]. Therefore, a study on Caesarean section and psychological development which did not take maternal psychiatric history into account may find an association between birth by Caesarean section and psychological development that is driven by maternal psychiatric history rather than mode of delivery. Confounding can be addressed either in the study design (through matching) or analysis (through adjustment or stratification)[80]. Notably, the concept of confounding is not a problem in study design, but is rather an intrinsic part of the exposure-outcome relationship. As it cannot be avoided, appropriate study designs and analyses must take potential confounding into account. When confounding is not fully measured or accounted for, it may still have an effect on the association even after adjustment. This issue, known as residual confounding, is impossible to rule out in observational research[83] and thus is important to take into account when interpreting results.

Confounding is particularly important when investigating perinatal risk factors for psychological development for two main reasons[84]. Firstly, it can be difficult to separate these risk factors from related post-natal factors which could also impact psychological development. For example, socioeconomic status and social adversity is often associated with perinatal risk factors and psychological development, and is notoriously difficult to measure and adjust for in health research[84,85]. Secondly, there is always the possibility of unmeasured genetic or inherited risk factors, which lead to perinatal risk factors are also independently associated with psychological development[84]. The research in the current thesis is also subject to an additional kind of confounding, known as confounding by indication[86]. It is possible that indications for performing a Caesarean section, such as breech presentation or foetal distress cause changes in psychological development. In this case, an association between birth by Caesarean section and psychological development would be present, but it would be driven by the indication for the procedure rather than a direct causal effect of the section.

1.2.3 Bias

Where confounding refers to potential mixing of effects inherent in the defined exposure and outcome relationship, bias refers to a systematic error in the study design or analysis[80]. The two main forms of bias are selection bias and information bias[80]. Selection bias refers to the potential effect of not having a representative sample with regards to your association of interest, and the effect this may have on your results. In other words, the people included in the study are meaningfully different than all who were eligible. For example, if a case-control study on Caesarean section and autism spectrum disorder in children were to recruit

cases from private health clinics, and controls from a public hospital, this may result in a biased estimate, as patients with private health insurance may be more likely to be delivered by Caesarean section.

Information bias refers to a situation where there is a problem in how data are collected or recorded. For example, if cases were recorded as controls in a systematic way or vice versa, this may result in a change in the measured association. This is a sub-group of information bias known as "misclassification bias." Misclassification bias can be either "differential" or "non-differential." If the misclassification is differential, that means one group is more likely to be misclassified than another. For example, if a study on smoking during pregnancy and attention-deficit/hyperactivity disorder were to involve psychological evaluations to determine case status, it's possible that researchers would be more likely to classify children they knew were exposed to smoking during pregnancy as cases. On the other hand, non-differential misclassification is not related to exposure and outcome but rather in the way that data are collected or recorded. For example, if the same hypothetical study were to use parent-reported smoking during pregnancy, it's possible mothers might under-report their smoking habits, but it's unlikely that this would be related to the child's determined attention-deficit/hyperactivity disorder status. The effect of non-differential misclassification bias is usually a shifting of the measured association towards the null, and a weaker measured association, whereas differential misclassification bias can shift the association in either direction[80]. Other examples of information bias include recall bias[87], where information is collected retrospectively and may be mis-remembered, or social desirability bias[88], where participants are more likely to report answers deemed socially acceptable rather than the true value.

Notably, there is the potential for bias in every epidemiological study and it must be considered not only in the design of a study, but also in interpreting the results[80]. It is important to at least acknowledge where bias may exist if it is not possible to eliminate or reduce it.

1.2.4 Confounding, bias, and sibling design studies

Matched analyses can be a useful way of controlling for particular confounders (e.g. age, sex etc.)[80]. Sibling designs are essentially an extension of this, where instead of being matched on specific variables, cases are matched within families[89]. This controls for genetics or static family environment which is shared amongst siblings and may confound the relationship of interest[90]. There are some limitations inherent in sibling designs. Firstly, they are only useful for familial confounders that remain static, or do not change from one sibling to another[90]. Secondly, in situations where exposures are more shared in families than confounders are, the resulting estimate will be biased[89]. With most studies, it is likely that both the exposure and confounders are correlated within families, affecting the estimated effect to some extent. However, if the exposure is correlated more strongly than confounders, then siblings discordant on exposure status will be more different with regards to unmeasured confounders than two discordant people in the general cohort would be, resulting in a more biased estimate. On the other hand, if confounders are correlated more strongly than exposure, not using sibling design studies results in a more biased estimate as unmeasured familial confounders are not accounted for. Thirdly, though non-differential misclassification leads to bias towards the null in traditional analysis, this effect can be magnified in sibling design

studies[89]. Therefore, if the exposure and outcome are not recorded accurately it could result in a false amelioration of the estimated effect.

Though not appropriate in all cases, when used correctly sibling design studies can be useful in controlling for un-measured confounding. Previous sibling design studies have provided evidence for familial confounding driving the previously documented associations between birth by Caesarean section and asthma[91] and type 1 diabetes[92].

1.2.5 Directed acyclic graphs

In any statistical analysis, assumptions must be made about the underlying causal relationship involving the exposure and outcome of interest. With the use of directed acyclic graphs (DAGs), researchers are able to be explicit about these assumptions[93]. Essentially, DAGs are a graphical representation of the potential causal relationship between exposure and outcome of interest, as well as any other factors which may be affecting this relationship. This method not only assists future researchers in understanding and interpreting the results of a study, but may also be used to identify confounding[93], interaction[94], or bias[14,95].

Though DAGs have several uses with regards to visualising an association, they are most commonly used to identify confounding and determine which co-variates to include in a statistical model. Traditionally, determination of a confounder involved assessing if it was associated with both exposure and outcome, and whether it changed the association when included in a regression model. However, this method alone may be insufficient and in some cases may even increase bias[14]. As a result, DAGs have been becoming an increasingly popular way to clarify the theorised causal structure behind a relationship and identify what co-

variates are true confounders and should be included in a regression model. An example DAG illustrating the potential causal structure behind birth by Caesarean section and psychological development is presented in **Figure 1-4**[96].

1.3 Study design and methods

1.3.1 Study design

Ideally, researching the effect of a particular exposure on an outcome in a human population would involve the use of a randomised controlled trial. However, this is often not feasible due to ethical concerns or constraints in budget or time. Instead, we must use other approaches to investigate these associations. Moreover similar results are provided by observational studies when well-designed and conducted[97]. Two common observational study designs include case-control and cohort studies. Case-control studies tend to be more affordable, more effective for rare outcomes, and good for researching multiple exposures at once[80]. Conversely, cohort studies are generally more expensive and time consuming, and are good for researching multiple outcomes[80]. In addition, cohort studies are generally more accurate as case-control studies may be more susceptible to bias, and give inflated estimates of effect sizes[80]. Notably, nested case-control studies (i.e. case-control studies based on a cohort) are thought to be similar in accuracy to cohort studies[80].

The research in this thesis is based on the analysis of data from two large cohort studies; the UK Millennium Cohort Study and the Swedish National Registers. These cohorts were chosen for several reasons. First, they are externally maintained and readily available. Second, they are either nationally representative (UK Millennium Cohort Study) or include national coverage (Swedish National Registers), providing evidence for accuracy and external validity of any results.

Third, both cohorts have been previously validated in several subjects and are regularly used for this kind of research. Finally, use of these cohorts allowed us to look at several outcomes, which would not have been feasible in a case-control study.

1.3.2 Statistical modelling

More detailed descriptions of the statistical modelling, including equations and assumptions, can be found in **Appendix 1**.

Logistic regression

Logistic regression, which was used for the UK Millennium Cohort Study analysis, is a common method of assessing an association with a dichotomous outcome. The result of a logistic regression is used to determine the odds ratio (OR) of an association[98]. An OR above one indicates that outcome Y is more likely with the exposure of interest (with larger values indicating a stronger association). Conversely, an OR below one indicates that Y is less likely with the exposure of interest (with smaller values indicating a stronger association). When the incidence of the outcome is rare (<10% prevalence), this value can be assumed to approximate the risk ratio (RR) in a population[99].

Two additional types of logistic regression used here are conditional logistic regression, and mixed effects logistic regression. Conditional logistic regression is used when the degrees of freedom are large compared to the number of observations included in an analysis, such as when observations are matched[98]. In the current thesis, conditional logistic regression was used when cases were matched with their siblings. Mixed effects logistic regression is used when data are clustered by group and thus observations are not independent[100]. This method was used in the current

thesis in analysis of the school performance data. As the grades are assigned by the teachers, and not standardised across Sweden, they were potentially correlated within schools. Use of mixed effects logistic regression allowed an adjustment for this correlation, without the loss of degrees of freedom that would come with traditional statistical adjustment.

Cox regression

Survival analysis is a method of measuring associations in longitudinal data including time to an event[101]. Though it is traditionally thought of in the context of time to death, such as in cancer survival research, it is widely used in research where individuals have experienced differing amounts of follow-up time. In the current thesis, survival analysis was used for the analysis on autism spectrum disorder and ADHD, as children were born and diagnosed at different times. Results from a Cox regression model can be used to estimate a hazard ratio (HR), which can be interpreted in the same way as a RR.

Quantile regression

Quantile regression is similar to an ordinary least squares (OLS) model. In a regular OLS model (i.e. linear regression), the sum of the square of the residuals is minimized, and the conditional mean (Y) is modelled given that X = x. Conversely, in quantile regression, the absolute value of the residuals is minimized, and the conditional quantile is modelled[102]. Though this is most commonly the median (50^{th} percentile), the equation can be weighted to account for any conditional quantile, allowing for estimates of the association across the entire distribution. Also, unlike OLS models, quantile regression does not require an assumption of normality or equal variance of residuals, making it particularly useful for modelling

heterogeneous distributions. This method was particularly relevant for the school performance analysis, as the school grade data was highly skewed in continuous form.

1.3.3 Confounding

There are multiple ways to account for confounding in observational epidemiological research. The current thesis used previous literature and directed acyclic graphs (DAGs) to identify which co-variates were likely confounders. These co-variates were then included in statistical models. Additional unmeasured confounding was investigated through the use of sibling study designs.

Propensity scores were considered as a potential additional way to interrogate observed associations for confounding. With the use of propensity scores, people in each exposure group are matched or weighted depending on their probability of being assigned to that group based on their previously measured characteristics[103]. In this way, it is thought that theoretically the main difference between the groups is the exposure itself. This re-enforces the counterfactual behind any association found[103]. In other words, the analysis better represents a random allocation of the exposure. Though more complicated with a multi-nomial exposure such as mode of delivery, it is possible to use propensity scores in this setting[103]. This would have been a valid method for analysis in the studies included in the current thesis, however propensity scores were not used for the present thesis. In the studies where an association was found, sibling studies were deemed a better way to adjust for unmeasured confounding, as propensity scores can only include measured co-variates. In the Chapters 3 and 6, where sibling designs were not used, no association was identified and propensity scores were deemed unnecessary.

1.4 Overall aims and objectives

The overall aim of this thesis was to assess the impact of birth by Caesarean section on psychological development (as outlined in **Figure 1-5**). In particular, the objectives of this thesis were:

1. Review of previous literature published on birth by Caesarean section and child psychological development
2. Estimate the association between birth by Caesarean section and parent-reported autism spectrum disorder, attention-deficit/hyperactivity disorder and behavioural difficulties in a British cohort (the Millennium Cohort Study).
3. Estimate the association between birth by Caesarean section and autism spectrum disorder in a cohort and amongst siblings, using the Swedish National Registers.
4. Estimate the association between birth by Caesarean section and attention-deficit/hyperactivity disorder in a cohort and amongst siblings, using the Swedish National Registers
5. Estimate the association between birth by Caesarean section and school performance using the Swedish National Registers.
6. Update the systematic review and meta-analysis for autism spectrum disorder and attention-deficit/hyperactivity disorder including the current results and any newly published studies.

Figure 1-1. Proposed biological mechanisms for the effect of birth by Caesarean section on psychological development.

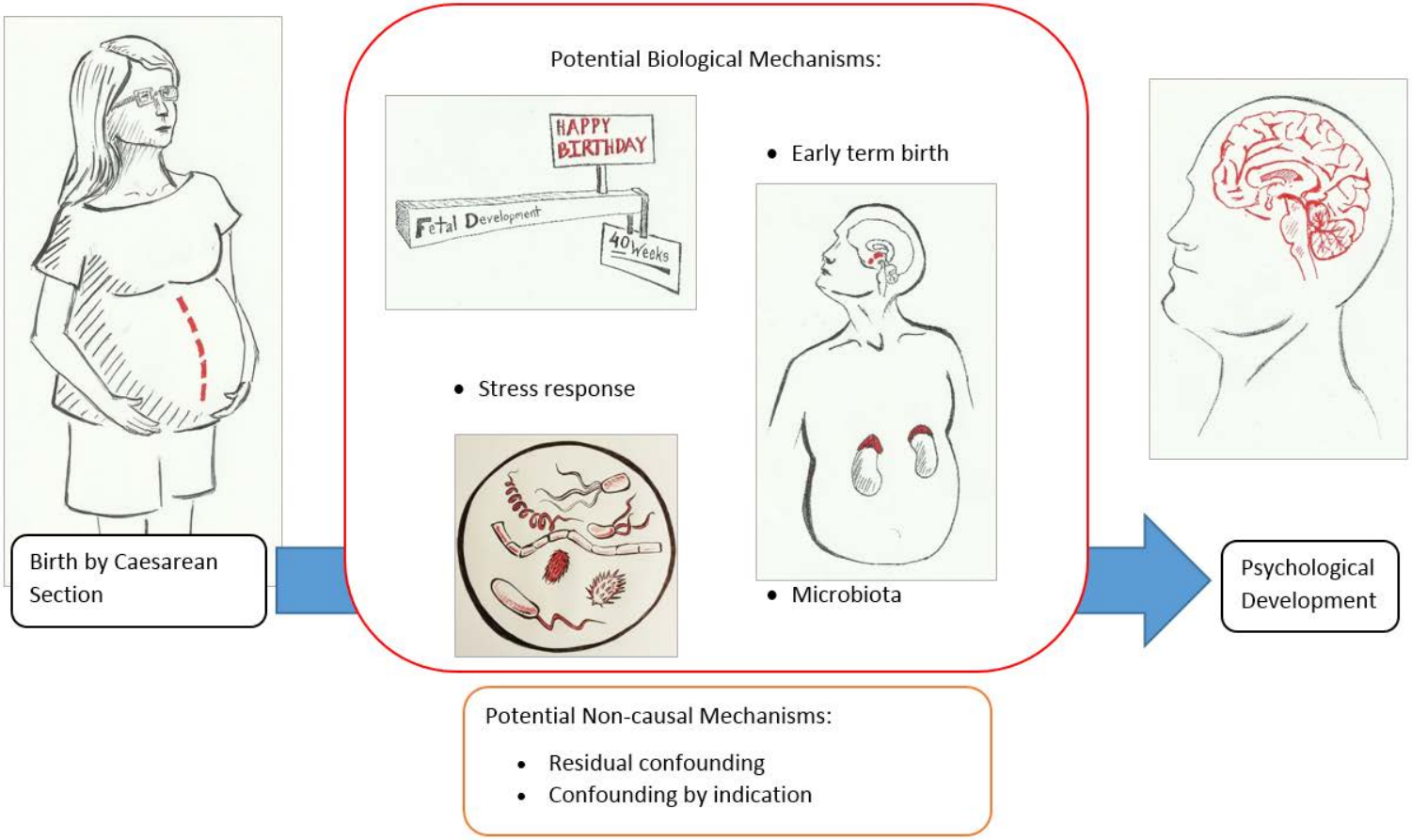


Figure 1-2. Timeline of the definition of autism spectrum disorder according to the Diagnostic and Statistical Manual of Mental Disorders (DSM).

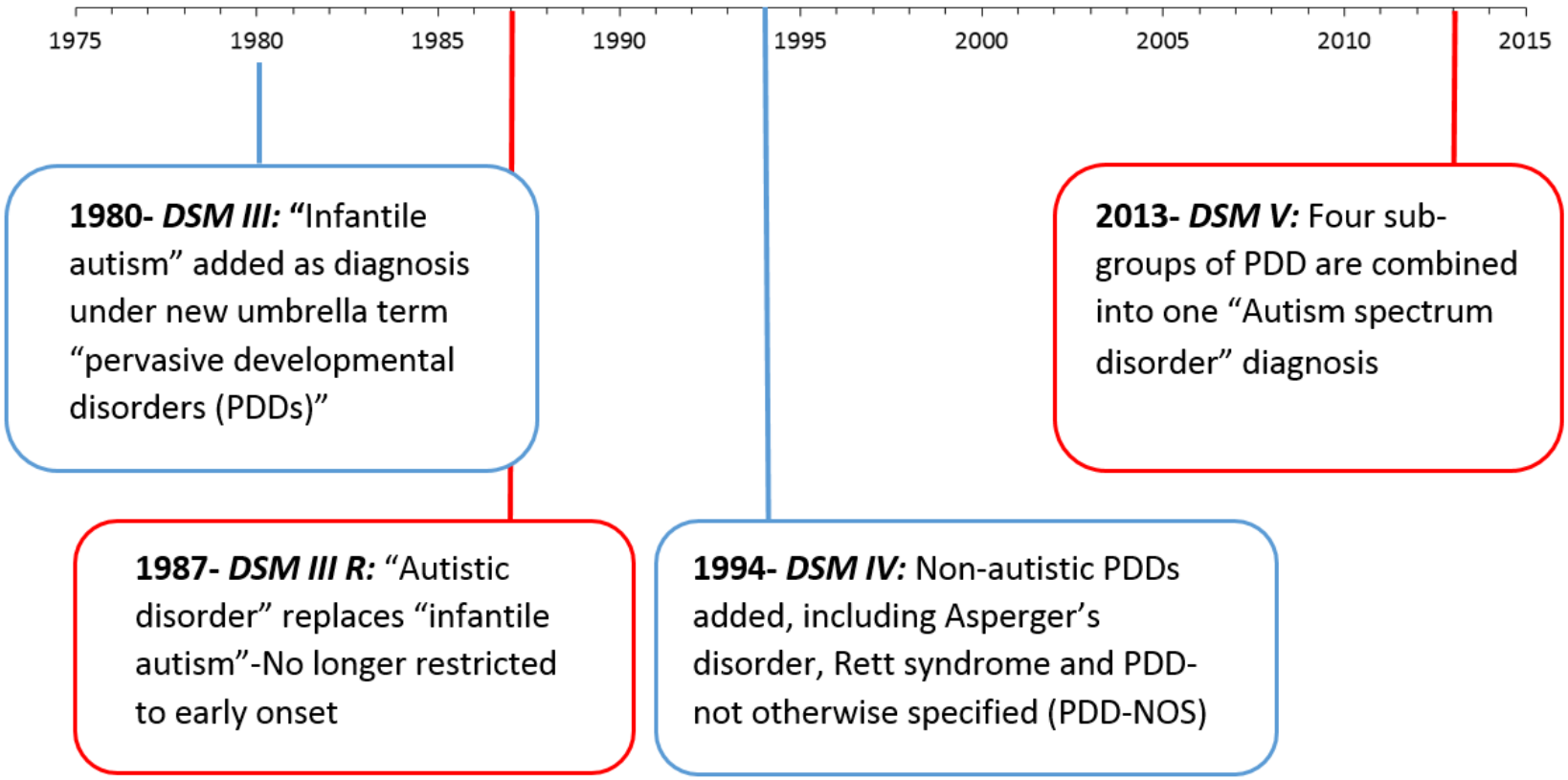


Figure 1-3. Timeline of the definition of attention-deficit/hyperactivity disorder (ADHD) according to the Diagnostic and Statistical Manual of Mental Disorders (DSM).

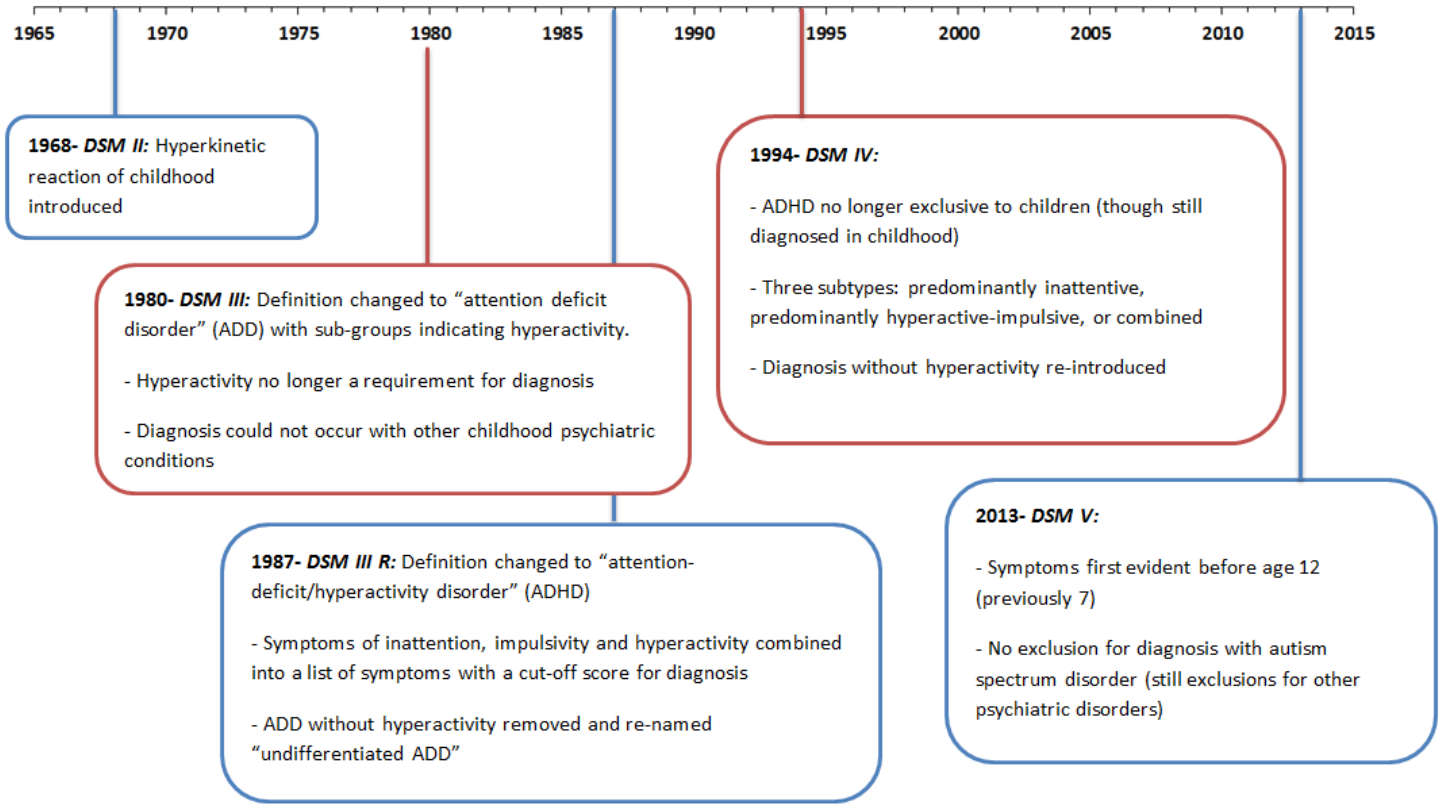


Figure 1-4. Directed acyclic graph representing the theorised association between birth by Caesarean section and psychological development.

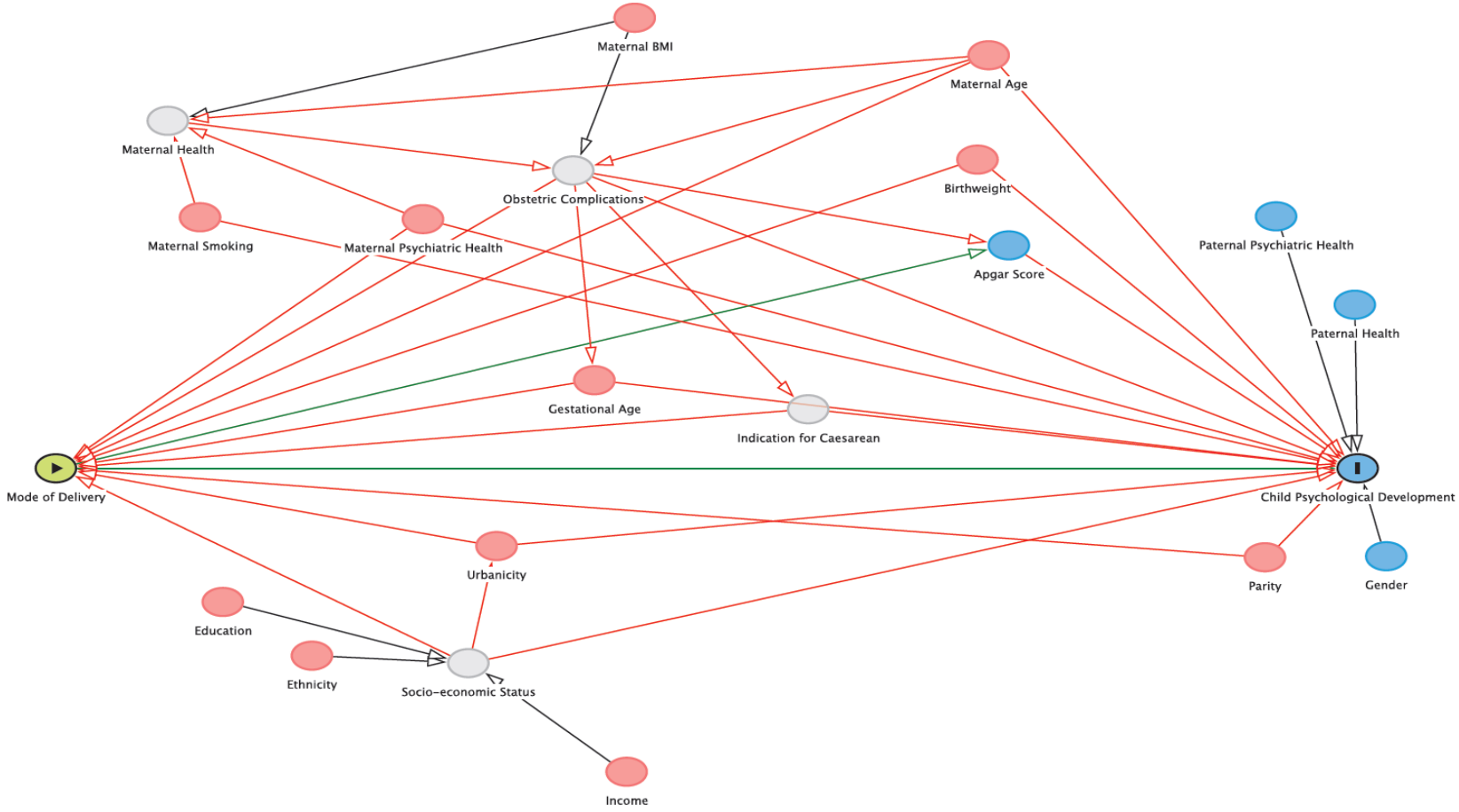
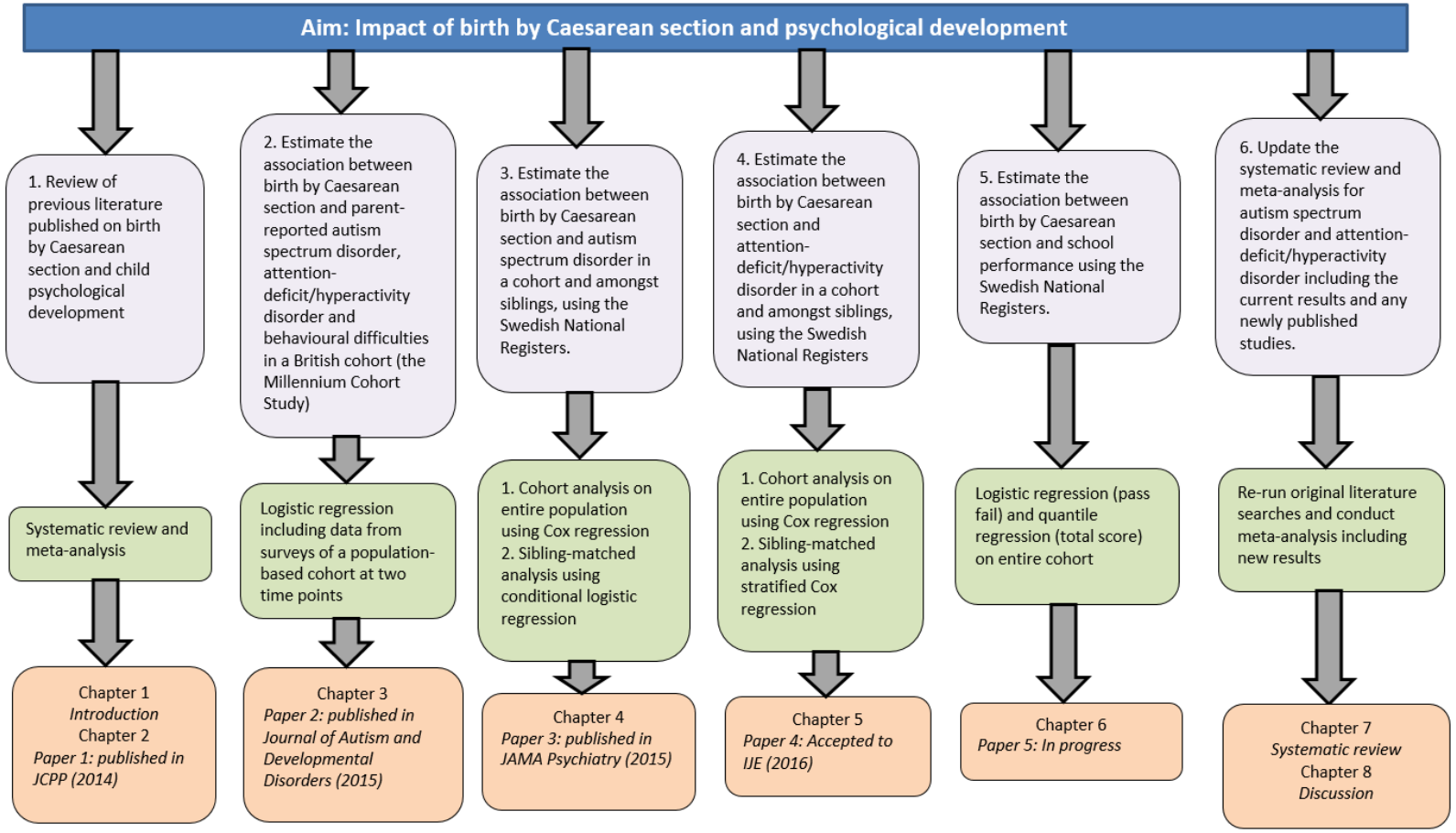


Figure 1-5. Thesis overview including overall aim as well as specific objectives and corresponding chapters.



CHAPTER 2. BIRTH BY CAESAREAN SECTION AND DEVELOPMENT OF AUTISM SPECTRUM DISORDER AND ATTENTION-DEFICIT/HYPERACTIVITY DISORDER: A SYSTEMATIC REVIEW AND META-ANALYSIS

Eileen A. Curran, MPH[1], Sinéad M. O'Neill, PhD[1,2], John F. Cryan, PhD[3], Louise C. Kenny, PhD, MRCOG[1], Timothy G. Dinan, PhD[3], Ali S. Khashan, PhD[1], Patricia M. Kearney, PhD[4]

[1]The Irish Centre for Fetal and Neonatal Translational Research (INFANT), University College Cork, Ireland.

[2]National Perinatal Epidemiology Centre, Cork University Maternity Hospital, Ireland

[3]APC Microbiome Institute, University College Cork, Ireland

[4]Department of Epidemiology and Public Health, University College Cork, Ireland

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(Appendix 42)

2.1 Abstract

Background: Given the growing prevalence of birth by Caesarean section (CS) worldwide, it is important to understand any long-term effects CS delivery may have on a child's development. We assessed the impact of mode of delivery on autism spectrum disorders (ASD) and attention-deficit/hyperactivity disorder (ADHD).

Methods: We conducted a systematic review of the literature in PubMed, Embase, CINAHL, PsycINFO and Web of Science until February 28th, 2014. No publication date, language, location, or age restrictions were employed.

Results: Thirteen studies reported an adjusted estimate for CS-ASD, producing a pooled odds ratio (OR) of 1.23 [95% CI: 1.07-1.40].Two studies reported an adjusted estimate for CS-ADHD, producing a pooled OR of 1.07 [95% CI: 0.86-1.33].

Conclusions: Delivery by CS is associated with a modest increased odds of ASD, and possibly ADHD, when compared to vaginal delivery. Although the effect may be due to residual confounding, the current and accelerating rate of CS implies that even a small increase in the odds of disorders, such as ASD or ADHD, may have a large impact on the society as a whole. This warrants further investigation.

2.2 Introduction

Prevalence of delivery by Caesarean section (CS) is estimated to be approximately 15% worldwide and over 20% in developed countries and growing annually[104]. The high prevalence of delivery by CS and the rate at which it is increasing are due to clinical, societal and economic realities[105]. Emergency CS are not scheduled ahead of time, and are performed as a result of complications, such as foetal distress[106]. In contrast, elective CS are scheduled beforehand, most commonly for obstetric or medical indications such as previous CS, malpresentation or pre-existing maternal medical complications[107]. In some countries, a significant minority of women undergo CS for maternal request[105].

Delivery by CS has previously been linked to offspring asthma, allergic rhinitis, diabetes, and gastrointestinal disease[50], and may be related to neurological development[108]. A 2014 study conducted using population-based registry data in Finland found a significant association between both emergency and elective CS and bipolar disorder in the offspring[109]. Such a relationship may be due to early-term birth[110] or altered microbiota[111], which are both associated with delivery by CS[52,111]. Animal models have shown delivery by CS to be associated with changes in dopamine response[112] and development of neurons in the prefrontal cortex and hippocampus[108]. Similarly, the microbiota has been found to be associated with social development[113] and behavioural development[55].

An estimated 0.62% of children worldwide are diagnosed with autism spectrum disorder (ASD)[114] and an estimated 5.3% are diagnosed with attention-deficit/hyperactivity disorder (ADHD)[115]. The prevalence of ASD has increased 20 fold since the 1980s [58] and the rate at which prevalence of pervasive developmental

disorders, including ASD, is increasing suggests factors other than improved detection and diagnosis are responsible[76].

ASD and ADHD are highly heritable, but there is evidence suggesting that environmental factors may be important for the development of either disorder[76,115]. It has been suggested that environmental and genetic factors may interact to increase a child's risk of developing ADHD[115]. In addition, genetic factors that have been associated with ASD are not unique, but are associated with other disorders. This implies environmental factors may also contribute to the development of ASD[76]. Recent papers have suggested that CS or induced labour may be associated with increased risk of ASD[76,116]. Considering the rising prevalence of delivery by CS, it is essential to understand any long-term effects delivery by CS might have on a child's development, including a possible impact on the incidence of either of these childhood disorders.

Potential Mechanisms

There are several hypotheses regarding the potential relationship between delivery by CS and psychological development. For example, children born by CS have different gut microbiota than those born vaginally, with the latter involving exposure to the mother's bacteria, whereas delivery by CS involves exposure to skin microbiota[57]. As altered microbiota has been shown to change behaviour in animal models, it is possible that this could be a factor in psychological development[55]. Notably, it has been hypothesized that children with ASD have different gut microbiota than the general population (though this could be as a result of increased antibiotic use and altered diet)[55,113], and a 2013 study found altered microbiota to be associated with ASD-like behaviour in mice[53]. In addition there is a growing

appreciation that the stress of being born vaginally primes the hypothalamic-pituitary adrenal axis and the immune system to better deal with future insults[50]. Another possible explanation for the impact of delivery by CS on psychological development is the fact that elective CS is normally scheduled for 37—39 weeks gestation, as opposed to the full 40 week gestation, in order to avoid spontaneous labour[52]. It is possible that the last few weeks are important for brain development, and therefore being born near rather than at term may lead to an increased risk of psychological problems. This is supported by a 2010 study, which found early-term birth, or birth between 37 and 39 weeks to be associated with a need for special education[110].

Though there has been a systematic review of delivery by CS and disorders related to immune function, to our knowledge there have been no previous reviews of CS and psychological development[50]. Similarly, several reviews have previously argued that pre- and perinatal factors may play a role in developing both ASD and ADHD[35,76,115,117,118]. However, to our knowledge, none of these prior reviews have focused on CS specifically. The objective of this systematic review and meta-analysis is to examine the impact of mode of delivery, specifically CS compared to vaginal delivery and the odds of subsequent ASD and ADHD using data from the published literature.

2.3 Methods

Primary exposure

The exposure of interest in this review was delivery by CS compared to vaginal birth. “Caesarean section” was considered to be any CS, including elective or emergency, as well as primary or repeat CS.

Primary outcomes

The outcomes of interest in this review were ASD and ADHD. "ASD" was defined as a diagnosis of autistic disorder, Asperger's disorder, or pervasive developmental disorder-not otherwise specified (PDD-NOS). "ADHD" was defined as a diagnosis of ADHD, attention deficit disorder (ADD), or hyperkinetic disorder.

Protocol and search strategy

The protocol for this systematic review and meta-analysis was registered on PROSPERO, the international prospective register of systematic reviews (CRD42014008778) and is available in full on the National Institute for Health Research (NIHR) website[119]. In accordance with the Preferred Reporting items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines[120], we searched the databases PubMed, Embase, CINAHL, PsycINFO and Web of Science until February 28th, 2014. We used search terms relating to ASD, ADHD, mode of delivery, perinatal factors and study design (Caesarean section OR mode of delivery AND autism spectrum disorders OR attention-deficit/hyperactivity disorder AND cohort study OR case-control study OR cross-sectional study), and limited all results to studies on humans . Searches were conducted according to the principles of Boolean logic and used medical subject headings (MeSH) where appropriate (**Appendix 2**). We further searched the reference lists of relevant papers for additional studies. No restrictions were placed on publication date, language, location of the study, or age of the participants. Study review and selection was completed independently by two reviewers (EAC and SMON), with any differences resolved by consensus and further discussion with a third reviewer where required (PMK). Eligibility criteria for inclusion in the meta-analysis included:

1. Data were from an original study using a cohort, case-control, or cross-sectional design.
2. An estimate (relative risk (RR), odds ratio (OR) or hazard ratio (HR) with 95% confidence intervals (CI)) of the impact of mode of delivery (CS compared to vaginal delivery) and the outcome of interest (ASD or ADHD), or sufficient data with which to compute an estimate were reported.
3. Adjustment for confounding (including at minimum parental age for ASD[76] and smoking during pregnancy for ADHD[35]) or the use of sibling controls to be included in the meta-analysis.

Data abstraction

Two reviewers (EAC and SMON) extracted data on study design, year and location of study, sample size, definition of exposure and outcome, type of CS (elective, emergency, if reported), and potential confounders adjusted for using a standardized data abstraction form. Studies written in a language other than English were translated using Google translate. As this was secondary data analysis, no ethical approval was needed.

Bias and quality assessment

A funnel plot of the overall OR and standard error (SE) was used to assess publication bias. Individual studies were reviewed for six types of bias common to observational studies (selection, exposure, outcome, analytic, attrition and confounding) and an overall level of bias for each paper was determined from a combination of these sub-groups (**Appendix 3**)[121]. Each type of bias was classified as “minimal,” “low,” “moderate” or “high,” and combined to form the overall level

of bias for the paper. This method of bias and quality appraisal is described in more detail elsewhere[122].

Primary analysis

We examined the overall CS-ASD and CS-ADHD association using random-effect models due to high heterogeneity and differences in study size, weighted by the inverse of the variance of the estimate. Where studies reported multiple estimates with separate ORs, for example for elective and emergency CSs, the estimates were pooled to provide using the same method one overall estimate. All analyses were performed using Review Manager software version 5.2[123].

Subgroup analyses

We planned *a priori* to look at estimates stratified by gender and by type of CS (elective vs. emergency), and conduct subgroup analyses based on study design (cohort vs. case-control), type of adjustment (sibling controls vs. other adjustment), and adjustment for operational or induced vaginal delivery. We decided *post-hoc* to also conduct subgroup analyses based on type of dataset (population-based, single institution, or interview/survey data). Finally, we estimated separate ORs for PDD, Asperger's, and autism, three disorders that make up ASD.

Population attributable risk

We calculated the population attributable risk (PAR) and PAR percent (PAR%) using the formula: PAR% = (P x (RR-1) / 1 + P x (RR-1)) x 100[124]. The PAR corresponds to the proportion of the population with the outcome (ASD) that can be attributed to the exposure of interest (delivery by CS).

Heterogeneity

The I^2 statistic was used to assess heterogeneity between studies, as recommended in the Cochrane Handbook for Systematic Reviews[125].

2.4 Results

Search results

Our original search produced 5,490 unique results after removal of duplicates (**Figure 2-1**). Of these, 123 full text articles were reviewed after screening of titles and abstracts. Several studies were excluded because they did not meet the criteria specified for the study, most frequently lack of information on the exposure or outcomes of interest, as well as including CS as part of a scale (for example "number of obstetric risk factors") or lack of original data. After reviewing reference lists, one additional study was found[126]. A total of 25 papers were included in the systematic review (21 for ASD and 4 for ADHD) and 15 were included in the meta-analyses (13 for ASD and 2 for ADHD).

Characteristics of studies included in the systematic review

A summary of the study characteristics can be found in **Appendix 4**. The unadjusted analysis included data on 1,384,278 children, 14,214 of whom had ASD, and the adjusted analysis included data on 1,192,806 children, 12,809 of whom had ASD. Four studies reported separate adjusted results by type of CS (i.e. for elective CS[30,32,127,128] alone, and an additional study reported adjusted results for emergency CS alone[129]). There were six cohort studies[30,31,130-132], and only three of these were included in the adjusted analysis[30,31,116].

Results of the meta-analysis

ASD

Primary Analysis

There were 21 studies that included an unadjusted estimate for CS-ASD (pooled OR = 1.36; [95% CI: 1.23-1.51]) (**Table 2-1**)[30-32,116,126-142]. Thirteen of these studies included an adjusted estimate[30-32,116,126-129,133-135,137,140]. The adjusted analysis included data on approximately 1.2 million participants, and 12,897 diagnoses of ASD. Using a random-effects model (due to heterogeneity between studies in the fixed-effect model I^2 = 66%) a pooled OR of 1.23 [95%CI: 1.07-1.40] was found (**Figure 2-2**). The unadjusted estimate among these 13 studies was 1.44 [95% CI: 1.27-1.63] (Data not shown).

Sub-group analysis

Study design

There were seven case-control studies which reported an adjusted estimate for the relationship between CS and ASD, all of which used matched controls[32,127,128,133-135,137]. The pooled OR for these studies was 1.23 [95% CI: 1.11-1.37] (**Table 2-1**). In contrast, the three cohort studies that reported an adjusted estimate provided a pooled OR of 1.07 [95% CI: 0.92-1.24][30,31,116].

The pooled estimate for the three studies which reported an adjusted estimate using interview or survey data was 2.73 [95% CI: 1.51-4.93][126,129,135]. In contrast, the seven studies which reported an adjusted estimate using a population-based dataset, including cohort and nested case-control studies, produced a pooled estimate of 1.14

[95%CI:1.01, 1.29][30-32,116,133,134]. No studies reported an adjusted estimate using data from a single institution.

Type of CS

Only one study reported primary and repeat CS separately[134] so a meta-analysis was not performed. It is worth noting that in this study, an association was detected for primary CS (OR = 1.67; [95% CI: 1.03-2.73]) but not for repeat CS (OR = 0.69; [95% CI: 0.03-1.58]), though that could be due to lower numbers and thus less power for repeat CS. The pooled OR from the four studies that reported an adjusted estimate for elective CS was not statistically significant (OR = 1.14; [95% CI: 1.00-1.31]). The five studies that reported an adjusted estimate for emergency CS however, resulted in a significant OR of 1.26 [95% CI: 1.05-1.51] (**Table 2-1**).

Confounder adjustment

Among the five studies that used sibling controls[126,128,129,140] the pooled OR was higher than the overall estimate, (OR = 1.35; [95% CI: 1.14-1.59]) whereas the eight studies that adjusted for confounding without the use of sibling controls[30-32,116,127,133-135] had a slightly lower estimate (OR =1.19; [95% CI: 1.04-1.37]) (**Table 2-1**).

Gender

Two studies reported unadjusted estimates stratified by gender[126,127]. The pooled OR for males was 1.18 [95% CI: 0.92-1.53] and for females was 1.89 [95% CI: 0.41, 8.68] (Data not shown). However, neither study reported an adjusted estimate by gender.

Definition of outcome

Two studies reported unadjusted estimates on PDD-NOS[127,128], and three reported unadjusted estimates on Asperger's[127,128,132]. However, only one study reported adjusted estimates[127], and no meta-analysis was performed on these disorders as a result. Five studies reported adjusted estimates specifically for autism[32,116,127,133,135], resulting in a pooled OR of 1.15 [95%CI: 0.93-1.42] (**Table 2-1**).

Mode of delivery confounders

The four studies that adjusted for assisted or operational vaginal delivery[30,116,127,134] produced a pooled OR of 1.14 [95%CI: 0.97, 1.33] (**Table 2-1**). The three studies that adjusted for induction of labour in their model[18,36,46] had a pooled OR of 1.19 [95%CI: 1.04-1.36] (**Table 2-1**). Among the eight studies which did not adjust for operational vaginal delivery or induction of labour the pooled OR was 1.55 [95% CI: 1.16-2.08] (Data not shown)[32,126,128,129,133,135,137,140].

Population attributable risk

One study did not report raw data with regards to delivery by CS[30], and so could not be included in the PAR% calculation. The remaining 12 studies included reported on 809,626 participants, 199,490 of whom were born by CS (24.6%), and 11,718 of whom had ASD (1.45%), resulting in a pooled OR of 1.23 [95% CI: 1.07-1.43] (Data not shown). This produced a PAR% of 5.36%, meaning 5.36% of cases of ASD may be attributable to delivery by CS assuming the observed association is causal.

ADHD

Four studies in total were found on CS and ADHD[38,143-145]. Three studies reported an unadjusted estimate[38,143,145], and an overall pooled OR of 1.12 [95% CI: 1.06-1.18] was generated in the random-effects model (Data not shown). Only two studies reported an adjusted estimate[38,144], producing a pooled estimate of 1.07 [95% CI: 0.86-1.33] (**Appendix 5**). Notably, one study[38] was much larger and contributed 80% of the pooled estimate. Both of these studies were matched case-control studies, and one of them[38] used data from a population-based dataset.

Bias and heterogeneity

Visual inspection of the funnel plot did not indicate publication bias (**Appendix 6**). There was moderate heterogeneity for ASD (I^2 = 66%) and low heterogeneity for ADHD (I^2=0%). Heterogeneity was possibly due to differences in location, study design, and variations in confounder adjustments and definitions of the outcomes used. The majority of studies were found to have "low" or "moderate" bias (**Appendix 3**).

2.5 Discussion

Our results indicate that delivery by CS is associated with a 23% increased odds of ASD in the pooled estimate where confounding effects of at minimum parental age were taken into account. In addition CS delivery may be attributable to over 5% of ASD cases. Notably, in the unadjusted analysis, the estimated increased odds is nearly doubled, highlighting the importance of controlling for confounders when examining the apparent relationship between CS and ASD. In addition, studies that adjusted for operational delivery or induction of labour produced a lower pooled

estimate, and so should be included in future analyses. Similarly, the estimate was lower for cohort studies, and studies that used population-based datasets which are generally considered to be less subject to bias when compared to case-control designs. This is consistent with previous results, which also indicate that it is difficult to estimate the true effect of delivery by CS on ASD without sufficient adjustment[76]. Our review indicates this may also be true for ADHD, but as there were not many studies on ADHD that included CS and no cohort studies which provided an adjusted estimate, further research is required to substantiate such a claim.

Although an association between delivery by CS and ASD was found in this meta-analysis (with an increase in odds by as much as 40%), it is unclear what may be driving this association and whether it is causal. As previously stated, it is possible that delivery by CS may be related to psychological development, for example though altered microbiota or early term birth. However, the association may also be due to unmeasured bias or residual confounding. For example, it is possible that the underlying indications for CS are leading to the increased odds of ASD, and not the CS per se, thus creating a spurious association between delivery by CS and ASD. This is known as confounding by indication[86]. However, among the studies that reported adjusted estimates for elective and emergency CS separately, the estimate for elective CS was lower and non-significant, which could indicate the possibility of confounding by indication. However, as only a few studies in this review have made this distinction, there was limited power to examine the association in the two groups separately. Moreover the sub-groups were not significantly different.

Sibling design studies have been used in studies of pre- or perinatal risk factors and development in order to control for genetic factors and family environment[90]. There are few studies that employed this method, the majority of which were published over 20 years ago. Notably, these studies potentially, based on obstetric practice at the time, could have included more emergency CS than elective CS and consequently may estimate different risks. As emergency CS is performed to save the life of the mother or infant, it is possible that any impact on child development that is detected may be related to the underlying cause of the CS. Given the difference in the ORs among adjusted and unadjusted estimates, future studies should account for such possible confounding. Sibling design studies may be an efficient way to adequately control for confounding in future studies of delivery by CS and ASD and ADHD.

Given the high prevalence of CS worldwide[104], it is important to understand any long-term effects delivery by CS may have on a child's neurological development. The studies included in this review were primarily published within the last five years, indicating a rising interest in this subject. However, there are still gaps in our knowledge. For example, the estimate for autism alone was lower than for ASD as a whole, implying that it is possible that delivery by CS may have more of an impact on the less serious disorders. As there were no adjusted estimates for PDD or Asperger's Syndrome alone however, it is difficult to make that claim. Also, few studies reported separate estimates for emergency and elective CS, and only one reported separate estimates for primary and repeat CS. In addition, no studies reported adjusted estimates by gender, which is an important distinction to make as boys and girls are known to have differential risk of ASD[146]. Though there is an association between delivery by CS and ASD, future research into CS and ASD will

need to assess whether this association is causal or whether it is due to residual or unmeasured confounding. Also, only two studies reported an adjusted estimate for CS and ADHD, and did not provide a clear picture of the possible association and should be investigated further in future studies.

Strengths and limitations

The strengths of this study include a comprehensive search conducted in five relevant databases, as well as several analyses of adjusted estimates, grouped by study design and characteristics. Unfortunately, few studies have made the distinction between elective and emergency CS, or primary and repeat CS, and no adjusted studies made the distinction between male and female risk. Ideally study designs designed studies, employing adjustments for key potential confounders and effect modifiers including type of CS, sex of the child, maternal obesity, socio-economic status as well as maternal age would provide a deeper understanding of the true relationship between CS and ASD and ADHD.

2.6 Conclusion

Our analyses indicate that delivery by CS is associated with a slightly increased risk odds of ASD diagnosis. This may also be true for ADHD, though this needs to be investigated further due to lack of adjusted estimates. The current and accelerating rate of CS implies that even a small increase in odds of disorders, such as ASD or ADHD, may have a large impact on society as a whole. The potential cause for such disorders urgently requires further investigation. As these are disorders with a strong genetic basis[76,115], clearly understanding the interrelationship between environmental factors such as mode of delivery and genetic susceptibility requires further attention. In order to draw more firm conclusions, future research

should distinguish between emergency and elective CS and include adequate adjustment for potential confounders and effect modifiers, perhaps through the use of sibling controls.

Table 2-1. Sub-group meta-analyses for birth by Caesarean section and autism spectrum disorder.

	Number of Studies	N	Outcomes	OR[a]	95% CI		I^2
Overall Unadjusted[b]	21	1,384,705	14,302	1.36	(1.23	,1.51)	65%
Overall Adjusted[c]	13	1,191,920	12,897	1.23	(1.07	,1.40)	66%
Included Sibling Controls	5	1,441	668	1.35	(1.14	,1.59)	63%
Adjusted without Sibling Controls	8	1,190,310	12,141	1.19	(1.04	,1.37)	74%
Elective Adjusted	4	405,343	5,456	1.14	(1.00	,1.31)	0%
Emergency Adjusted	5	406,185	5,599	1.26	(1.05	,1.51)	38%
Cohort	3	1,147,941	7,226	1.07	(0.92	,1.24)	74%
Case-Control	7	43,345	5,395	1.23	(1.11	,1.37)	0%
Matched Controls	7	43,345	5,395	1.23	(1.11	,1.37)	0%
Population-based Dataset	7	1,190,129	12,055	1.14	(1.01	,1.29)	66%
Interview or survey data	3	545	150	2.73	(1.51	,4.93)	34%
Autism Alone	5	559,253	6,839	1.15	(0.93	,1.42)	62%
Included induction	3	784,780	9,867	1.19	(1.04	,1.36)	71%
Included assisted/operational VD	4	963,740	10,056	1.14	(0.97	,1.33)	77%

[a]OR: Odds ratio
[b]Crude estimate of association
[c]Estimate of association among studies that either used sibling controls or adjusted for at minimum parental age

Figure 2-1. Flow diagram of studies selected for systematic review and meta-analysis.

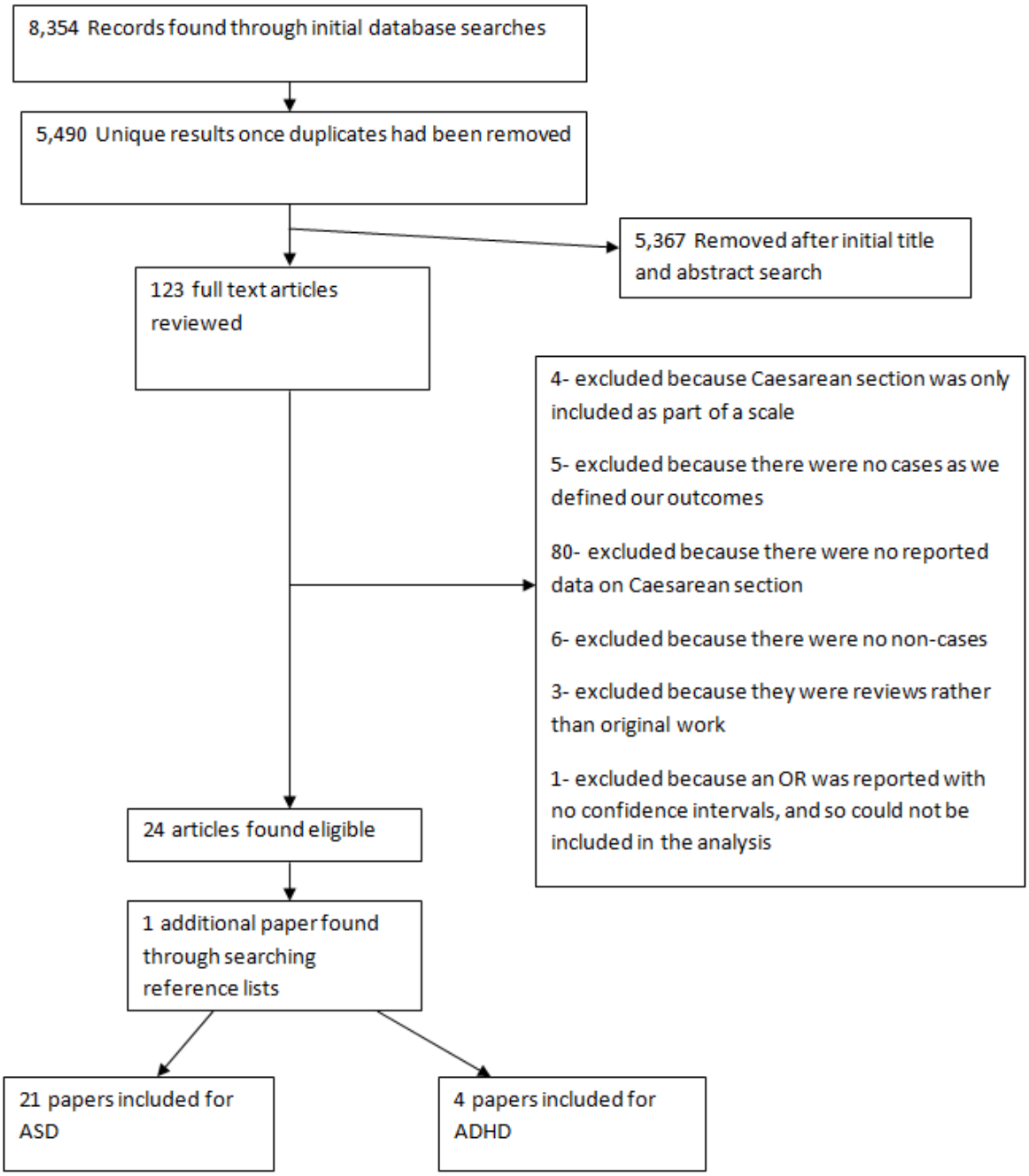
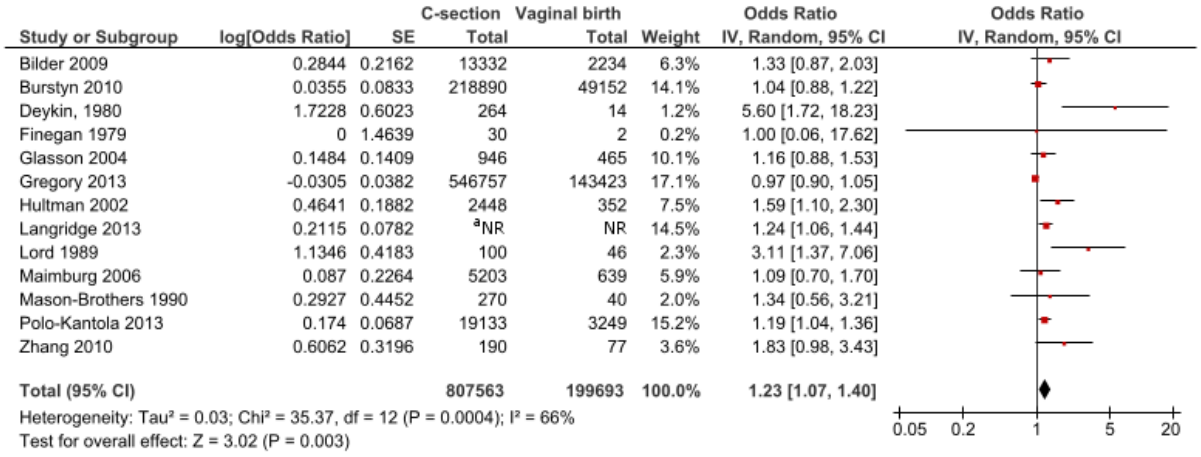


Figure 2-2. Forest plot of adjusted estimates of the association between Caesarean section and autistic spectrum disorder.



[a]NR: Not recorded

CHAPTER 3. OBSTETRICAL MODE OF DELIVERY AND CHILDHOOD BEHAVIOUR AND PSYCHOLOGICAL DEVELOPMENT IN A BRITISH COHORT

Eileen A. Curran[1], John F. Cryan[2], Louise C. Kenny[1], Timothy G. Dinan[2], Patricia M. Kearney[3], Ali S. Khashan[1,3]

[1]The Irish Centre for Fetal and Neonatal Translational Research (INFANT), University College Cork, Ireland.

[2]APC Microbiome Institute, University College Cork, Ireland

[3]Department of Epidemiology and Public Health, University College Cork, Ireland

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(Appendix 42)

3.1 Abstract

The association between mode of delivery (specifically birth by Caesarean section (CS) and induction of labour (IOL)) psychological development at age 7 was assessed (including autism spectrum disorders (ASD), attention-deficit/hyperactivity disorder (ADHD) and behavioural difficulties). The Millennium cohort study, a nationally representative UK cohort of children (including 13,141 children), was used. There was no association between planned CS and ASD (adjusted OR = 0.58; [95% CI: 0.19-1.79]) or ADHD (adjusted OR = 0.54; [95% CI: 0.18-1.64]) analyses. Induced vaginal delivery was significantly associated with behavioural difficulties in unadjusted (OR = 1.26; [95% CI: 1.03-1.54]), but not adjusted analysis (OR = 1.15; [95% CI: 0.82-1.60]). There was no association between mode of delivery and ASD or ADHD in this cohort. Further research is needed to understand the relationship between mode of delivery and IOL and psychological development.

3.2 Background

Worldwide, the Caesarean section (CS) rate is estimated to be 15% but there is considerable variation between countries[104]. In the United Kingdom (UK) for example, the rate of birth by CS was 23% in 2007[11], almost twice the estimated 12.5% reported in 1990[147]. Increasing rate of birth by CS is even higher elsewhere, such as China and Brazil[148]. Similarly, induction of labour (IOL) is becoming more common[149] and in 2011, it was estimated that 25% of all births in developed countries were induced[150]. As a result, the possibility of long-term effects of the mode of delivery on child development is of increasing interest[36].

Birth by CS has been linked to psychological development through adulthood in animal models[108,112]. Some studies have linked birth by CS to an increased risk of autism spectrum disorders (ASD)[133], attention-deficit/hyperactivity disorder (ADHD)[38] and behavioural difficulties[41] but results have been inconsistent[31,144]. A recent meta-analysis reported that CS was associated with a 23% increased risk of ASD compared to vaginal delivery (VD)[151], however, the included studies were limited. For example, few studies distinguished between elective and emergency CS. Though, a more recent population-based sibling control study reported that any association may be caused by familial confounding rather than be a causal effect[152]. A review of the association between CS and ADHD found the evidence was very limited and inconclusive[151]. There are several hypotheses regarding how CS may affect behavioural development, including "early term" birth [48,52], exposure to altered microbiota[50,53,55,56,113], or possibly through changes in maternal-child attachment [153-155]. Notably, while ASD and ADHD are both highly inheritable disorders, environmental factors have also been found to be important[36,115].

IOL might also be associated with psychological development, including ASD[116]. One hypothesis is that this association may be related to exposure to induction agents such as oxytocin or prostaglandins[156]. Oxytocin in particular is important for normal brain development and social behaviour[157,158]. This association may also be explained by underlying indications for IOL, such as prolonged labour or pre-labour rupture of membranes, rather than be a result of the induction itself[159].

The objective of this study is to assess the impact of mode of delivery and IOL on psychological and behavioural development through measures of ASD, ADHD and parent-rated behavioural difficulty at age 7, using data from a large, representative UK cohort.

3.3 Methods

Study cohort

The Millennium Cohort Study (MCS) is a nationally representative sample of 18,827 children born in the UK between 2000 and 2002[160]. Sampling was stratified by region, electoral ward, ethnic composition of the ward, and social disadvantage. There have been five surveys of the MCS participants to date; conducted at 9 months and 3, 5, 7, and 11 years of age. More detailed explanation of the MCS can be found in **Appendix 7**. Data on mode of delivery and potential confounders for the present study were obtained from the first survey (conducted at 9 months)[160]. The majority of main respondents (99.7%) were the infant's biological mother, and majority of partner respondents (85.7%) were the infant's biological father.

The London Multicentre Research Ethics Committee granted ethical approval for these surveys, and as the current investigation is a secondary data analysis no

further approval was needed. All survey materials are available online (www.cls.ioe.ac.uk/mcs).

Definition of mode of delivery

Mode of delivery was categorized into "spontaneous VD," "assisted VD," "induced VD," "emergency CS," "planned CS" and "induced CS." The variable was defined this way to assess the effect of any intervention on labour as compared to no intervention (i.e. spontaneous, non-assisted VD). Respondents who reported a planned or emergency CS were recorded as such. Those that reported "assisted with forceps," "assisted vacuum extraction," "assisted breach" or "other assisted delivery" were considered "assisted VD". Those that reported "normal delivery" or "water birth," but responded "yes" to the question "was the labour induced or attempted to be induced" were considered "induced VD" or "induced CS" depending on the final mode of delivery. Attempted and successful inductions were grouped together, as the women were exposed to the same intervention. Women who had a vaginal delivery and did not report induction of labour were considered "spontaneous VD." Answers that were recorded as "other" or "irrelevant" were considered missing. Consistent with previous studies, only singleton births were included[161].

Outcome variables

In surveys 3 and 4, i.e. at age 5 and 7 years respectively, respondents were asked, in two separate questions, if a doctor or health professional had ever told them that their child had ADHD or ASD. We considered children of parents that responded "yes" to either question in survey 4 as cases for that particular disorder. A chi-squared test was performed on those that responded "yes" in both surveys 3 (age 5 years) and 4 (age 7 years) as a sub-group of "confirmed cases" to see if it was

meaningfully different from the population of all cases reported in survey 4 with regards to mode of delivery.

Behavioural difficulty was measured using the Strengths and Difficulties Questionnaire (SDQ) that was completed by parents in survey 4. The SDQ measures behavioural difficulties in five areas: conduct problems, hyperactivity, emotional symptoms, peer problems and pro-social behaviours. All scores except pro-social can be combined to create a "total difficulties" score. Consistent with the recommended cut-off for parent-scored SDQ in the UK, a total difficulties score of 17 was used to indicate an "abnormal" score[57]. This has previously been found to correspond to the top 10% of scorers, and is associated with an increased overall risk of being diagnosed with a psychiatric disorder[162], including not only ASD/ADHD, but also anxiety disorders, conduct-oppositional disorders, and obsessive-compulsive disorder, among others[163].

Potential confounders

Included variables were determined *a priori* based on previous literature: small for gestational age (SGA)[76], gestational age at birth[76], maternal high blood pressure/pre-eclampsia/eclampsia[76], mother smoking more than 1 cigarette a day while pregnant[118], being the first born child[76], bleeding or threatened miscarriage during pregnancy[76], poverty, white ethnicity, maternal age[76], maternal education, maternal depression[117] and maternal body mass index (BMI)[117].

Additional variables that were assessed included: infant age when he/she came home from the hospital[164], infant gender[131], urbanicity[165], single parent household at time of first survey[131], paternal age[142], paternal education, whether

pregnancy was unplanned[164], and maternal irritable bowel syndrome (IBS)[55,166]. (Variable definitions in **Appendix 8**).

Statistical analysis

Logistic regression was used to evaluate all associations. Survey commands were used and estimates were weighted to account for complex survey design[167]. First survey weights were used as it has been previously shown that the original sample weights are not meaningfully different from the combined sample and loss to follow-up weights at age 7[167].

Univariate analyses were performed to measure the association of all variables with ASD, ADHD, and abnormal SDQ scores, variables were adjusted for in each of three categories (**Table 3-1**), with each group of variables modelled separately (Models 1-3). Two additional models were then used, which adjusted for pregnancy and delivery and demographic/socio-economic factors together (Model 4), and all three categories (Model 5). All variables identified *a priori* were included, as well as other predictors that were significant at 95% confidence in Model 1, 2, or 3. To account for gender differences in behavioural difficulty[168], interaction between mode of delivery and gender was tested. SDQ scores for each of the five sub-groups measured were also modelled.

Sensitivity analyses were performed excluding babies who were SGA, pre-term, female, or in a breech presentation, as well as babies with mothers over the age of 40 and mothers who reported gestational hypertension to examine what impact these factors might have had on results. Though analyses were originally performed comparing children with ASD to the entire cohort of children without ASD, a sensitivity analysis was conducted excluding children with ADHD and behavioural

difficulties from the control group. Similar sensitivity analyses were performed for ADHD and behavioural difficulties. As maternal depression, maternal IBS and gestational age may have played a mediating role in this association, these variables were not only included in the main analysis, but also tested separately to examine their effect on the association between mode of delivery and the outcome measures. Likewise, breastfeeding was included in a separate analysis to assess a potential mediating effect.

Maternal attachment

The impact of maternal attachment[153,154] on the relationship between mode of delivery and ASD, ADHD and SDQ scores was assessed by comparing fully adjusted estimates (Model 5) to estimates excluding children with low maternal attachment, and high maternal attachment separately. Maternal attachment was measured using a subset of six questions from the Condon Maternal Attachment Questionnaire[169]. The Condon Maternal Attachment Questionnaire has been previously shown to have acceptably high construct validity and test-retest reliability[169], and has been used to measure postpartum attachment in a variety of populations[170]. Consistent with previous studies, the lowest 25% of scores were considered "low maternal attachment"[171]. The highest 25% of scores was also considered "high maternal attachment."

Post-hoc analyses

Models 1-5 were repeated using mode of delivery and IOL as two separate exposures to determine the impact of induction alone, irrespective of the final mode of delivery, and vice versa. For this analysis, mode of delivery was divided into four

categories: emergency CS, planned CS, assisted VD and unassisted VD. IOL was divided into two categories: induced or non-induced.

The validity of ADHD diagnoses can be questionable, especially in younger children, in part because some health professionals diagnose ADHD solely in terms of behavioural difficulties[172]. Therefore, in the *post-hoc* analysis children with ADHD and/or children with abnormal SDQ scores were grouped into one "behavioural difficulties" category.

3.4 Results

Descriptive results

There were 13,141 singletons that participated in both the 2001 and the 2008 survey with data on mode of delivery (**Appendix 9**). Of these, 13,049 answered the question on ASD (99.3%), 13,038 answered the question on ADHD (99.2%) and 12,683 had complete data for SDQ scores (96.5%). Planned CS was reported by 8-9% of participants, regardless of ASD, ADHD, or SDQ status (**Table 3-2**). Induced VD was reported by 20% of participants. There were 209 (1.5%) reported cases of ASD and 173 (1.3%) reported cases of ADHD. Abnormal SDQ scores were present in 951 (8%) children, 338 of whom were girls and 613 of which were boys. There were 80 "confirmed cases" of ASD (i.e. also reported at age 5), and 47 "confirmed cases" of ADHD. The "confirmed cases" were not different from survey 4 cases with regards to mode of delivery for either ASD (p=0.97) or ADHD (p=0.64).

Logistic regression results

No significant association was found between mode of delivery and ASD or ADHD in unadjusted or adjusted analyses (**Table 3-3**). In unadjusted analysis, the

odds ratio (OR) for planned CS was close to 1 for both ASD (OR = 1.08; [95% CI: 0.54-2.17]) and ADHD (OR = 0.79; [95% CI: 0.37-1.69]). The estimates remained non-significant for both ASD (OR = 0.58; [95% CI: 0.19-1.79]) and ADHD (OR = 0.54; [95% CI: 0.18-1.64]) in the adjusted models. There was a non-significant association between emergency CS and ADHD in both unadjusted (OR = 1.70; [95% CI: 0.93-3.11]) and adjusted (OR = 1.28; [95% CI: 0.61-2.66]) models.

In the unadjusted analysis, induced VD was significantly associated with SDQ scores (OR = 1.26; [95% CI: 1.03-1.54]) (Table 4). This association persisted in Model 1(OR = 1.26; [95% CI: 1.02-1.55]), but was no longer significant in the fully adjusted model (OR = 1.15; [95% CI: 0.82-1.60]). As the statistical interaction between mode of delivery and gender was not significant (p = 0.19), results were not stratified by gender. Adjustment for maternal depression, maternal IBS, gestational age, and breastfeeding in separate models did not change the results materially suggesting that these variables did not have confounding or mediating effect (**Appendix 10**).

When SDQ scores were broken down by sub-group, IOL was associated with peer problems in the unadjusted (OR = 1.29; [95% CI: 1.07-1.55]) and adjusted models (OR = 1.36; [95% CI: 1.04-1.78]) (**Appendix 11**). Assisted VD, emergency CS, elective CS were not associated with any SDQ sub-groups.

Excluding children with high or low maternal attachment did not change the association between mode of delivery and ASD, ADHD and abnormal SDQ scores (**Table 3-5**). Similarly, sensitivity analyses by SGA, pre-term birth, infant gender, breech presentation, mother's age and maternal hypertension in pregnancy had no impact on the results (**Appendix 12**). Excluding children with any outcome

(ASD/ADHD/behavioural difficulties) from each analysis also had no effect (**Appendix 13**).

Post-hoc analyses

Results were similar when we separated IOL from the overall mode of delivery, and combined ADHD with abnormal SDQ scores. Planned CS was not associated with ASD (OR = 1.05; [95% CI: 0.56-1.97]) or combined behavioural difficulties/ADHD (OR = 0.85; [95% CI: 0.63-1.13]) (**Appendix 14**). Similarly, IOL was not associated with ASD (OR = 1.10; [95% CI: 0.76-1.57]) or behavioural difficulties/ADHD (OR = 1.18; [95% CI: 1.00-1.38]). There remained no significant associations in the adjusted model.

3.5 Discussion

In this study, the impact of mode of delivery on psychological and behavioural development was investigated. No association was found between CS and ASD, ADHD or behaviour. Recent results indicate that birth by CS is associated with a 23% increased risk of ASD[151]. However, further analysis shows an attenuation of this association when siblings are used as controls, implying it can likely be explained by residual familial confounding[152]. Results from the current study are also in line with a nested case-control study published last year by Polo-Kantola et al, which did not find an association with either CS or IOL and autism or Asperger syndrome[29]. Notably, the same study found an association between both CS and IOL with pervasive developmental disorders (PDD)[29] (atypical autism[173]). This may have been partly due to the over-diagnosis of PDD which occurred prior to the revision of the *Diagnostic and Statistical Manual of Mental Disorders,* fourth edition (DSM-IV) in May 2000[174]. As the current study only had data on ASD as a whole,

and not PDD specifically, it was not feasible to investigate this in our cohort. Our ADHD results are in line with a recent population-based study[38], which found no association between CS or IOL and ADHD in adjusted analysis.

No association was found between IOL and ASD or ADHD.A recent study by Gregory et al indicated that IOL may be associated with ASD[116], which appears to be at odds with current results. This investigation differs from that of Gregory et al.[116] in that we excluded multiple births (though they report a sensitivity analysis excluding non-singleton births which did not change the results), and included IOL and mode of delivery in the same variable, thus investigating possible interaction between the two. Also, as previously noted[174], the results reported by Gregory et al may have also been influenced by the over-diagnosis of PDD prior to the year 2000, whereas our cohort was born in the years 2000-2001. In contrast to ASD, previous studies have reported no association between IOL and ADHD or developmental delay[38,175].

No association was found between CS and abnormal SDQ scores in the current investigation. Previous studies have found maternally requested CS to be associated with lower externalizing behaviour problems (e.g. aggression)[40], but higher internalizing behaviours problems (e.g. anxiety/depression)[41]. These studies used the Child Behaviour Checklist, rather than the SDQ, which may account for the difference. Though results from the Child Behaviour Checklist and the SDQ are highly correlated, the SDQ is much shorter and has better specificity, whereas the Child Behaviour Checklist has a higher sensitivity[176,177]. In unadjusted analyses, there was a small association between IOL and abnormal SDQ scores. However, this association did not remain significant in the adjusted model, and thus was likely due to confounding. Some evidence was found that IOL or assisted VD increases the risk

of peer problems at age 7 years. This could indicate that IOL may be associated with specific outcomes, such as peer problems, rather than composite outcomes, such as ASD, ADHD, or overall behavioural difficulty. Consequently, future research may focus on cognitive and developmental assessments, as well as behavioural problems in order to achieve a more complete understanding of the impact of mode of delivery and IOL on development.

Previous results would indicate that CS is associated with ASD[151]. However, the majority of these studies were case-control studies which are generally considered to be more subject to bias[32,128,133-135,137]. There have been four cohort studies that have reported an adjusted estimate of the association between CS and ASD[30,31,116]. Three of these cohort studies did not report a significant association between CS and ASD, however they were subject to some limitations. The studies by Langridge et al.[30] and Gregory et al.[116] included children that may have been diagnosed with ASD prior to the changes in diagnostic criteria in 2000. Notably, though Gregory et al. was unable to limit results by birth year due to low numbers, they did later report stratified results that showed no significant association in those born after 1996 and thus posited results may have not been influenced by this change in diagnostic criteria[178]. Burstyn et al.[31] included participants born in the years 1998-2004 and so was not subject to this over-diagnosis of PDD. However they did not distinguish between planned and emergency CS, making it difficult to determine the true effect of CS. Curran et al. reported an association between CS and ASD in the cohort as a whole, but not amongst siblings, indicating that this association may have been related to unmeasured confounding[152]. There have only been two previous studies that reported an adjusted estimate of the association between CS and ADHD, neither of which found a significant association[38,144]. However, both studies were

case-control (though one was nested and used a population-based dataset[38]). The current investigation addressed these limitations by including children born in the years 2000-2001, as well as a detailed definition of mode of delivery, and confirmed previous findings of no association between CS and ASD or ADHD.

Strengths and limitations

The present study has several strengths. First, the study was based on a large, contemporary cohort of children representing all births in the UK in 2000. As randomized trials on mode of delivery would be unethical, epidemiological research using large population-based studies provides invaluable evidence on the effect of mode of delivery on child mental health morbidity. Second, the current study classified CS into planned or emergency, which was a limitation in several previous studies. Third, this cohort contains information on a wide variety of variables, including detailed information on economic and socio-demographic variables, and maternal attachment, which have been missing from previous investigations on this topic. Data from the MCS have been used for several studies on various topics including mode of delivery, ASD/ADHD, and SDQ scores[31,32,43]. The richness of the dataset allowed for inclusion of previously validated measures, including the SDQ and Condon attachment score, leading to a more thorough examination of the impact of mode of delivery than may have otherwise been possible. For example, as a parent-rated abnormal SDQ score is associated with over 15 times higher odds of having a psychiatric disorder[162], use of the SDQ allowed for examination not only of behavioural difficulties in general, but also psychiatric outcomes that were not specifically measured in this population.

There are some limitations, however, that should be considered when interpreting the present findings. Though one of the strengths of this study is the ability to assess the impact of mode of delivery on development, and possible confounding effects on this association through an extensive list of variables, spurious associations may have arisen due to the use of multiple models. All variables, including exposures and outcomes, were self-reported, and as external validation of responses through medical records was not possible, these may be subject to recall bias, or social desirability bias. However, mode of delivery has been shown to be accurately reported in this dataset compared to medical records[179]. Also, the question regarding ASD/ADHD- "has a doctor or health professional ever told you that your child has…"- may have been overly inclusive, as parents could mis-report cases, or report cases which had been diagnosed previously, but were not currently considered cases. Data on validity of parent-reported neurodevelopmental disorders is limited, though surveys including parent-reported ASD and ADHD have been used in previous research studies[161,180]. Notably, the prevalence of ASD in this cohort was similar to recent prevalence estimates by the Centers for Disease Control and Prevention[181]. In addition, it has been noted that though prevalence of ADHD is low in this cohort when compared to the United States or other European countries, it is comparable to previous estimates in the UK, and may be reflective of more stringent diagnostic criteria, or older age of diagnosis[161]. Regardless, possible misclassification bias was addressed by conducting a sub-group analysis on cases that were reported at both age 5 and age 7. Another limitation is the inability to adjust for potential confounders which were not measured in this cohort, for example parental history of ASD/ADHD, or behavioural difficulties (although maternal depression and depression treatment were included in the full model). Similarly,

there was no measure for IQ, and thus it was not possible to stratify cases of ASD by intellectual disability as some previous papers have done[30]. However, this is consistent with how ASD has been analysed in this dataset[161], and as no overall association was found, stratification may not have changed the results or interpretation. In addition, previous results indicate that stratification by IQ may not impact the association between mode of delivery and ASD[152] (though it is possible that this is different in different populations). Also, as with all cohort studies, loss to follow-up is a potential limitation of this study although the original weights used at 9 months appeared to be valid at 7 years[167].

3.6 Conclusion

No association was found between mode of delivery and ASD/ADHD in this cohort. IOL was associated with behavioural difficulties in unadjusted analysis, but this relationship was not significant in the adjusted model. Further research is needed to understand the relationship between mode of delivery and IOL and psychological development in children.

Table 3-1. Categories of variables adjusted for in logistic regression analysis.

Category of variable	
Pregnancy and Delivery (Model 1)	Small for gestational age, gestational age, maternal high blood pressure/pre-eclampsia, maternal smoking during pregnancy, being the first born child, bleeding or threatened miscarriage during pregnancy, and infant age when he/she came home from the hospital
Demographic/socio-economic (Model 2)	poverty, ethnicity, maternal age, maternal education, urbanicity, single parent household at time of first survey, paternal age, and paternal education.
Maternal health/mental health (Model 3)	maternal depression, maternal BMI, whether the pregnancy was a surprise, and maternal irritable bowel syndrome

Table 3-2.Characteristics of the population in relation to mode of delivery.

	Spontaneous vaginal delivery N(%)	**Assisted vaginal delivery** N(%)	**Emergency C-section** N(%)	**Induced vaginal delivery** N(%)	**Planned C-section** N(%)	**Induced C-section** N(%)
Total	6317(48.36)	1305(9.99)	916(7.01)	2625(20.10)	1050(8.04)	849(6.50)
ASD	93(1.47)	20(1.53)	15(1.64)	40(1.53)	16(1.52)	14(1.65)
ADHD	77(1.22)	14(1.07)	17(1.86)	35(1.34)	13(1.24)	11(1.30)
Abnormal SDQ Scores	448(7.33)	83(6.46)	66(6.46)	223(8.74)	66(6.46)	57(6.91)
Pregnancy and Delivery						
Small for gestational age	1323(15.04)	264(14.86)	275(21.57)	638(17.21)	180(12.41)	214(17.85)
Gestational age at birth						
40 weeks	2971(34.14)	559(31.8)	317(25.06)	868(23.66)	136(9.47)	241(20.39)
24-36 weeks	493(5.67)	74(4.21)	290(22.92)	179(4.88)	88(6.13)	115(9.73)
37 weeks	429(4.93)	73(4.15)	83(6.56)	205(5.59)	144(10.03)	70(5.92)
38 weeks	1011(11.620	140(7.96)	144(11.38)	453(12.35)	589(41.02)	151(12.77)
39 weeks	2165(24.88)	336(19.11)	215(17.00)	569(15.51)	398(27.72)	170(14.38)
41+ weeks	1633(18.77)	576(32.76)	216(17.08)	1384(38.02)	81(5.64)	435(36.8)
Maternal high blood pressure/pre-eclampsia while pregnant	323(3.67)	160(9.01)	153(12.00)	343(9.25)	123(8.48)	193(16.10)
Smoke more than 1 cigarette a day while pregnant	1364(15.51)	242(13.63)	198(15.53)	651(17.56)	176(12.13)	149(12.430
First born	2999(34.09)	1385(77.98)	711(55.76)	1399(37.74)	358(24.67)	794(66.22)
Age when came home from the hospital						
< 1 day	1618(18.39)	92(5.18)	5(0.39)	531(14.32)	1(0.07)	1(0.08)
1- 7 days	6562(74.59)	1496(84.23)	862(97.61)	2852(76.94)	1190(82.01)	880(73.39)
8 - 30 days	525(5.97)	172(9.68)	319(25.02)	302(8.15)	235(16.20)	280(23.35)
> 30 days	92(1.05)	16(0.900	89(6.98)	22(0.59)	25(1.72)	38(3.17)
Bleeding/threatened miscarriage	468(5.32)	99(5.57)	98(7.69)	236(6.37)	119(8.20)	84(7.01)
Demographic and socio-economic						
Infant Sex (Male)	4442(50.49)	983(55.35)	735(57.65)	1850(49.91)	712(49.07)	642(53.54)
Poverty	3526(40.10)	471(26.55)	389(30.70)	1564(42.44)	432(29.81)	355(29.63)
White Ethnicity	7050(80.14)	1587(89.36)	1004(78.75)	3133(84.52)	1251(86.22)	983(81.98)
Urbanicity						
Urban	6116(86.70)	1091(84.31)	892(86.94)	2326(86.92)	915(84.72)	769(85.92)
Mixed urban/rural	517(7.33)	113(8.73)	74(7.21)	200(7.47)	90(8.33)	73(8.16)
Rural	421(5.97)	90(6.96)	60(5.85)	150(5.61)	75(6.94)	83(5.92)

Table 3-2 Continued

	Spontaneous vaginal delivery N(%)	**Assisted vaginal delivery** N(%)	**Emergency C-section** N(%)	**Induced vaginal delivery** N(%)	**Planned C-section** N(%)	**Induced C-section** N(%)
Maternal age						
14 to 19	797(9.06)	174(9.80)	85(6.67)	413(11.14)	36(2.48)	71(5.92)
20 to 29	4332(49.26)	841(47.35)	557(43.69)	1780(48.03)	521(35.91)	516(43.04)
30 to 39	3506(39.86)	739(41.61)	592(46.43)	1447(39.04)	840(57.89)	573(47.79)
40 plus	160(1.82)	22(1.24)	41(3.22)	66(1.78)	54(3.72)	39(3.25)
Paternal age						
14 to 19	134(1.87)	19(1.25)	13(1.21)	60(2.02)	9(0.71)	16(1.57)
20 to 29	2475(34.49)	541(35.52)	318(29.64)	1104(37.22)	310(24.29)	314(30.88)
30 to 39	3890(54.21)	830(54.50)	605(56.38)	1514(51.05)	795(62.30)	570(56.05)
40 plus	677(9.43)	133(8.73)	137(12.77)	288(9.71)	162(12.70)	117(11.50)
Maternal education						
Secondary school	4668(56.36)	958(55.57)	657(54.25)	2047(59.04)	756(55.67)	634(55.52)
Higher degree	2263(27.32)	614(35.61)	402(33.20)	807(23.28)	445(32.77)	372(32.57)
No degree	1352(16.32)	152(8.82)	152(12.55)	613(17.68)	157(11.56)	136(11.91)
Paternal education						
Secondary school	2979(48.28)	634(47.38)	437(48.01)	1292(52.35)	536(48.51)	462(50.88)
Higher degree	1894(30.70)	493(36.85)	324(35.60)	651(25.87)	368(33.30)	284(31.28)
No degree	1297(21.02)	211(15.77)	149(16.37)	573(22.77)	201(18.19)	162(17.84)
Single Parent	1597(18.15)	253(14.25)	197(15.45)	735(19.83)	174(11.99)	181(15.10)
Maternal health and mental health						
Maternal depression						
Not Depressed	6745(76.7)	1372(77.30)	969(76.00)	2698(72.86)	1052(72.50)	894(74.56)
Depressed, not treated	1345(15.29)	267(15.04)	192(15.06)	628(16.96)	222(15.30)	181(15.10)
Depressed, treated	705(8.02)	136(7.66)	114(8.94)	267(15.04)	177(12.20)	124(10.34)
Pregnancy unplanned	4175(47.53)	704(39.71)	560(44.06)	1854(50.07)	588(40.58)	494(41.27)
IBS	470(5.35)	127(7.15)	98(7.69)	199(5.37)	111(7.65)	68(5.67)
Maternal BMI						
Normal weight	5616(68.36)	1200(70.13)	714(59.75)	2166(62.35)	787(58.25)	644(57.24)
Underweight	538(6.55)	89(5.20)	77(6.44)	226(6.51)	47(3.48)	46(4.09)
Overweight	1503(18.30)	311(18.18)	270(22.59)	736(21.19)	328(24.28)	268(23.83)
Obese	558(6.79)	111(6.49)	134(11.21)	346(9.96)	189(13.99)	167(14.84)
Maternal attachment						
Low maternal attachment	2213(30.63)	468(30.12)	313(29.58)	913(29.36)	357(30.00)	308(30.74)
High maternal attachment	1795(24.84)	394(25.35)	275(25.99)	820(26.37)	299(25.13)	252(25.15)

[a] Autism spectrum disorder
[b] Attention-deficit/hyperactivity disorder
[c] Strengths and Difficulties Questionnaire
[d] Irritable bowel syndrome

Table 3-3. The association between mode of delivery and autism spectrum disorders and attention-deficit/hyperactivity disorder at age 7.

	Exposed Cases	Unadjusted OR OR (95% CI)	Model 1 Pregnancy and delivery factors OR (95% CI)	Model 2 Maternal health/mental health OR (95% CI)	Model 3 Demographic /Socioeconomic factors OR (95% CI)	Model 4 Model 1 + 3 OR (95% CI)	Model 5 Model 1 + 2 + 3 OR (95% CI)
ASD[a]							
Spontaneous vaginal delivery	93	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	20	1.06(0.56,2.01)	1.01(0.52,1.96)	1.09(0.56,2.11)	0.92(0.45,1.89)	0.78(0.37,1.65)	0.76(0.34,1.67)
Emergency C-section	15	1.31(0.68,2.49)	0.96(0.49,1.90)	1.37(0.71,2.64)	1.35(0.64,2.86)	0.94(0.45,1.97)	0.96(0.45,2.05)
Induced vaginal delivery	40	1.05(0.69,1.62)	0.97(0.61,1.53)	0.95(0.60,1.51)	1.08(0.62,1.87)	1.00(0.54,1.84)	0.89(0.47,1.70)
Planned C-section	16	1.08(0.54,2.17)	0.81(0.39,1.68)	1.15(0.57,2.32)	0.89(0.32,2.42)	0.57(0.19,1.74)	0.58(0.19,1.79)
Induced C-section	14	0.98(0.50,1.93)	0.69(0.32,1.48)	1.06(0.53,2.11)	0.93(0.38,2.31)	0.57(0.20,1.62)	0.67(0.24,1.82)
ADHD[b]							
Spontaneous vaginal delivery	77	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	14	0.88(0.45,1.71)	0.79(0.39,1.60)	0.98(0.50,1.93)	0.81(0.37,1.79)	0.77(0.34,1.78)	0.76(0.32,1.80)
Emergency C-section	17	1.70(0.93,3.11)	1.30(0.71,2.38)	1.69(0.90,3.19)	1.62(0.80,3.31)	1.42(0.71,2.87)	1.28(0.61,2.66)
Induced vaginal delivery	35	1.04(0.65,1.66)	0.95(0.58,1.54)	0.99(0.62,1.59)	0.93(0.54,1.61)	0.88(0.50,1.58)	0.84(0.46,1.51)
Planned C-section	13	0.79(0.37,1.69)	0.59(0.26,1.33)	0.86(0.40,1.86)	0.84(0.31,2.29)	0.57(0.20,1.64)	0.54(0.18,1.64)
Induced C-section	11	1.27(0.60,2.69)	0.92(0.42,2.03)	1.36(0.63,2.93)	1.69(0.77,3.73)	1.37(0.58,3.20)	1.31(0.54,3.15)

Pregnancy and delivery factors: Small for gestational age, gestational age, maternal high blood pressure/pre-eclampsia, maternal smoking during pregnancy, being the first born child, bleeding or threatened miscarriage during pregnancy, and infant age when he or she came home from the hospital

Demographic/socio-economic factors: poverty, ethnicity, maternal age, maternal education, child gender

Maternal health/mental health: maternal depression, maternal BMI

[a] Autism spectrum disorder

[b] Attention-deficit/hyperactivity disorder

Table 3-4. The association between mode of delivery and abnormal scores on the strengths and difficulties questionnaire at age 7.

	Exposed Cases	**Unadjusted OR** **OR (95% CI)**	**Model 1** **Pregnancy and delivery factors OR (95% CI)**	**Model 2** **Maternal health/mental health** **OR (95% CI)**	**Model 3** **Demographic /Socioeconomic factors** **OR (95% CI)**	**Model 4** **Model 1 + 3** **OR (95% CI)**	**Model 5** **Model 1 + 2 + 3** **OR (95% CI)**
Spontaneous vaginal delivery	448	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	83	0.77(0.57,1.03)	0.78(0.57,1.06)	0.87(0.64,1.18)	0.77(0.49,1.19)	0.76(0.48,1.22)	0.80(0.49,1.31)
Emergency C-section	66	0.88(0.63,1.23)	0.72(0.50,1.03)	0.93(0.65,1.31)	0.90(0.54,1.51)	0.71(0.41,1.21)	0.76(0.44,1.33)
Induced vaginal delivery	223	**1.26(1.03,1.54)**	**1.26(1.02,1.55)**	1.19(0.96,1.46)	1.16(0.86,1.57)	1.19(0.86,1.63)	1.15(0.82,1.60)
Planned C-section	66	0.83(0.60,1.17)	0.74(0.53,1.05)	0.79(0.56,1.13)	1.00(0.61,1.65)	0.75(0.45,1.24)	0.73(0.43,1.24)
Induced C-section	57	0.83(0.59,1.17)	0.77(0.54,1.12)	0.85(0.60,1.22)	0.93(0.55,1.55)	0.81(0.47,1.41)	0.82(0.47,1.45)

Pregnancy and delivery factors: Small for gestational age, gestational age, maternal high blood pressure/pre-eclampsia, maternal smoking during pregnancy, being the first born child, bleeding or threatened miscarriage during pregnancy, and infant age when he or she came home from the hospital

Demographic/socio-economic factors: poverty, ethnicity, maternal age, maternal education, single parent household at time of first survey

Maternal health/mental health: maternal depression, maternal BMI and whether or not pregnancy was planned

Table 3-5. The effect of maternal attachment on the association between mode of delivery and child development at age 7.

	Exposed Cases	Entire Population	Excluding low-attachment	Excluding high-attachment
ASD[a]				
Spontaneous vaginal delivery	93	Ref	Ref	Ref
Assisted vaginal delivery	20	0.76(0.34,1.67)	0.76(0.30,1.93)	0.54(0.20,1.45)
Emergency C-section	15	0.96(0.45,2.05)	0.98(0.42,2.29)	0.93(0.39,2.22)
Induced vaginal delivery	40	0.89(0.47,1.70)	0.57(0.25,1.30)	0.95(0.47,1.92)
Planned C-section	16	0.58(0.19,1.79)	0.71(0.20,2.47)	0.70(0.21,2.37)
Induced C-section	14	0.67(0.24,1.82)	0.60(0.19,1.85)	0.64(0.21,1.89)
ADHD[b]				
Spontaneous vaginal delivery	77	Ref	Ref	Ref
Assisted vaginal delivery	14	0.76(0.32,1.80)	0.43(0.12,1.55)	0.66(0.25,1.76)
Emergency C-section	17	1.28(0.61,2.66)	1.00(0.44,2.29)	1.35(0.62,2.95)
Induced vaginal delivery	35	0.84(0.46,1.51)	0.69(0.34,1.42)	0.77(0.41,1.47)
Planned C-section	13	0.54(0.18,1.64)	0.44(0.13,1.51)	0.34(0.08,1.44)
Induced C-section	11			
Overall SDQ[c]				
Spontaneous vaginal delivery	448	Ref	Ref	Ref
Assisted vaginal delivery	83	0.80(0.49,1.31)	0.87(0.48,1.56)	0.75(0.43,1.31)
Emergency C-section	66	0.76(0.44,1.33)	0.47(0.23,0.96)	0.82(0.44,1.53)
Induced vaginal delivery	223	1.15(0.82,1.60)	1.08(0.72,1.63)	1.20(0.84,1.71)
Planned C-section	66	0.73(0.43,1.24)	0.88(0.47,1.62)	0.62(0.34,1.13)
Induced C-section	57	0.82(0.47,1.45)	0.69(0.36,1.35)	0.82(0.44,1.53)

[a] Autism spectrum disorder
[b] Attention-deficit/hyperactivity disorder
[c] Strengths and Difficulties Questionnaire

CHAPTER 4. THE ASSOCIATION BETWEEN OBSTETRIC MODE OF DELIVERY AND AUTISM SPECTRUM DISORDER: SIBLING STUDY SUGGESTING COMPLETE CONFOUNDING BY FAMILIAL FACTORS

Eileen A. Curran[1], Christina Dalman[2], Patricia M. Kearney[3], Louise Kenny[1],

John F. Cryan[4], Timothy G. Dinan[4], Ali S. Khashan[1,3]

[1]The Irish Centre for Fetal and Neonatal Translational Research (INFANT), University College Cork, Ireland.

[2]Division of Public Health Epidemiology, Karolinska Institutet, Sweden.

[3]Department of Epidemiology and Public Health, University College Cork, Ireland

[4]APC Microbiome Institute, University College Cork, Ireland

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4.1 Abstract

Importance: As rates of Caesarean section (CS) are rising worldwide, it is becoming increasingly important to understand long-term effects mode of delivery may have on child development.

Objective: To investigate the association between obstetrical mode of delivery and autism spectrum disorder (ASD) using a large, population-based cohort.

Design: Perinatal factors and ASD diagnoses based on the *International Classification of Disease* (ICD) were identified from the Swedish Medical Birth Register and the National Patient Register. We conducted stratified Cox regression analysis to examine the impact of mode of delivery on ASD. This was followed by sibling design study, which consisted of sibling pairs discordant on ASD status, and was performed using conditional logistic regression. Analyses were adjusted for year of birth (i.e. partially adjusted) then "fully adjusted" for various perinatal and socio-demographic factors.

Setting and participants: The study cohort consisted of all singleton live births in Sweden between 1982 -2010. The full cohort consisted of 2,697,315 children, and 28,290 cases of ASD. Sibling control analysis consisted of 13,411 sibling pairs.

Exposure: Obstetrical mode of delivery defined as: unassisted vaginal delivery (VD), assisted VD, elective CS, and emergency CS (as defined by before or after onset of labour).

Main outcome measures: ASD status as defined using codes from the ICD, versions 9 (code 299) and 10 (code F84).

Results: In adjusted Cox regression, both elective (HR = 1.21; [95% CI: 1.15-1.27]) and emergency (HR = 1.15; [95% CI: 1.10-1.20]) CS were associated with ASD when compared to unassisted VD. In the sibling control analysis, elective CS was not associated with ASD in partially (OR = 0.97; [95% CI: 0.85-1.11]) or fully adjusted models (OR = 0.89; [95% CI: 0.76-1.04]). Emergency CS was significantly associated with ASD in partially adjusted analysis (OR = 1.20; [95% CI: 1.06-1.36]), but this effect disappeared in the fully adjusted model (OR =0.97; [95% CI: 0.85-1.11]).

Conclusions and relevance: This study confirms previous findings that children born by CS are about 20% more likely to be diagnosed with ASD. However, the association did not persist when using sibling controls implying that this relationship is due to familial confounding by genetic and/or environmental factors.

4.2 Background

Autism spectrum disorder (ASD) is a disorder characterized by impairment in social interaction and communication with the presence of restricted interest and repetitive behaviours[26]. ASD is thought to affect an estimated 0.62% of children worldwide[114], though recent estimates in the United States have been closer to 1.5% [182]. The prevalence of ASD has increased drastically since 1980, yet changes in diagnostic criteria might only explain 60% of this increase[27].

Though ASD is highly heritable[36], it has previously been linked to numerous perinatal factors[133], possibly including birth by Caesarean section (CS)[151]. There are several possible mechanisms underlying this association including "early term" (i.e. 37-39 weeks' gestation) birth[110], exposure to altered microbiota[55], changes in stress response[50], and type of anaesthesia[182]. Alternatively the observed association may be due to residual confounding, or confounding by indication, meaning ASD could be associated with the indication for CS rather than the CS itself[151], or an unknown genetic factor that is associated with increased risk of CS and increased risk of ASD. The prevalence of birth by CS is estimated to be 15% worldwide, and is over 20% in developed countries[104]. As it is increasing worldwide, for a variety of reasons[11,105], it is becoming increasingly important to fully understand any possible long-term effects of birth by CS.

Birth by CS has previously been linked to several long-term outcomes including asthma, allergic rhinitis, diabetes and gastro-intestinal disease[50]. Notably, recent population-based studies that used siblings as controls found that these reported associations could be explained by genetic factors or family environment for both asthma[91] and type 1 diabetes[92]. Thus it is important to consider residual confounding from both environmental and genetic factors using appropriate methods.

The objective of this investigation was to assess the impact of mode of delivery, specifically birth by CS, on ASD using data from a large Swedish population-based registry. To our knowledge, this is the largest study on this subject to date[151], and the only one to use the combination of adjusted cohort and sibling control study designs. Using this combination, taking both familial environmental and genetic confounding factors into consideration, we aimed to robustly assess any association between mode of delivery and ASD.

4.3 Methods

Study population

The study cohort consisted of all singleton live births in Sweden from 1982-2010 using data from the Swedish national registers including the Medical Birth Register, the National Patient Register, and the Multi-Generation Register. Data from these registers can be linked using the personal identification number (PIN) given to each Swedish resident. Over 99% of all births in Sweden are recorded in the Medical Birth Register[183,184].

We started follow-up from the 1st birthday (left censored), consistent with previous studies on ASD in this population[36]. Prior to 1987 there was no code for autism available in the register[36], so for those persons who turned one year of age earlier than 1987, follow-up began on January 1st, 1987. Children were followed up until first diagnosis of ASD, death, migration or December 31, 2011 (end of study period), whichever came first.

Sibling design studies have been used in investigations of pre- or perinatal risk factors and were developed as a way to control for shared genetic factors and

familial environment[90]. For the sibling study, we included all sibling pairs that included the first two children for each mother, where one was diagnosed with ASD and one was not. As conditional logistic regression does not adjust for differential follow-up time, follow-up time during the study period was longer for the control sibling (i.e. the control died, emigrated, or left the study at an older age than when their sibling was diagnosed with ASD). The case sibling could be the younger or older sibling. This is consistent with previous sibling control studies using this dataset[92].

Exposure

Mode of delivery was divided into four categories; unassisted vaginal delivery (VD), assisted VD, elective CS, and emergency CS. Assisted VD was defined as VD with the use of forceps or vacuum extraction. Unassisted VD included both spontaneous and induced VD. Elective and emergency CS were defined as CS before and after onset of labour respectively. Though the Medical Birth Register began in 1973, variables indicating emergency and elective CS are available from 1982, which marks the beginning of our investigation.

Outcome

Information on ASD status and date of first diagnosis was obtained from the National Patient Register. In line with the most recent Diagnostic and Statistical Manual of Mental Disorders (DSM-5), we included all pervasive developmental disorders (PDD) as cases of ASD[185], including codes from the *International Classification of Diseases* (ICD), versions 9 (code 299) and 10 (code F84)[73,186,187]. The National Patient Register includes inpatient data from 1973, and outpatient data from 2001[188], with good outpatient coverage from 2006. Children in Sweden undergo

a mandatory developmental assessment at age 4, and children with suspected developmental disorders are referred for further assessment by a child psychiatry unit[36]. The procedures for this assessment are standardized across Sweden. To account for the possibility of increased diagnosis using this method, we conducted a sensitivity analysis on children born on or before 2007 to allow everyone to be followed at least until age 4. Previous results have indicated that risk factors may differ for ASD with and without intellectual disability (ID)[30]. Therefore, we looked at ASD overall, as well as ASD with and without ID (defined as IQ<70 and functional impairment[189]).

Statistical analysis

First, we performed Cox regression to estimate hazard ratios (HRs) and 95% CIs. We assessed the proportional hazards assumption using graphical displays of the empirical score process. Though originally violated, when analyses were adjusted for year of birth and stratified by every 4th birth year, the assumption was no longer violated. Therefore all analyses were partially adjusted in this way. In fully adjusted models, we also included infant gender[76], maternal age[76], gestational age[76], maternal and paternal citizenship[76], SGA[76], LGA, 5 minute Apgar score[76], parity[76], social welfare status, family disposable income[131], and maternal and paternal depression[187], bipolar disorder, and non-affective disorder[186] in the total population (**Appendix 15**). We used robust confidence intervals, with analysis clustered on maternal ID, to account for increased likelihood of ASD diagnosis within families. Sensitivity analyses are detailed in supplementary material (**Appendix 16**).

For the sibling study, we conducted conditional logistic regression using non-affected siblings as controls, matched on maternal ID. Estimates for the association

between mode of delivery and ASD were obtained using pairs discordant on both ASD and mode of delivery, although pairs concordant on mode of delivery were included in the analysis as they contribute to the covariate estimates. The conditional logistic regression analysis included adjustment for the same covariates as the Cox regression model, with the exception of maternal country of birth, which was the same for each sibling pair. We conducted a sensitivity analysis including only full siblings (i.e. same father as well as mother).

All analyses were conducted using SAS 9.4.

4.4 Results

Descriptive statistics

There were 2,941,629 live births in Sweden between January 1, 1982 and December 31, 2010. After excluding 77,209 multiple births, 17,968 that died or emigrated before 1 year of age, 87 whose first diagnosis of ASD was before 1 year of age, and 149,050 children with unknown mode of delivery, 2,697,315 remained in the final cohort. Approximately 80% of the study population were born through non-assisted VD (n = 2,161,148); 6.09% for elective CS (n = 164,305); 6.52% for emergency CS; and 7.27% for assisted VD (n = 196,058) (**Appendix 17**). About 1% of our population was diagnosed with ASD (n = 28,290) (**Table 4-1**). Median age at diagnosis was 12 years (IQR: 7-16), among boys it was 11 (IQR: 7-16) and among girls it was 14 (IQR: 9-17).

Cox regression

In partially adjusted analyses (i.e. adjusted for year of birth), elective (HR = 1.39; [95% CI: 1.33-1.45]) and emergency CS (HR = 1.40; [95% CI: 1.34-1.46]) as

well as assisted VD (HR =1.18; [95%CI: 1.13-1.23]) were all associated with ASD, when compared to unassisted VD (**Table 4-2**). After full adjustment the adjusted HRs (aHR) were reduced but the association remained significant for elective (aHR = 1.21; [95% CI: 1.15-1.27]) and emergency CS (aHR = 1.15; [95% CI: 1.10-1.20]) but not assisted VD (aHR =1.03; [95%CI: 0.98-1.07]). Sensitivity analyses excluding children who were preterm, female, SGA, LGA, were born after 2007 and children whose mothers had ever been diagnosed with depression did not materially change the results (**Appendix 18**). Including children diagnosed prior to 1 year of age also had no effect. Excluding children who had been born prior to 1990 and 2000 similarly did not materially change the results (**Table 4-2**). Including parental education after excluding those born prior to 1990 had no effect on the results (Data not shown). Sensitivity analyses on birth order, CS order, and family size had no effect on results (**Appendix 19**). Similarly, including induction of labour also did not change results (**Appendix 20**). Sensitivity analyses on maternal age, obesity, diabetes and hypertension did not have an effect on results (**Appendix 20**). Likewise, analyses regarding breech presentation did not have an impact (**Appendices 22-24**).

When cases of ASD were stratified by the presence of intellectual disability (ID), assisted VD was not significantly associated with ASD with ID (aHR = 1.11; [95% CI: 1.00-1.22]) or without ID (aHR = 1.01; [95% CI: 0.96-1.06]) (**Table 4-2**). Elective CS was significantly associated with both ASD with (aHR = 1.26; [95% CI: 1.14-1.39]) and without ID (aHR = 1.19; [95% CI: 1.12-1.26]). Similarly, emergency CS was associated with both ASD with (aHR = 1.25; [95% CI: 1.14-1.37]) and without ID (aHR = 1.12; [95% CI: 1.06-1.18]). Excluding children born before 1990 and 2000 did not change results materially.

Sibling control

In the sibling control study, there were 13,411 sibling pairs discordant on ASD, 2,555 pairs were also discordant on mode of delivery (with one sibling born by unassisted VD). In the partially adjusted analysis there was no association between assisted VD (OR = 1.04; [95% CI: 0.91-1.15]), or elective CS (OR = 0.97; [95% CI: 0.85-1.11]) and ASD (**Table 4-3**). After full adjustment assisted VD (OR = 0.91; [95% CI: 0.82 -1.02]) and elective CS (OR = 0.89; [95% CI: 0.76 -1.04]) were not associated with ASD. Conversely, though emergency CS was associated with ASD in partially adjusted analysis (OR = 1.20; [95% CI: 1.06 -1.36]), this association did not persist after full adjustment (OR = 0.97; [95% CI: 0.85 -1.11]). Due to the lack of overall ASD association, we did not further analyse the impact of mode of delivery on ASD stratified by ID status. Excluding children born before 1990 and 2000 did not materially change the results. Sensitivity analysis including only the 11,634 full biological siblings (i.e. shared both father and mother) did not change results (Data not shown).

4.5 Discussion

The current study combines two approaches to assess the impact of obstetric mode of delivery on ASD; a conventional cohort analysis, which we used to test the association in a large population, and a sibling control analysis, which we used to control for familial confounding and assess causality. In the conventional cohort analysis, children born by elective CS were 21% more likely to be diagnosed with ASD after controlling for known confounders, similar to previous findings regarding birth by CS and ASD[151]. However, in the sibling control analysis, there was no association between mode of delivery and ASD.

Though the traditional cohort analysis showed birth by CS to be associated with ASD, it is not necessarily a cause, as the association could be due to residual confounding. As randomizing mode of delivery would be unethical, large population-based cohort studies are the most robust way to assess an association. In such cases, the use of siblings as controls is seen as a way to test the causality of a relationship when randomized experiments are not feasible[190]. This use of siblings as controls adjusts for shared factors relating to genetics and family environment that may not be measured or fully understood[191]. Therefore, as the association between birth by CS and ASD did not persist in the sibling control analysis, we can conclude that there is no causal relationship. It is more likely that birth by CS is related to some unknown genetic or environmental factor that leads to increased risk of both CS and ASD.

There have been recent developments in understanding genetic risk factors for ASD[151,192], but it is likely that even amongst inherited cases, genetic and environmental factors interact[76]. Therefore, though birth by CS may not have a causal relationship with ASD, future research into environmental risk factors for ASD is warranted. It is worth noting, however, that though CS may not have a causal relationship with ASD, it is not without health risks for both mother and foetus and should not be undertaken without due regard for those risks.

Previous studies

A recent systematic review and meta-analysis on mode of delivery and ASD reported a 23% increased risk of ASD in relation to CS although studies have been limited[151]. Only one investigation in recent years, conducted in Australia by Glasson et al.[128], has made use of sibling design. Glasson et al. detected no significant effect

of birth by elective CS on ASD overall, but did find a significant effect on Autism, Asperger and PDD-NOS each separately. However, the study did not provide adjusted estimates, beyond the use of sibling controls, and was much smaller than the current study (n = 473 sibling pairs). There have been three previous cohort studies to report an adjusted estimate of the association between CS and ASD[30,31,116], however all were subject to some limitation. Burstyn et al.[31] and Gregory et al.[116] did not provide separate estimates for emergency and elective CS, making it difficult to distinguish between the effect of CS and possible confounding by indication, as an association with emergency CS may be more related to complications during labour rather than the CS itself. Langridge et al. did distinguish between emergency and elective CS, and also stratified by ID status, similar to this study[30]. However, the present study accounts for known confounders that were not included in Langridge et al, such as family income, and parental psychiatric health.

Strengths and limitations

Our study is the largest on this subject to date, with 2.6 million births included in our analysis. In addition, as data were prospectively obtained from national registries they were not subject to recall bias or selection bias. Also, due to the nature of the dataset, we were able to adjust for a variety of known potential confounders. Even after controlling for these variables we found birth by elective CS to be associated with a 20% increased risk of ASD, which is comparable to previous estimates[151]. We were also able to conduct sensitivity analyses by birth cohort to determine if the association changed in recent years, which was a limitation in some previous studies[116]. This also allowed us to address possible bias due to changes in ASD reporting practices over time. That we saw no change over time in the traditional cohort analysis or sibling analysis leads us to conclude that changes in

ASD definition and reporting did not affect the association. Finally, due to such large numbers and quality of data, we were able to conduct a sibling design study, which helped control for unknown genetic and environmental confounders, after which the association did not persist.

There are also limitations to the present investigation. Although we were able to differentiate between "emergency" and "elective" CS based on timing of labour, we did not have access to the indication for CS. In addition, it is possible that parents who stopped having children after their first was diagnosed with ASD were different than parents with multiple children, one or more of whom were diagnosed with ASD. Though our sensitivity analysis on only children did not show a difference in the overall association, we were unable to include these children in the sibling analysis, and so were unable to see if the association was attenuated in a similar manner when genetic and family environment were controlled for. Also, as we have mostly inpatient data until 2001, severe cases of ASD were likely over-represented, and the association between mode of delivery and ASD may be different for milder forms of ASD. However, as sensitivity analysis from 2000 onwards showed no difference in results, this likely did not affect our findings. Moreover, though this is the largest study to date on this subject, we did reduce the sample size in the sibling control analysis due to strict inclusion criteria, which is reflected in the relatively wider confidence intervals. Also, modes of delivery were likely not independent between siblings as women who have given birth by CS are more likely to give birth by CS[3], and it's possible that such relatedness with the exposure could lead to increased bias in the sibling control design[89]. However, the results from our sensitivity analyses based on the cohort analysis, conducted by birth order, CS order and family size would imply that the difference in results was in fact due to the

comparison to siblings rather than other factors such as cohort size or clustering of exposure. There are several known limitations to sibling control analyses[89,90], including an inability to distinguish whether the confounding is due to genetics, family environment, or both[90]. For example, we were unable to measure any epigenetic changes related to CS that differed amongst siblings and may have been associated with ASD. In addition, in the presence of measurement error of the exposure, sibling control analysis could lead to an artificial decrease in the association[89]. A limitation of the use of anonymized registry data is that we were not able to validate cases. However, the positive predictive value (PPV) of psychiatric disorders in this registry has previously been estimated to be between 85 and 95%[188], and a review of the medical records for 88 cases of ASD found a PPV of 94.3%[193].

4.6 Conclusion

This study confirms previous results that children born by CS are about 20% more likely to be diagnosed with ASD after adjustment for known confounders. However, the lack of association in analyses using sibling controls implies this relationship is not causal, and therefore is due to unknown genetic or environmental factors.

Table 4-1. Perinatal and socio-demographic variables related to autism spectrum disorder and mode of delivery among those born in Sweden from 1982 to 2010.

	Total Population N(%)	**Non-assisted Vaginal Delivery N(%)**	**Assisted Vaginal Delivery N(%)**	**Elective Caesarean section N(%)**	**Emergency Caesarean section N(%)**
Total Population		2,161,148(80.12)	196,058(7.27)	164,305(6.09)	175,803(6.52)
ASD[a]	28,290(1.05)	21,757 (1.01)	2,203(1.12)	2,035 (1.24)	2,295 (1.31)
SGA[b]	65,208 (2.44)	39,523 (1.84)	5,198 (2.66)	9,627 (5.89)	10,860 (6.22)
LGA[c]	93,617 (3.49)	66,918(3.11)	5,476(2.81)	11,950(7.31)	9,273(5.31)
First born child	1,148,649 (42.58)	826,564(38.25)	156,405(79.77)	55,768(33.94)	109,912(62.52)
Induced	183,319(9.45)	129,640(8.55)	20,348(13.75)	N/A	33,331(23.27)
Sex*(male)*	1,386,889(51.42)	10,993,170(50.58)	112,850(57.56)	83,614(50.89)	97,255(55.32)
Maternal age					
<20 years	63,572(2.36)	53,837(2.49)	4,843(2.47)	1,722(1.05)	3,170(1.80)
20-29 Years	1,423,741(52.78)	1,173,448(54.30)	108,085(55.13)	59,985(36.51)	82,223(46.77)
30-39 years	1,144,909(42.45)	889,416(41.15)	79,148(40.37)	92,648(56.39)	83,697(47.61)
40+ years	65,092(2.41)	44,447(2.06)	3,982(2.03)	9,950(6.06)	6,713(3.82)
Gestational age					
<37 weeks	130,484(4.85)	81,132(3.76)	5,244(2.68)	21,804(13.29)	22,305(12.71)
37 weeks	133,330(4.95)	98,600(4.57)	6,265(3.25)	16,793(10.24)	11,572(6.59)
38 weeks	366,171(13.60)	251,075(11.64)	16,217(8.28)	78,142(47.63)	20,737(11.82)
39 weeks	624,225(23.18)	529,513(24.54)	37,408(19.11)	32,201(19.63)	25,013(14.30)
40 weeks	760,038(28.22)	658,128(30.50)	58,140(29.70)	7,641(4.66)	36,129(20.59)
>40 weeks	678,571(25.20)	539,049(24.98)	72,387(39.98)	7,481(4.56)	59,654(33.99)
5 Min Apgar					
Low *(0-3)*	5,207(0.10)	2,711(0.13)	736(0.38)	478(0.29)	2,182(0.74)
Intermediate *(4-6)*	19,530(0.74)	7,744(0.36)	4,355(2.24)	2,170(1.34)	5,261(3.03)
High *(7-10)*	2,631,702(99.07)	2,115,971(99.51)	189,213(97.38)	159,424(98.37)	167,094(96.23)
Mother Country of birth					
Sweden	2,273,396(84.29)	1,827,303(84.56)	165,868(84.61)	136,590(83.14)	143,635(81.72)

Other Nordic	87,097(3.23)	70,762(3.27)	5,637(2.88)	5,279(3.21)	5,419(3.08)
Other	336,634(12.48)	262,952(12.17)	24,853(12.52)	22,424(13.65)	26,720(15.20)
Father Country of birth					
Sweden	2,245,361(83.86)	1,799,637(83.84)	164,406(84.63)	137,033(84.12)	144,285(82.92)
Other Nordic	77,491(2.89)	63,128(2.94)	5,012(2.58)	4,613(2.83)	4,738(2.72)
Other	354,723(13.25)	283,632(13.21)	24,853(12.79)	2,156(13.05)	24,982(14.36)
Maternal Depression					
Never	2,475,676(91.78)	1,988,761(92.02)	147,389(89.70)	180,163(91.89)	1,988,761(92.02)
Before birth	4,443(1.65)	31,876(1.47)	5,009(6.05)	3,375(1.72)	31,876(1.47)
After birth	177,195(6.57)	140,511(6.50)	11,907(7.25)	12,520(6.39)	170,511(6.50)
Maternal Bipolar Disorder					
Never	2,672,402(99.08)	2,141,549(99.09)	194,366(99.14)	162,469(98.88)	174,018(98.98)
Before birth	3,527(0.13)	2,529(0.12)	276(0.14)	392(0.24)	330(0.19)
After birth	21,385(0.79)	1,707(0.79)	1,416(0.72)	1,444(0.88)	1,455(0.83)
Maternal Non-affective Disorder					
Never	2,676,778(99.24)	2,145,090(99.26)	194,575(99.24)	162,810(99.09)	174,303(99.15)
Before birth	6,903(0.26)	5,114(0.24)	535(0.27)	669(0.41)	585(0.33)
After birth	13,633(0.51)	10,944(0.51)	948(0.48)	826(0.50)	915(0.52)

Table 4-1 Continued

	Total Population N(%)	**Non-assisted Vaginal Delivery N(%)**	**Assisted Vaginal Delivery N(%)**	**Elective Caesarean section N(%)**	**Emergency Caesarean section N(%)**
Paternal Depression					

Never	2,566,589(95.15)	2,056,415(95.15)	187,010(95.39)	156,048(94.97)	167,116(95.06)
Before birth	24,630(0.91)	18,677(0.86)	1,848(0.94)	2,089(1.27)	2,016(1.15)
After birth	106,095(3.93)	86,056(3.98)	7,200(3.67)	6,168(3.75)	6,671(3.79)
Paternal Bipolar Disorder					
Never	2,681,863(99.43)	2,148,738(99.43)	195,029(99.48)	163,313(99.40)	174,783(99.42)
Before birth	2,661(0.10)	2,065(0.10)	200(0.10)	204(0.12)	192(0.11)
After birth	12,790(0.47)	10,345(0.48)	829(0.42)	788(0.48)	828(0.47)
Paternal Non-affective Disorder					
Never	2,678,375(99.30)	2,145,820(99.29)	194,742(99.33)	163,245(99.35)	174,568(99.30)
Before birth	7,163(0.27)	5,588(0.26)	553(0.28)	486(0.30)	536(0.30)
After birth	11,776(0.44)	9,740(0.45)	763(0.39)	574(0.35)	699(0.40)
Income Quintile					
First	513,647(19.29)	431,504(20.24)	25,970(13.40)	27,913(17.14)	28,260(16.25)
Second	632,841(20.01)	44,518(20.80)	27,872(14.38)	32,010(19.66)	29,442(16.93)
Third	538,145(20.21)	435,310(20.42)	36,420(18.79)	32,755(20.12)	33,660(19.36)
Fourth	540,963(20.32)	423,450(19.86)	45,981(23.72)	33,412(20.52)	38,120(21.92)
Fifth	537,003(20.17)	398,255(18.68)	57,617(29.72)	36,734(22.56)	44,397(25.53)

[a]Autism spectrum disorder
[b]Small for gestational age
[c]Large for gestational age

Table 4-2. The association between mode of delivery and autism spectrum disorder with and without intellectual disability in the offspring.

	Exposed Cases	Partially Adjusted[a]	Adjusted[b]	Exposed Cases	Born 1990 or later[b]	Exposed Cases	Born 2000 or later[b]
All ASD[c]							
Unassisted VD [d]	21,757	Ref	Ref	15,765	Ref	4,259	Ref
Assisted VD	2,203	**1.18(1.13-1.23)**	1.03(0.98-1.07)	1,698	1.02(0.97-1.08)	575	1.00(0.91-1.09)
Elective CS[e]	2,035	**1.39(1.33-1.45)**	**1.21(1.15-1.27)**	1,696	**1.24(1.18-1.32)**	683	**1.26(1.15-1.38)**
Emergency CS	2,295	**1.40(1.34-1.46)**	**1.15(1.10-1.20)**	1,867	**1.15(1.09-1.21)**	661	**1.10(1.01-1.20)**
ASD With ID[f]							
Unassisted VD	4,678	Ref	Ref	3,425	Ref	901	Ref
Assisted VD	463	**1.16(1.05-1.27)**	1.11(1.00-1.22)	351	1.08(0.97-1.21)	111	1.07(0.87-1.32)
Elective CS	518	**1.66(1.51-1.82)**	**1.26(1.14-1.39)**	437	**1.17(1.17-1.47)**	166	**1.26(1.05-1.52)**
Emergency CS	566	**1.61(1.47-1.76)**	**1.25(1.14-1.37)**	457	**1.14(1.14-1.40)**	133	1.07(0.89-1.30)
ASD Without ID							
Unassisted VD	17,079	Ref	Ref	12,340	Ref	3,358	Ref

Assisted VD	1,740	**1.19(1.13-1.25)**	1.01(0.96-1.06)	1,347	1.01(0.95-1.07)	464	0.98(0.89-1.09)
Elective CS	1,517	**1.32(1.25-1.39)**	**1.19(1.12-1.26)**	1,259	**1.22(1.15-1.31)**	517	**1.26(1.13-1.40)**
Emergency CS	1,729	**1.34(1.28-1.41)**	**1.12(1.06-1.18)**	1,410	**1.12(1.05-1.18)**	528	**1.11(1.01-1.22)**

[a]Adjusted for year of birth
[b]Adjusted for year of birth, infant gender, maternal age, gestational age, 5 minute Apgar score, maternal and paternal country of birth, small for gestational age, large for gestational age, first born, family income, maternal and paternal depression, bipolar disorder, and non-affective disorder
[c]Autism spectrum disorder
[d]Vaginal delivery
[e]Caesarean section
[f]Intellectual disability

Table 4-3. The association between mode of delivery and autism spectrum disorder among sibling pairs.

	Exposed Cases	Partially Adjusted[a]	Adjusted[b]	Exposed Cases	Born From 1990[b]	Exposed Cases	Born from 2000[b]
All ASD[c]							
Unassisted VD [d]	10,733	Ref	Ref	7509	Ref	1685	Ref
Assisted VD	893	1.04(0.91-1.15)	0.91(0.82-1.02)	644	0.91(0.80-1.05)	178	0.92(0.68-1.24)
Elective CS[e]	856	0.97(0.85-1.11)	0.89(0.76-1.04)	701	0.94(0.78-1.13)	246	0.93(0.63-1.36)
Emergency CS	929	**1.20(1.06-1.36)**	0.97(0.85-1.11)	728	0.96(0.81-1.13)	214	0.85(0.59-1.24)

[a]Adjusted for year of birth

[b]Adjusted for year of birth, infant gender, maternal age, gestational age, 5 minute Apgar score, paternal country of birth, small for gestational age, large for gestational age, first born, family income, maternal and paternal depression, bipolar disorder, and non-affective disorder
[c]Autism spectrum disorder
[d]Vaginal delivery
[e]Caesarean section

CHAPTER 5. OBSTETRIC MODE OF DELIVERY AND ATTENTION-DEFICIT/HYPERACTIVITY DISORDER: A SIBLING-MATCHED STUDY

Eileen A. Curran[1], Ali S. Khashan[1,2], Christina Dalman[3], Louise C. Kenny[1], John F. Cryan[4], Timothy G. Dinan[5], Patricia M. Kearney[2]

[1]The Irish Centre for Fetal and Neonatal Translational Research (INFANT), University College Cork, Ireland.

[2]Department of Epidemiology and Public Health, University College Cork, Ireland

[3]Division of Public Health Epidemiology, Karolinska Institutet, Sweden.

[4]APC Microbiome Institute, University College Cork, Ireland

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5.1 Abstract

Background: It has been suggested that birth by Caesarean section (CS) may affect psychological development through changes in microbiota or stress response. We assessed the impact of mode of delivery, specifically CS on the development of attention-deficit/hyperactivity disorder (ADHD), using a large, population-based cohort.

Methods: The study cohort consisted of all singleton live births in Sweden from 1990-2008 using data from Swedish national registers. Mode of delivery included: unassisted vaginal delivery (VD), assisted VD, elective CS or emergency CS. ADHD was determined using International Classification of Diseases version 10 (F90 or F98.8), or prescription for ADHD medication. We used Cox regression to assess the association between birth by CS and ADHD in the total study population, adjusting for perinatal and socio-demographic factors, then stratified Cox regression analysis on maternal ID to assess the association among siblings.

Results: Our cohort consisted of 1,722,548 children, and 47,778 cases of ADHD. The HR of the association between elective CS, compared to unassisted VD, and ADHD was 1.15 [95% CI: 1.11-1.20] in the cohort, and 1.05 [95% CI: 0.93-1.18] in the stratified analysis. The HR of the association between emergency CS and ADHD was 1.16 [95% CI: 1.12-1.20] in the cohort and 1.13 [95% CI: 1.01-1.26] in the stratified analysis.

Conclusion: Birth by CS is associated with a small increased risk of ADHD. However among siblings the association only remained for emergency CS. If this were a causal effect of CS, the association would be expected to persist for both types of CS, suggesting the observed association is due to confounding.

5.2 Background

Attention-deficit/hyperactivity disorder (ADHD), which is characterized by difficulty with attention, hyperactivity and impulsivity, affects an estimated 5.9-7.1% of children and adolescents worldwide[33]. While some studies have suggested an increasing prevalence of ADHD[194,195], a recent paper has demonstrated that the reported variability in prevalence estimates is due to methodological differences, and the reported increasing prevalence among clinical populations may reflect increased awareness, access to services or changes in diagnostic practices rather than true increases[34]. ADHD is the most common and longest recognized neurodevelopmental disorder in children, and often persists into adulthood[196]. It not only affects health outcomes directly, but is associated with various co-morbidities such as anxiety, mood disorders, antisocial behaviours and addiction[197,198]. In addition, ADHD leads to loss of productivity, increased costs for healthcare and education, and increased conviction rates[68]. In the United States alone, ADHD costs an estimated $143-266 billion annually[68]. Estimates of costs in Europe are lower, for example around €1,041-€1,529 million for the Netherlands, though these did not include costs relating to adult patients, which could account for the difference[69]. ADHD is highly heritable[37], but emerging evidence suggests that environmental factors and genetic factors interact to contribute to the development of ADHD[35]. Moreover, perinatal factors such as low birth weight and pre-term birth have been associated with increased risk of ADHD[35].

It has been hypothesized that birth by Caesarean section (CS) may affect psychological development in children through a variety of mechanisms, including exposure to altered microbiota[54], and inadequate priming of the stress response[50]. In animal models, birth by CS has been shown to alter dopamine response into

adulthood, which is of particular interest as changes in dopamine have also been linked to ADHD[112]. Notably, psychological development could be associated with CS through residual confounding, or what's known as confounding by indication[86], meaning the measured association is related to the underlying indication for CS rather than due to a causal effect of the CS. Elective CS is scheduled before labour, usually due to a combination of maternal and/or foetal characteristics (e.g. malpresentation, multiple gestation etc.) or as a result of clinician or maternal choice. Emergency CS is defined as delivery by CS after a woman is in labour, when there are complications meaning vaginal delivery is no longer safe[49]. Unlike with emergency CS, with elective CS mothers and infants are not exposed to excessive distress, and infants are not exposed to maternal microbiota in the birth canal[49]. When medically indicated, CS can be a lifesaving procedure for both mothers and infants[13], but can cause significant and sometimes permanent complications[199]. The WHO have estimated that the ideal rate of CS is 10-15%[13]. However, in 2009 the average rate of CS among countries in the Organisation for Economic Co-operation and Development (OECD) was 25.8%, and this is increasing worldwide[54]. With such a high exposure prevalence, even a small absolute increase in risk of harm on child development could have a large impact on public health.

Despite the biological plausibility of a potential causal relationship between birth by CS and ADHD, our recent systematic review found previous research in this area to be inconsistent and limited[151]. Only two studies reported an adjusted association between birth by CS and ADHD[38,39], neither of which distinguished between elective and emergency CS in their fully adjusted models, and neither of which included socio-demographic variables specific to the family, or parental mental health (with the exception of maternal ADHD). The association between

perinatal risk factors and psychological development is complex, as many factors are associated both with such risk factors and psychological development, including parental mental health and social adversity[84].

The objectives of the present study are to assess the association between mode of delivery and ADHD using a large, population-based cohort and to conduct a sibling-matched analysis to interrogate potential causality of any association detected.

5.3 Methods

Study population

The study cohort consisted of all singleton live births in Sweden from 1990-2008 using data from the Swedish national registers including the Medical Birth Register, the National Patient Register, and the Multi-generation Register. Data from these registers can be linked using the personal identification number (PIN) given to each Swedish resident. Over 99% of all births in Sweden are recorded in the Medical Birth Register[184]. A more detailed description of the Swedish National Registers can be found in **Appendix 7**. An outline of our study population is provided in **Figure 5-1**.

Exposure variable: mode of delivery

Mode of delivery was divided into four categories; unassisted VD, assisted VD, elective CS, and emergency CS. Assisted VD was defined as VD with the use of forceps or vacuum extraction. Unassisted VD included both spontaneous and induced VD. Elective and emergency CS were defined as CS before and after onset of labour respectively. Onset of labour, as recorded in medical records, was defined as water

departure, bleeding, or regular and sustained pain. Though the Medical Birth Register began in 1973, variables indicating emergency and elective CS are only available from 1982. More detailed information on birth, including induction of labour, as well as additional socio-demographic data, are available beginning in 1990.

Outcome variable: ADHD diagnosis

To determine an ADHD diagnosis we used *International Classification of Diseases* version 10 (ICD-10) codes F90 and F98.8, or prescription of the psychostimulants methyphenidate (ATC code: N06BA04), amphetamine (N06BA01), dexamphetamine (N06BA02) or the noradrenergic reuptake inhibitor atomoxetine (N06BA09). Inpatient ICD-10 codes are available beginning in 1997, and outpatient available from 2001. Prescriptions are available beginning in July, 2005[200].

Other covariates

Potential confounders were identified using previous literature and examined for inclusion using a directed acyclic graph (DAG) (**Appendix 25**)[96].We obtained data on small for gestational age (SGA), large for gestational age (LGA), infant sex, maternal age at birth, maternal smoking during pregnancy, gestational age, birth order, 5 minute Apgar score, and maternal and paternal country of birth from the Medical Birth Register. Maternal smoking during pregnancy was measured at the time of the first antenatal visit and was divided into three categories: "no smoking," "smoking 1-9 cigarettes a day," and "smoking 10 or more cigarettes a day." For socio-economic factors we included variables on social welfare, disposable income, and parental highest education level. The variable on social welfare indicated

whether either parent received social welfare the year the index child was born. The variable on disposable income indicated the disposable income of the household the year the child was born, and was divided into quintiles, ranging from "low income" to "high income". Parental highest education level was divided into three categories: "pre-high school," "high school," and "post high school".

We obtained data on maternal and paternal depression (ICD-8: 29600, 3004; ICD-9: 296B, 311, 300E; ICD-10: F32, F33), bipolar disorder (ICD-8: 29610-29630; ICD-9: 296A,C-E; ICD-10: F30-31), and non-affective psychiatric disorders (ICD-8: 295, 297, 298 [excluding 29800 and 29810], 29999; ICD-9: 295, 297, 298 [excluding 298A and 298B]; ICD-10: F20-29) from the National Patient Register, which has data on inpatient care from 1964 and outpatient care from 2001. Using date of birth and date of first diagnosis, maternal and paternal depression, bipolar disorder and non-affective psychiatric disorder were divided into three categories: never diagnosed, first diagnosis before birth of each child and first diagnosis after birth of each child.

Analysis

First, we performed Cox regression to estimate hazard ratios (HRs) and 95% CIs. Diagnosis of ADHD is difficult in infancy, as most children are inattentive and hyperactive in that time[201]. Therefore, we began follow-up of participants on their 3rd birthday for any child born after January 1 1994. As ICD-10 was available beginning in 1997, for children who turned three prior to 1997 we began follow-up on January 1, 1997. Participants were followed until ADHD diagnosis or medication, death, emigration, or the end of study period (December 31, 2011). We assessed the proportional hazards assumption using graphical displays of the empirical score of martingale residuals, and found it to be violated. Therefore, we partially adjusted all

analyses for year of birth. In fully adjusted models, we also included infant gender, maternal age, gestational age, maternal and paternal citizenship, SGA, LGA, 5 minute Apgar score, parity, maternal smoking during pregnancy, social welfare status, parental education, family disposable income, and maternal and paternal depression, bipolar disorder, and non-affective disorder.

We then repeated the analysis starting follow-up on January 1, 2001, including only ADHD diagnoses entered since outpatient data became available in Sweden (**Appendix 26**). We also conducted sensitivity analyses by excluding children who were preterm (<37 weeks' gestation), female, SGA, LGA, born outside of Stockholm County (due to possible differences in diagnostic validity and ADHD medication use), induced labour, and children whose mothers had ever been diagnosed with depression, non-affective disorder or bipolar disorder. We restricted the analysis separately to the first child, second child, children with birth order 3 or higher, one-child families, and primary CS. We also conducted a sensitivity analysis, re-running the model including children diagnosed before 3 years of age.

For the sibling-matched analyses, we conducted Cox regression stratified on maternal ID. Though the entire population was included for the stratified Cox regression and thus contributed to covariate estimates, only siblings discordant on both mode of delivery and ADHD were considered "informative" and contributed to the overall estimate. The sibling-matched analysis included adjustment for the same covariates as the total cohort analysis, with the exception of maternal country of birth, which was the same for each stratum. Similar to the total cohort analysis, we repeated analysis with follow-up beginning on January 1, 2001, and conducted a sensitivity analysis on medicated cases only. Additionally, we conducted conditional logistic regression on matched sibling pairs (methods detailed in **Appendix 27**).

All analyses were conducted using SAS 9.4, and we used robust confidence intervals clustered on maternal ID to account for differential risk of ADHD within families.

5.4 Results

Descriptive statistics

There were 1,921,793 live births in Sweden between January 1, 1990 and December 31, 2008. After excluding multiple births (n =54,919), unclear modes of delivery (n = 115,445), children who died before age 3 or the year 1997 (n = 6,799), children who emigrated before age 3 or the year 1997 (n = 21,773) and children who were diagnosed with ADHD before age 3 or the year 1997 (n = 308), there were 1,722,548 in the cohort (**Figure 5-2**). The majority of these were delivered through non-assisted VD (n = 1,353,845; 78.6%), followed by assisted VD (n = 130,007; 7.6%), emergency CS (n = 124,621; 7.2%) and elective CS (n = 114,066; 6.6%) (**Table 5-1**).

Overall, we included 47,778 cases of ADHD (2.8%), the majority of whom were prescribed medication at some point (n = 36,694; 76.8%). The majority of cases were recorded both with ICD code and medication (n = 30,607; 64.0%), however there were 6,387 (13.4%) cases with only medication and 10,784 (22.6%) with only ICD diagnosis. Prevalence of ADHD rose at an increasing rate from 1997-2011, with a dramatic increase in 2001 when outpatient data became available, and again in 2005 when the Prescribed Drug Register became available (**Figure 5-1**).

Cohort analysis

There was little evidence of an association between assisted VD, compared to unassisted VD, and ADHD in the partially (adjusted only for birth year) (HR = 1.04; [95% CI: 1.01-1.08]) or fully adjusted analysis (HR = 1.02; [95% CI: 0.98-1.06]). For elective CS, the HR was 1.27 [95%CI: 1.22-1.32] in partially adjusted and 1.15 [95%CI: 1.11-1.20] in fully adjusted analysis, when compared to unassisted VD. For emergency CS the HR was 1.27 [95%CI: 1.23-1.31] in partially adjusted and 1.16 [95%CI: 1.12-1.20] in fully adjusted analysis. Results were similar when follow-up was started in 2001 (**Table 5-2**).

Sensitivity analyses excluding preterm babies, females, children with maternal history of depression, bipolar disorder or non-affective psychiatric disorder, children born outside Stockholm County, children with low Apgar scores, induced deliveries, and non-medicated cases of ADHD showed no material change in the association (**Appendix 28**). Similarly, sensitivity analyses by birth order, CS order, and one-child families showed no changes in association (**Appendix 29**).

Of the 1,241,366 families included in the stratified Cox regression, 6,976 were discordant on both ADHD and mode of delivery (including a total of 17,382 children and 7,291 cases). The HR of the association between assisted VD, compared to unassisted VD, and ADHD was 1.10 [95% CI: 1.08-1.27] in partially adjusted and 1.04 [95% CI: 0.94-1.15] in fully adjusted analysis (**Table 5-3**). For elective CS, the HR was 1.16 [95% CI: 1.48-1.29] in partially adjusted and 1.05 [95% CI: 0.93-1.18] in fully adjusted analysis. For emergency CS, the HR was 1.24 [95%CI: 1.12-1.37] in partially adjusted and 1.13 [95%CI: 1.01-1.26] in fully adjusted analysis. Results were similar when follow-up was started in 2000 (**Table 5-3**). Sensitivity analysis excluding ADHD cases that were not first-born and were not

medicated also provided similar results (**Appendix 30**). Similar results were received from the conditional logistic regression performed on matched sibling pairs (**Appendix 31**).

5.5 Discussion

This study examined the association between mode of delivery and ADHD in a large, population-based cohort with survival analysis both in the general population, and amongst siblings. The survival analysis indicated that birth by CS was associated with a 14-16% increased risk of ADHD compared to vaginal delivery, and this association persisted after controlling for a large number of known confounders and in an extensive range of sensitivity analyses. However, the association was largely ameliorated in the sibling-matched analyses suggesting that this association may not be causal but is more likely due to familial or environmental confounding. As emergency CS is started after the onset of labour, children are likely exposed to their mother's microbiota, and elevated stress hormones associated with vaginal delivery[49,50]. Therefore, while an association between emergency CS and ADHD did persist in the sibling-matched analyses, such an association with emergency and not elective CS is not consistent with a causal relationship through altered microbiota or stress response, and is more likely due to confounding by indication[49].

Ideally, in epidemiological investigation, we rely on randomization to assess the potential causality of an association. However, when such designs would be unethical or not feasible, as is the case with mode of delivery and child psychological development, we must rely on large cohorts with a wide range of measured confounders to assess associations. The use of sibling-matched designs can be seen as an additional way to control for unmeasured confounders that are shared between

siblings, such as genetics or family environment[90]. This is particularly important for pre-, peri- or postnatal exposures and psychiatric outcomes as both are likely to have a genetic component, making causality difficult to determine[84].

Previous sibling-control designs have found an attenuation of the association between CS and asthma[91], type 1 diabetes[92] and autism spectrum disorder[152]. For ADHD, sibling-control designs have previously provided evidence for potential causal relationships with preterm birth[202] and family income in early childhood[203], but not maternal age at birth[204], maternal pre-pregnancy body mass index[205], or maternal smoking during pregnancy[191,206]. An attenuation of a previously measured association with sibling-matched analysis, such as is seen in the present study, provides evidence for a non-casual association[190]. For example it is possible that women with ADHD were more likely to give birth through CS and maternal ADHD is a known risk factor for ADHD in the offspring (and women with psychiatric illness have previously been found to be more likely to request a CS[81]). Similarly, given the increased preference for CS among women with anxiety, and the high co-morbidity rate of anxiety disorders with ADHD[196], the increased risk could be associated with maternal anxiety. Therefore, from our results we can infer that though birth by CS is associated with ADHD, it is likely due to genetic or environmental influences, and is not a causal relationship.

Notably, while birth by CS may not increase the risk of ADHD, CS without a medical indication is associated with increased morbidity in mothers and children[199]. Clinicians and expectant mothers should fully consider all risks before undertaking this mode of delivery.

Previous studies

Our recent systematic review identified four studies have estimated the association between birth by CS and ADHD[151]. Only two of these studies reported adjusted estimates[38,39]. The adjusted OR by Ketzer et al. (adjusted for IQ, maternal ADHD and smoking during pregnancy, and matched on gender and age) was 1.3, in line with our partially adjusted HR (no unadjusted estimate was reported)[39]. The investigation by Silva et al. found no association between CS and ADHD in their full model, but in their partially adjusted model (adjusted for year of birth and socioeconomic status of the residential area) they reported ORs for elective CS of 1.15 and 1.17 for males and females, respectively[38], similar to the estimate in our fully adjusted model. Two additional papers by our group examined birth by CS and ADHD, one reported an association between parent-rated hyperactivity and emergency CS only[42], the other reported no association, either with parent-reported ADHD or parent-rated hyperactivity[207]. However, as the outcomes in these papers were parent-reported rather than clinically diagnosed, they may be more indicative of specific outcomes such as disruptive behaviour or hyperactivity rather than the wider range of behaviours associated with a clinical diagnosis of ADHD.

Strengths and limitations

The current investigation has several strengths. To our knowledge, this is the largest study conducted on the relationship between mode of delivery and ADHD, and the first to use sibling-matched analysis. In addition, as we used data from a national registry, recall and selection biases were limited. Moreover, we were able to control for a large variety of potential confounders, including demographic and socio-economic information, and parental mental health, which were missing from

previous investigations. We were also able to address the concern about generalizability of sibling-control design (i.e. concern that the first two children may have been meaningfully different than others, or one-child families may have been meaningfully different than families with at least 2 children) through our sensitivity analyses on birth order, CS order, and family size, none of which showed any change in results.

This study is also subject to some limitations. Sibling-matched analysis assumes that siblings are more similar in confounders than exposure[89], though we were able to address possible concerns with relatedness in mode of delivery with sensitivity analyses examining CS order, birth order, and family size, which implied that any clustering of exposure did not impact our results. In addition, measurement error within families could lead to an artificially attenuated estimate in sibling-control designs[89]. This register has previously been shown to be accurate in reporting mode of delivery for term babies (though in preterm births distinguishing between elective and emergency CS is less accurate)[208]. However, with regards to measurement error in our outcome, data were anonymized and thus we were unable to validate individual cases. High correlation between our definition of ADHD in this cohort and parent-reported diagnoses suggests this is a valid way to determine cases[209]. Moreover, we addressed this limitation through our sensitivity analysis excluding non-medicated cases which did not change our findings. Also, due to the strict criteria of ICD-10, lack of outpatient data until 2001 (with increasingly better coverage until 2006), lack of medication data until 2005, and restrictive recommendations for medication in the guideline from the National Board of Health and Welfare, it is likely we have an over-representation of more severe cases of ADHD. However, our sensitivity analysis excluding non-medicated cases, as well as

our cohort analysis beginning in 2001 (when outpatient data became available) showed no changes in results, indicating that including less severe cases may have not had a large impact on our findings. In addition, if the attenuation of the association in sibling-matched analysis does indeed indicate confounding, there is no way to determine how much of the association was due to genetic vs. environmental influences.

5.6 Conclusion

Birth by CS is associated with a small increased risk of ADHD after controlling for known confounders. However among siblings the association only remained for emergency CS. If this were a causal effect, we would have expected the association to also remain with elective CS, indicating this association is likely due to confounding.

Table 5-1. Perinatal and socio-demographic variables related to attention-deficit/hyperactivity disorder and mode of delivery among those born in Sweden from 1990 to 2008.

	Total Population	Unassisted VD	Assisted VD	Elective CS	Emergency CS
Total Population	1,722,548	1,353,854 (78.60)	130,007 (7.55)	114,066 (6.62)	124,621(7.23)
ADHD	47,778 (2.77)	37,099 (2.74)	3,449 (2.65)	3,340 (2.93)	3,890(3.12)
Small for gestational age	39,616 (2.31)	22,562 (1.68)	3,335 (2.58)	7,222 (6.38)	6,497(5.26)
Large for gestational age	62,355 (3.64)	43,019 (3.19)	3,620 (2.80)	8,714 (7.70)	7,002(5.66)
First born child	735,450 (42.82)	515,542 (38.19)	102,443 (79.04)	39,290(34.55)	78,175 (62.93)
Induced	155,503 (9.05)	110,607 (8.19)	17,220 (13.29)	N/A	27,676 (22.28)
Sex*(male)*	883,115 (51.42)	681,669 (50.49)	74,565 (57.53)	58,042 (51.05)	68,839 (55.42)
Maternal age					
<25 years	314,165 (18.24)	259,798 (19.19)	24,575 (18.90)	11,183 (9.80)	18,609 (14.93)
25-34 Years	1,131,441 (65.68)	893,887 (66.03)	86,467 (66.56)	70,418 (61.73)	80,669 (64.73)
35-44 years	275,280 (15.98)	199,167 (14.71)	18,871 (14.52)	32,104 (28.15)	25,138 (20.17)
45+ years	1,662 (0.10)	1,002 (0.07)	94 (0.07)	361 (0.32)	205 (0.16)
Gestational age					
<37 weeks	82,553 (4.81)	48,890 (3.62)	3,493 (2.70)	16,995 (14.95)	13,175 (10.61)
37 weeks	83,279 (4.85)	59,565 (4.41)	4,102 (3.17)	11,838 (10.41)	7,774 (6.26)
38 weeks	233,493 (13.59)	154,220 (11.42)	10,543 (8.13)	53,899 (47.40)	14,831 (11.94)
39 weeks	394,298 (22.96)	331,590 (24.56)	24,530 (18.93)	20,331 (17.88)	17,847 (14.37)
40 weeks	485,516 (28.27)	415,589 (30.78)	38,556 (29.75)	5,157 (4.54)	26,214 (21.10)
>40 weeks	436,711 (25.43)	338,849 (25.10)	48,275 (37.25)	5,359 (4.71)	44,228 (35.61)
Mother smoking at time of first antenatal visit					
None	1,532,937(89.25)	1,200,792 (88.94)	117,930 (90.99)	103,196 (90.76)	111,019 (89.38)
1-9 cigarettes/day	173,398 (10.10)	140,039 (10.37)	10,958 (8.46)	10,011 (8.80)	12,390 (9.97)
10+ cigarettes/day	11,273 (0.66)	9,252 (0.69)	714 (0.55)	500 (0.44)	807 (0.65)
Apgar score at 5 minutes					
Low *(0-3)*	2,881 (0.17)	1,258 (0.09)	465 (0.36)	331 (0.29)	827 (0.67)
Intermediate *(4-6)*	12,849 (0.75)	4,675 (0.35)	2,956 (2.29)	1,644 (1.46)	3,574 (2.90)
High *(7-10)*	1,688,879 (99.08)	1,333,825 (99.56)	125,579 (97.35)	110,681 (98.25)	118,794 (96.43)
Mother Country of birth					
Sweden	1,439,426 (83.80)	1,133,497(83.96)	109,482(84.48)	94,860(83.42)	101,587(81.78)
Other Nordic	41,687 (2.43)	32,797(2.43)	2,787(2.15)	3,019(2.66)	3,084(2.48)
Other	236,368 (13.76)	183,710(13.61)	17,320(13.36)	15,818(13.91)	19,520(15.71)

Table 5-1 Continued

	Total Population	Unassisted VD	Assisted VD	Elective CS	Emergency CS
Father Country of birth					
Sweden	1,421,454(82.76)	1,115,657(82.64)	108,503(83.73)	95,154(83.68)	102,130(82.22)
Other Nordic	39,507(2.30)	31,314 (2.32)	2,701(2.08)	2,725(2.40)	2,767 2.23)
Other	246,028(14.32)	195,313(14.47)	17,403(13.43)	15,027(13.22)	18,285(14.72)
Maternal Depression					
Never	1,575,21 (91.71)	1,241,893 (91.99)	119,101 (91.90)	101,762(89.49)	112,463(90.54)
Diagnosed before birth	26,510 (1.54)	18,709 (1.39)	2,094 (1.62)	3,150 (2.77)	2,557(2.06)
Diagnosed after birth	115,879 (6.75)	89,481 (6.63)	8,407 (6.49)	8,795 (7.73)	9,196 (7.40)
Maternal Bipolar Disorder					
Never	1,701,904 (99.09)	1,338,040 (99.11)	128,512 (99.16)	112,387 (98.84)	122,965 (98.99)
Diagnosed before birth	1,912 (0.11)	1,336 (0.10)	158 (0.12)	227 (0.20)	191(0.15)
Diagnosed after birth	13,792 (0.80)	10,707 (0.79)	932 (0.72)	1,093 (0.96)	1,060 (0.85)
Maternal Non-affective Disorder					
Never	1,706,145 (99.33)	1,341,378 (99.36)	128,777 (99.36)	112,744 (99.15)	123,246 (99.22)
Diagnosed before birth	4,261 (0.25)	3,086 (0.23)	314 (0.24)	460 (0.40)	401 (0.32)
Diagnosed after birth	569 (0.42)	5,619 (0.42)	511 (0.39)	503 (0.44)	569 (0.46)
Paternal Depression					
Never	1,634,972 (95.19)	1,285,170 (95.19)	123,749 (95.48)	108,006 (94.99)	118,047 (95.03)
Diagnosed before birth	14,635 (0.85)	10,984 (0.81)	1,142 (0.88)	1,259 (1.11)	1,250 (1.01)
Diagnosed after birth	68,001 (3.96)	53,929 (3.99)	4,711 (3.63)	4,442 (3.91)	4,919 (3.96)
Paternal Bipolar Disorder					
Never	1,708,334 (99.46)	1,342,818 (99.46)	128,968 (99.51)	113,039 (99.41)	123,509 (99.43)
Diagnosed before birth	1,537 (0.09)	1,172 (0.09)	119 (0.09)	125 (0.11)	121 (0.10)
Diagnosed after birth	7,737 (0.45)	6,093 (0.45)	515 (0.40)	543 (0.48)	586 (0.47)
Paternal Non-affective Disorder					
Never	1,706,261 (99.34)	1,341,097 (99.33)	128,782 (99.37)	112,986 (99.37)	123,396 (99.34)
Diagnosed before birth	4,543 (0.26)	3,479 (0.26)	374 (0.29)	324 (0.28)	366 (0.29)
Diagnosed after birth	6,804 (0.40)	5,507 (0.41)	446 (0.34)	397 (0.35)	454 (0.37)

Table 5-1 Continued

	Total Population	Unassisted VD	Assisted VD	Elective CS	Emergency CS
Income Quintile					
First	324,287 (18.88)	267,897 (19.84)	17,380 (13.41)	19,217 (16.90)	19,793 (15.93)
Second	342,870 (19.96)	280,608 (20.78)	18,681 (14.41)	22,624 (19.90)	20,957 (16.87)
Third	347,057 (20.21)	275,614 (20.41)	24,334 (18.78)	23,027 (20.25)	24,082 (19.39)
Fourth	349,073 (20.32)	267,664 (19.83)	30,858 (23.81)	23,228 (20.43)	27,323 (22.00)
Fifth	343,030 (19.97)	249,029 (18.45)	37,635 (29.04)	25,112 (22.08)	31,254 (25.16)
Highest Parental Education					
Pre-high school	118,898 (6.92)	96,966 (7.18)	6,819 (5.26)	7,126 (6.27)	7,987 (6.43)
High school	816,138 (47.52)	650,105 (48.15)	57,459 (44.33)	51,374 (45.18)	57,200 (46.05)
Post high school	759,177 (44.20)	583,937 (43.25)	63,787 (49.22)	54,141 (47.61)	57,302 (46.14)
Parents on welfare	173,398 (10.10)	140,039 (10.37)	10,958 (8.46)	10,011 (8.80)	12,390 (9.97)

Abbreviations: VD-vaginal delivery; CS-Caesarean section; ADHD-attention-deficit/hyperactivity disorder

Table 5-2. The association between mode of delivery and attention-deficit/hyperactivity disorder.

	Exposed Cases	Partially Adjusted HR from 1997			Fully Adjusted HR from 1997			Exposed Cases	Partially Adjusted HR from 2001			Fully Adjusted HR from 2001		
Unassisted VD	37,099	Ref			Ref			36,524	Ref			Ref		
Assisted VD	3,449	1.04	(1.01	1.08)	1.02	(0.98	1.06)	3,383	1.04	(1.00	1.08)	1.02	(0.98	1.06)
Elective CS	3,340	1.27	(1.22	1.32)	1.15	(1.11	1.20)	3,285	1.26	(1.22	1.31)	1.15	(1.10	1.19)
Emergency CS	3,890	1.27	(1.23	1.31)	1.16	(1.12	1.20)	3,814	1.26	(1.22	1.30)	1.16	(1.12	1.20)

Abbreviations: HR-Hazard ratio; VD-vaginal delivery; CS-Caesarean section
Partially adjusted-Adjusted for year of birth; *Fully adjusted*-Adjusted for year of birth, infant gender, maternal age, maternal smoke during pregnancy, gestational age, 5 minute Apgar score, maternal and paternal country of birth, small for gestational age, large for gestational age, first born, family income, maternal and paternal depression, bipolar disorder, and non-affective disorder

Table 5-3. The association between mode of delivery and attention-deficit/hyperactivity disorder using sibling controls.

	Exposed Cases[a]	Partially Adjusted HR from 1997			Fully Adjusted HR from 1997			Exposed Cases	Partially Adjusted HR from 2001			Fully Adjusted HR from 2001		
Unassisted VD	3,094	Ref			Ref			3,055	Ref			Ref		
Assisted VD	1,560	1.10	(1.08	1.27)	1.04	(0.94	1.15)	1,528	1.11	(1.01	1.23)	1.03	(0.92	1.14)
Elective CS	1,182	1.16	(1.05	1.29)	1.05	(0.93	1.18)	1,162	1.10	(0.98	1.23)	0.99	(0.87	1.12)
Emergency CS	1,455	1.24	(1.12	1.37)	1.13	(1.01	1.26)	1,426	1.25	(1.13	1.39)	1.13	(1.00	1.27)

Abbreviations: OR-Odds ratio; VD-vaginal delivery; CS-Caesarean section

Partially adjusted-Adjusted for year of birth; *Fully adjusted*-Adjusted for year of birth, infant gender, maternal age, maternal smoke during pregnancy, gestational age, 5 minute Apgar score, paternal country of birth, small for gestational age, large for gestational age, first born, family income, maternal and paternal depression, bipolar disorder, and non-affective disorder

[a]Note: Number of exposed cases reflects the number in strata discordant on both mode of delivery and ADHD

Figure 5-1. Timeline of study design follow-up and cases of attention-deficit/hyperactivity disorder (ADHD) by year.

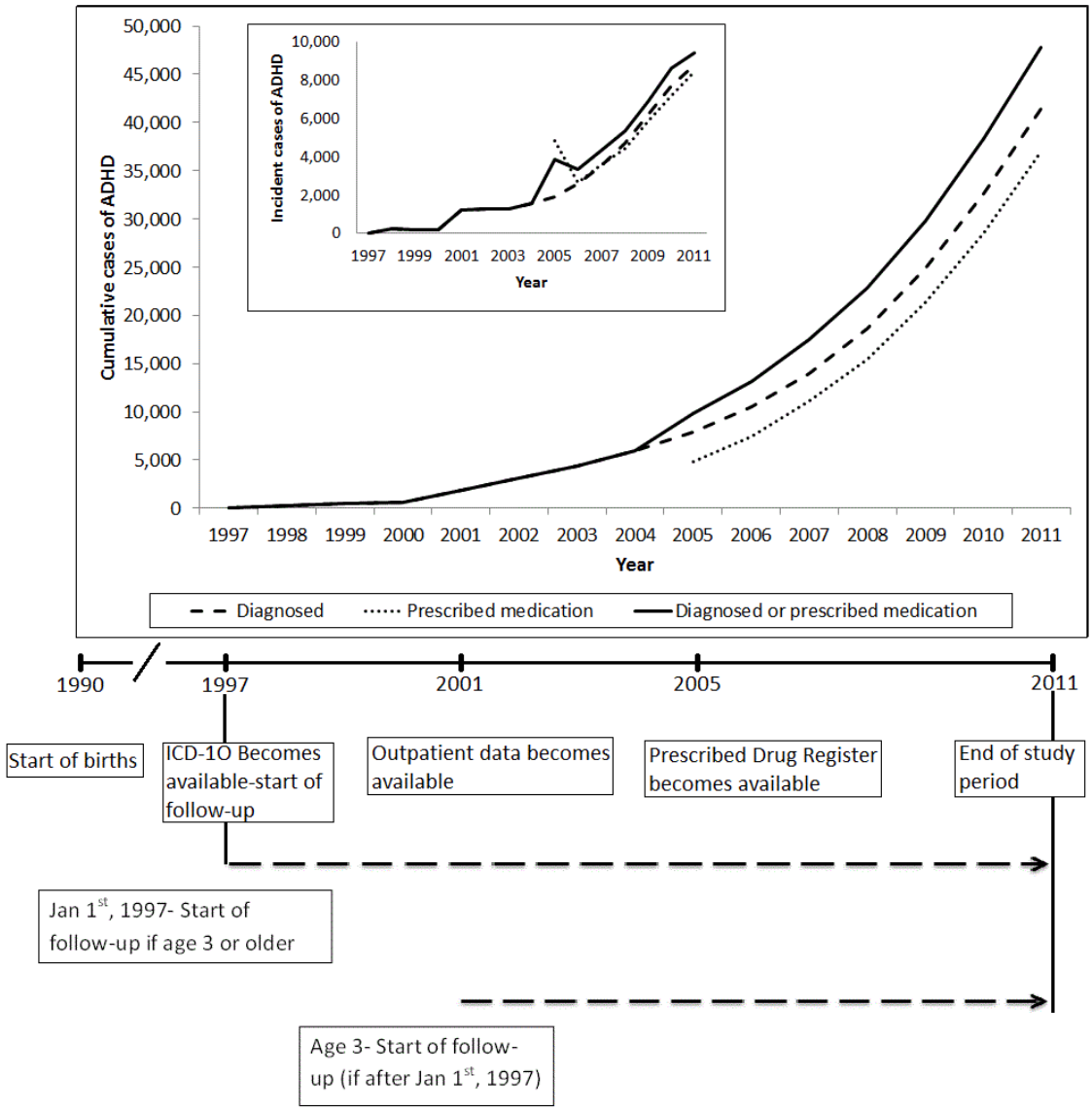
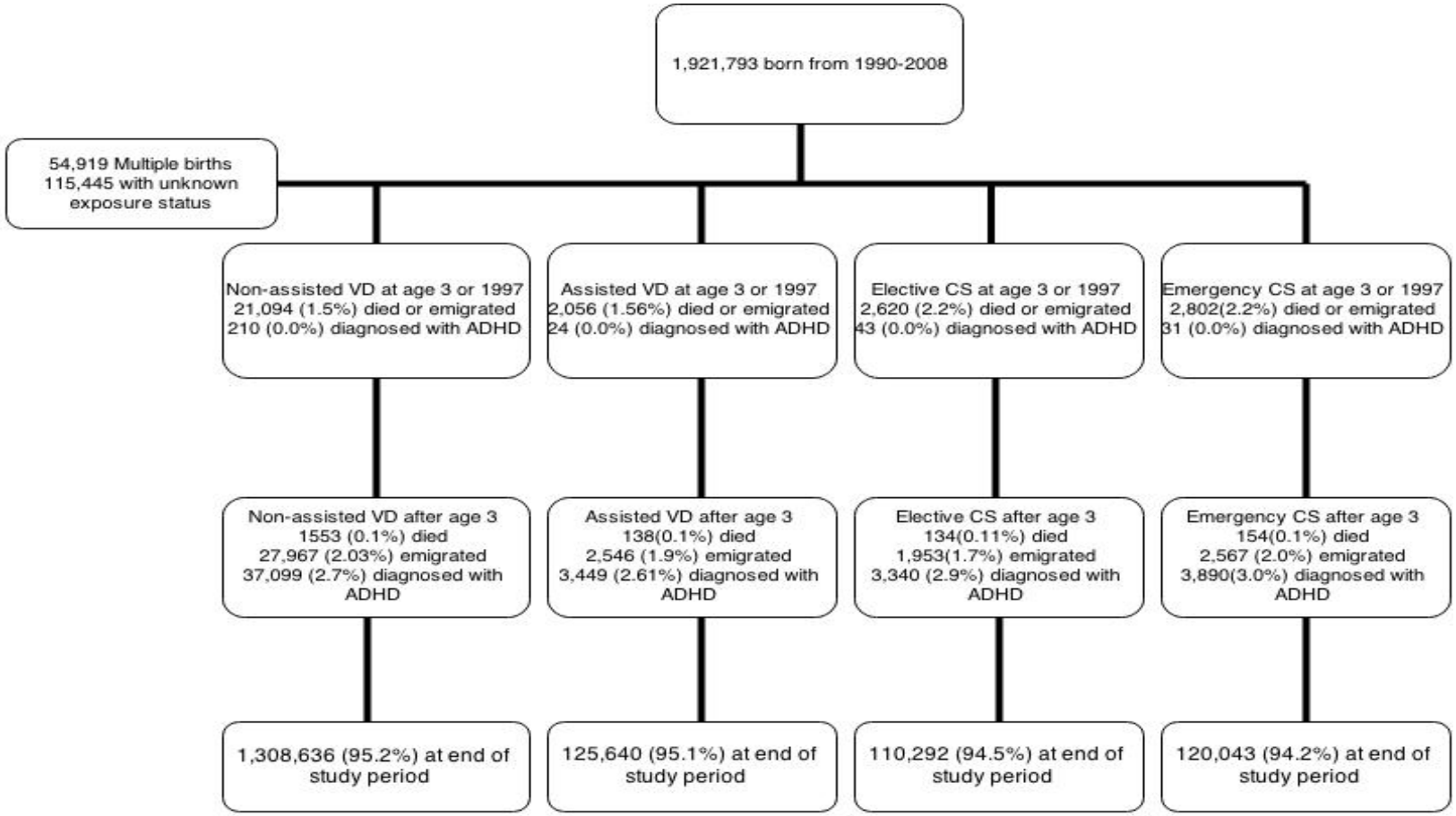


Figure 5-2. Reasons for censoring study participants by mode of delivery.



CHAPTER 6. BIRTH BY CAESAREN SECTION AND SCHOOL PERFORMANCE IN SWEDISH ADOLESCENTS

Eileen A. Curran[1], Louise C. Kenny[1], Christina Dalman[2], Patricia M Kearney[3], John F. Cryan[4], Timothy Dinan[4], Ali S. Khashan[1,3]

[1]The Irish Centre for Fetal and Neonatal Translational Research (INFANT), University College Cork, Ireland.

[2]Division of Public Health Epidemiology, Karolinska Institutet, Sweden.

[3]Department of Epidemiology and Public Health, University College Cork, Ireland

[4]APC Microbiome Institute, University College Cork, Ireland

6.1 Abstract

Background: As rates of birth by Caesarean section (CS) continue to rise globally, it is becoming increasingly important to investigate any potential long-term effects it may have on child development. Our objective was to assess the impact of obstetric mode of delivery, and in particular birth by CS, on school performance in adolescents using a large, population-based cohort.

Methods: We extracted data from the Swedish Medical Birth Register and National School Register. Mode of delivery was defined as: unassisted vaginal delivery (VD), assisted VD, elective CS and emergency CS. School grades were reported on a scale from 0 to 320, scores less than 160 (i.e. "pass") were considered to be "poor school performance." We measured the association between mode of delivery and poor school performance using logistic regression, with a random intercept for school ID. We then used quantile regression to assess the association between mode of delivery and school performance across the distribution of scores. We adjusted for maternal age, parity, small and large for gestational age, gestational age, maternal country of birth, maternal depression, non-affective disorder or bipolar disorder, parental income at time of birth, and parental social welfare at time of birth. We also conducted several sensitivity analyses to investigate the association further.

Results: With logistic regression analysis, the adjusted odds ratio (aOR) of assisted VD and poor school performance, when compared to unassisted VD, was 1.06 (95% CI: 1.03-1.08). For elective CS it was 1.06 (95% CI: 1.03-1.09) and for emergency CS it was 1.12 (95% CI: 1.09-1.15). With quantile regression, assisted VD showed little difference in scores, when compared to unassisted VD, at any point across the distribution. Elective CS was associated with a 1-3 point decrease in scores, and emergency CS was associated with a 2-5 point decrease in scores.

Conclusion: Birth by CS is associated with a slight reduction in school performance. Possible explanations include an effect on a more specific outcome such as anxiety, or a non-causal association driven by confounding by indication or residual confounding.

6.2 Background

Rates of Caesarean section (CS) are rising globally. A recent study including data from thirty one European countries, reported the median rate of CS in those countries to be 25%[10]. However, high prevalence of CS is by no means limited to Europe or other high-income countries. Another recent study reported that in 2010-2011, among the 21 countries included, the rate of CS in countries with a high Human Development Index (HDI) was 40.0%, in countries with moderate HDI it was 32.4% and in countries with low HDI it was 20.3%[12]. In fact, only one country (Japan) did not report an increasing rate of CS over the study period. Given such a large and growing rate of CS, it is becoming increasingly important to understand potential long-term effects of birth by CS, as even a small increase in risk could potentially have a large impact globally.

It has been hypothesised that birth by CS can lead to changes in psychological development, due to "early term" birth[48,210] or alterations in microbiota or stress response[50]. In animal models, birth by CS and changes in microbiota have been associated with changes in behaviour, stress response, and anxiety[55]. Despite these theories, previous evidence suggests the association between CS and neurodevelopmental disorders in human populations may be primarily driven by confounding[152]. However, it is also possible that birth by CS may lead to sub-clinical changes in behavioural development that are not included in official diagnoses, for example increased anxiety. One way to potentially assess the overall impact on psychological well-being is through school performance.

School performance has been associated with several psychological outcomes including attention problems[211], substance abuse[211], and insomnia[44]. In addition, school performance as a teenager has been linked to psychological well-being as an

adult, including depression and self-harm[46,212]. Furthermore, behavioural difficulties and specific personality traits have been associated with school performance in children[43], making school performance a potential way to assess overall behavioural development.

While elective CS has been associated with delays in personal social skills and gross motor function at age 9 months[42], and early term birth has been associated with increased special education requirements[48], to our knowledge, no one has examined the potential association between obstetric mode of delivery, more specifically birth by CS, on school performance. To that end, the objective of the current study was to investigate the possible impact of mode of delivery on school performance in a large, population-based Swedish cohort.

6.3 Methods

Data

Study Population

We included data from 4 Swedish Registers: the Medical Birth Register, the National School Register, the Multi-Generation Register, and the National Patient Register. Each resident of Sweden is given a personal identification number (PIN) which is the same in each of these registers, and can be used to link data across registers. The Medical Birth Register was established in 1973 and includes data on over 98% of all births in Sweden[213]. For our cohort, we included all live singleton births in the Swedish Medical Birth Register that occurred between 1982 and 1995.

Exposure-obstetric mode of delivery

Obstetric mode of delivery, extracted from the Medical Birth Register, consisted of "unassisted vaginal delivery (VD);" "assisted VD," "elective Caesarean section (CS)" and "emergency CS". Unassisted VD was defined as VD without the use of forceps or vacuum extraction, and assisted VD was VD with the use of forceps or vacuum extraction. Unassisted and assisted VD included both spontaneous and induced VD. Timing of onset of labour (as indicated on medical charts by water departure, bleeding or regular and sustained pain) determined type of CS. Elective CS was defined as CS which started before onset of labour and emergency CS was defined as CS which started after onset of labour. Variables detailing the timing of onset of labour and CS are available from 1982, thus marking the beginning of our cohort births.

Outcome-school performance

Data on school performance were extracted from the National School Register. Data from the National School Register are available for public schools beginning in 1988, and private schools beginning in 1993. In Sweden, upon finishing the compulsory years of school (generally at age 16), grades in 16 subjects are recorded. Starting in 1998, these grades were categorized into 4 levels for each subject: not pass (score of 0), pass (score of 10), pass with distinction (score of 15), and pass with great distinction (score of 20). This allowed for a maximum total score of 320 (i.e. a score of 20 in each of the 16 subjects). Prior to 1998 there was a different grading system, but as the oldest children in our cohort turned 16 in 1998 only the current method was included. Children that "drop out" of school before compulsory grading still technically graduate but are recorded as having received a

total of 0 for their final grade, and are not able to continue on to high school. These children remained in our population and were recorded as having a total score of 0. Scores were assessed in both categorical and continuous form[74,214].

Co-Variates

Based on previous literature and the use of a directed acyclic graph (DAG)[96] (**Appendix 32**), the following *a priori* co-variates were included in analysis: maternal age at time of birth (<20 years, 21-29 years, 31-39 years, 40+ years)[214], parity (first born)[214], small for gestational age (SGA)[214], large for gestational age (LGA) (birth weight less or greater than 2 standard deviations from the mean for gestational age, respectively), gestational age (<37 weeks, 37, 38, 39, or 40 weeks, >40 weeks)[214], maternal country of birth (Swedish, other Nordic, other)[215], maternal depression, non-affective disorder, or bipolar disorder (never diagnosed, diagnosed before birth, diagnosed after birth), parental income at time of birth (in quintiles), and parental social welfare at time of birth (yes/no, note: available from 1983)[215].

Further co-variates that were assessed included: year of birth[216], year of school completion, smoking at time of first antenatal visit (none, 1-9 cigarettes/day, 10+ cigarettes/day)[214,216], infant gender[214,215], Apgar score at 5 minutes ("low" [0-3], "intermediate" [4-6], "high" [7-10])[78,214], paternal country of birth (Swedish, other Nordic, other)[215], paternal depression, non-affective disorder, and bi-polar disorder (never diagnosed, diagnosed before birth, diagnosed after birth), parental co-habitation at time of birth[215,216], and parental highest education (pre-high school, high school, post-high school)[214-216].

All co-variates were tested individually in the logistic regression analysis to assess the potential impact on the association between mode of delivery and school

performance. As no variable changed the estimate by more than 10% (and only maternal age changed the estimate by more than 5%), only the variables decided on *a priori* were included in final analysis. Notably, though parental education was noted as a potential confounder both in previous literature and our DAG, the variable was only available from 1990. Therefore, though it was tested individually, it was not considered an *a priori* co-variate and thus was not included in the model. Distribution of each variable by mode of delivery is outlined in **Table 6-1**.

Statistical analysis

Logistic Regression

For the dichotomous analysis, we considered "poor school performance" to be a total score of less than160[74,214,217], meaning the individual did not have an average of at least 10 (i.e. "pass") for the 16 subjects. To determine the association between mode of delivery and poor school performance, we used logistic regression. In Sweden, scores are assigned by teachers rather than a standardised test, and thus standards for a particular grade could vary school-to-school. To account for this, we used mixed effects modelling with a random intercept for school ID.

Quantile Regression

The data on school performance have been previously reported to be highly skewed[214,216]. We used quantile regression to analyse school performance in its continuous form. Quantile regression is similar to an ordinary least squares (OLS) model, except the model regresses on the quantile of interest (such as the median), instead of the mean. Quantile regression also does not require an assumption of normality or equal variance, and it allows for assessment across the distribution (i.e.

at every quantile). In this way we were able to determine if there was an effect of mode of delivery across the distribution of scores (for example, a possible effect only on the high or low scores), rather than an effect only on average or passing scores. We first plotted quantile regression coefficients for every fifth quantile from the 5th to the 95th using the kernel-based method for estimating standard errors[218]. We also looked at coefficient estimates for specifically the 5th, 25th, 50th, 75th, and 95th quantiles. In adjusted analysis we included the same co-variates from the fully adjusted logistic regression.

Additional analyses

To investigate this association further, we conducted several sub-group analyses. In the logistic regression, we restricted to births from 1990 onwards (the year data on parental education became available), and assessed the association with and without adjustment for parental education. We assessed the association among male babies only to account for potential gender differences in school performance. We further excluded children born through a secondary CS (children born by CS whose mothers' had previously given birth through CS), and children with a low Apgar score at 5 minutes. Though the vast majority of the population finishes compulsory years of school at age 16, there are some students who finish younger or older. To that end, we also restricted the population to those who were 16 at the time they finished compulsory school to determine what effect age may have had on school performance. To account for potential clustering of academic performance within families, we restricted the population to one-child families and first born children. We then repeated overall analysis with a random intercept for maternal ID instead of school ID. For both logistic and quantile regression we conducted

sensitivity analyses by excluding children who received a 0 as a grade to see if the association was different among children who completed the compulsory years of schooling.

The logistic regression analysis was conducted in SAS v9.3 (Cary, N.C) using PROC GLIMMIX[40] and quantile regression analysis was conducted in R v3.2.2 using the QUANTREG package[218].

6.4 Results

Descriptive statistics

There were 1,489,925 live births in Sweden from 1982-1995. We excluded 34,199 multiple births, and 52,562 births missing mode of delivery, leaving a total of 1,403,164 births. Of these, 1,243,876 (88.6%) children had grades recorded in the National School Register upon finishing the compulsory years of school. Possible reasons for not being recorded in the National School Register include death, emigration, and attendance at a specialized school (for example if the child had a disability)[78]. Among the 1,243,876 included in our cohort, 1,036,424 were born by unassisted VD (84.5%), 78,441 by assisted VD (6.4%), 42,107 by elective CS (4.3%) and 59,357 by emergency CS (4.8%) (Table 1). 162,349 (13.2%) Children had poor school performance. Ages at time of grading ranged from 14-21, with 95% of children receiving grades at 16 years of age. The median score was 210 (Inter-quartile range: 175-250) (**Figure 6-1**).

Logistic regression

In unadjusted analysis, the OR for assisted VD compared to unassisted VD was 0.84 [95% CI: 0.82-0.86] (**Table 6-2**). After adjustment it was 1.06 [95% CI:

1.03-1.08]. For elective CS the unadjusted OR was 1.05 [95% CI: 1.03-1.08] and the adjusted OR was 1.06 [95% CI: 1.03-1.09]. For emergency CS the unadjusted OR was 1.05 [95% CI: 1.02-1.07] and increased to 1.12 [95% CI: 1.09-1.15] after adjustment (Chi^2 for heterogeneity p = 0.001). Adjusting for birth year, year of receiving grades, and parental education (for those born after 1990) had no effect on results (**Appendices 33 and 34**). Results were consistent among male babies, children born in Stockholm County, children who were 16 years old when they received grades, children without a low Apgar score, and children with a score above 0 (**Appendix 35**). Similarly, there seemed to be no family effect seen by creating a random intercept for maternal ID, or restricting the population based on family size and birth order (**Appendices 36 and 37**).

Quantile regression

There was little difference in the distribution of grades by mode of delivery (**Figure 6-2**). The plots of estimates across the distribution, as well an explanation for how to interpret them, is shown in **Figure 6-2**. Scores for children born through assisted VD were slightly higher than unassisted VD, especially at lower quantiles. There was no association with elective or emergency CS. After adjustment, there was no association between assisted VD and school performance (**Figure 6-3** and **Appendix 38**). Elective and emergency CS were each associated with a slight decrease (2-5 points) in scores across the distribution.

Specific coefficients from the quantile regression models can be found in **Appendix 39**. In unadjusted analysis, the 5^{th}, 25^{th}, 50^{th}, 75^{th} and 95^{th} percentiles for unassisted VD were 70, 175, 210, 250 and 300, respectively. Assisted VD was 20 points higher for the 5^{th} percentile, and 5 points higher for the 50^{th} and 75^{th}

percentiles. Elective CS was the same as unassisted VD at all five percentiles. Emergency CS was 5 points lower than unassisted VD at the 25th and 50th percentiles.

In adjusted analysis, the 5th, 25th, 50th, 75th, and 95th percentiles for unassisted VD were 17.2, 132.8, 180, 215, and 274.7, respectively (**Appendix 40**). Assisted VD was largely equal to unassisted VD. Elective CS showed a slight decrease (ranging from -1.4 to -2.78) in scores across the distribution, with the exception of the 75th percentile where it was equal. Similarly, emergency CS also showed a slight decrease in scores (ranging from -2 to -5) with the exception of the 75th percentile, which showed no change. Excluding scores of 0 did not change results (**Appendix 41**).

6.5 Discussion

We assessed the impact of obstetric mode of delivery, and in particular birth by CS, on school performance at age 16 using a large, population-based cohort. Two separate analyses were conducted, using both logistic regression and quantile regression, assessing school performance in both dichotomous and continuous form. There was a slight association between birth by CS and a reduction in school performance in both analyses. In logistic regression, elective CS was significantly associated with increased likelihood of poor school performance. However, the OR was only 1.06, meaning that children born by CS had a 6% increased odds of receiving poor grade when compared to children born through unassisted VD. The association between emergency CS and poor school performance was somewhat stronger (OR = 1.12). Similarly, with the quantile regression analysis, there was a slight association between birth by CS on school performance, primarily in adjusted

results. Children born by both elective and emergency CS had a 1-5 point decrease in score across the distribution, which translates to a 0.31-1.56% decrease.

There are several possible explanations for the small association between birth by CS and decreased school performance. A range of characteristics influence school performance including not only behaviour[43] and personality[219], but also cognitive ability[219], and external factors such as ethnic diversity in the district[215]. It is possible that rather than having an effect across this wide range of factors and behaviours, birth by CS is having an effect on only one aspect, such as anxiety. A more probable explanation, however, is that this result is being driven by confounding, such as confounding by indication or residual confounding. Confounding by indication occurs when an outcome is causally associated with an indication for the exposure of interest[83]. For example, foetal distress is a common indication for emergency CS[4], and could also cause poor school performance[220], leading to a potential measured association between emergency CS and poor school performance that is non-causal. Additionally, the association could be driven by residual confounding[83]. The relationship between pre and perinatal risk factors and psychological development is complex, and it can be difficult to rule out the effect of hard to measure confounders, such as social adversity[84].

Regardless of what is driving the association, the decrease in score is very slight. A previous study on this population found other conditions, including current asthma, rhinitis, eczema to have similar associations with school performance (change in mean score ranging from -3.1 to 4.1) and similarly concluded that though they were statistically significant they were likely not causally associated[221]. For comparison, the same study reported that severe nasal symptoms is associated with a

12.1 point decrease in mean grade[221], and another study reported consumption of fish at least once a week is associated with a 14.5 to 19.9 point increase in mean grade[222].

Strengths & limitations

The strengths of the present study include the use of population-based registries, limiting selection and information bias and measurement error, as we were able to include the entire population, and received data from official records. Also, due to the extensive nature of these registries, we were able to assess the effect of a wide variety of co-variates and potential confounders including not only obstetric information, but also demographic and socio-economic factors. In addition, due to the grading system in Sweden and the use of quantile regression, we were able to assess the impact of mode of delivery across a wide range of school grades, rather than merely assessing the likelihood of a "passing" grade.

The present study also has several limitations. First, we had no data on breast feeding, which has been linked to both mode of delivery[223] and school performance[224]. It is unlikely breast feeding played a confounding role in this association, though it may have been of interest to investigate potential effect modification. However, Sweden has a very high rate of breast feeding and close to 100% of Swedish-born children have ever been breast fed[225]. Second, as a range of factors affect school performance, we can conclude that mode of delivery does not appear to have a large impact on school performance, but cannot rule out a potential effect on more specific outcomes, such as anxiety, disruptive behaviour, or cognition. However, previous results would indicate that mode of delivery does not have an impact on behavioural difficulties[207], or childhood neurodevelopment[152]. Finally, it is worth noting that birth by CS in Sweden may not be generalizable to countries with

higher prevalence of CS. Access to medical care in Sweden is egalitarian, and the associations between social class and CS seen in other countries are not as prevalent[226]. Additionally, Sweden has a very low rate of birth by CS compared to other European countries[10], and it is probable that we had a low incidence of non-medically indicated CS. Though we did not have access to information on indications for CS, we were able to separate pre- and post-labour CS, which had no impact on results.

6.6 Conclusion

To our knowledge, the present study is the first to investigate mode of delivery and school performance. We used two analysis methods, adjusted for a wide variety of potential confounders, and conducted several sensitivity analyses to further investigate a potential association. With the results of these robust analytical methods, we have concluded that there is a slight association between birth by CS and poor school performance. However, this should be interpreted with caution as the association is very small and residual confounding cannot be ruled out.

Birth by CS is associated with a slight reduction in school performance. Possible explanations include an effect on a more specific outcome such as anxiety, or a non-causal association driven by confounding by indication or residual confounding.

Table 6-1. Distribution of descriptive variables by mode of delivery.

	Unassisted VD		Assisted VD		Elective CS		Emergency CS	
Total Population	1036424	(84.51)	78441	(6.40)	52107	(4.25)	59357	(4.84)
Poor grade	138202	(13.33)	8880	(11.32)	7064	(13.56)	8203	(13.82)
Gender (Male)	522988	(50.46)	45361	(57.83)	26165	(50.21)	32357	(54.51)
Maternal age								
<20	30367	(2.93)	2679	(3.42)	698	(1.34)	1551	(2.61)
21-29	633991	(61.17)	50482	(64.36)	24004	(46.07)	33689	(56.76)
31-39	356370	(34.38)	24176	(30.82)	2486	(47.71)	22406	(37.75)
40+	15696	(1.51)	1104	(1.41)	2545	(4.88)	1711	(2.88)
First born child	599463	(57.84)	69919	(89.14)	31350	(60.16)	44922	(75.68)
Smoking at time of first antenatal visit								
None	704557	(67.98)	52772	(67.28)	35328	(67.80)	39483	(66.52)
1-9 Cigarettes/day	148178	(14.30)	11924	(15.20)	7746	(14.87)	9586	(16.15)
10+ Cigarettes/day	91075	(8.79)	6401	(8.16)	4979	(9.56)	5864	(9.88)
Missing	92614	(8.94)	7344	(9.36)	4054	(7.78)	4424	(7.45)
SGA	20383	(1.97)	2178	(2.78)	2969	(5.70)	5017	(8.45)
LGA	31455	(3.03)	2279	(2.91)	2996	(5.75)	2605	(4.39)
Parental education								
Pre-Highschool	34151	(3.30)	209	(2.67)	1878	(3.60)	2342	(3.95)
Highschool	232551	(22.44)	17107	(21.81)	11862	(22.76)	15568	(26.23)
Post-Highschool	151078	(14.58)	12916	(16.47)	8555	(16.42)	10506	(17.70)
Missing	9145	(0.88)	680	(0.87)	344	(0.66)	703	(1.18)
Born Prior to 1990	609499	(58.81)	45647	(58.19)	29468	(56.55)	30238	(50.94)
Parental income								
First	201292	(19.42)	8839	(11.27)	8943	(17.16)	9095	(15.32)
Second	210046	(20.27)	9836	(12.54)	10020	(19.23)	9407	(15.85)
Third	209679	(20.23)	13499	(17.21)	10215	(19.60)	10713	(18.05)
Fourth	204240	(19.71)	19267	(24.56)	10316	(19.80)	13159	(22.17)
Fifth	191366	(18.46)	25526	(32.54)	11730	(22.51)	15816	(26.65)
Missing	1981	(1.91)	1474	(1.88)	883	(1.69)	1167	(1.97)
Parental social welfare status								
Yes	84609	(8.16)	5597	(7.14)	4173	(8.10)	5490	(9.25)
No	864190	(83.38)	65913	(83.03)	44174	(84.78)	50000	(84.24)
Missing	19132	(1.85)	1416	(1.81)	858	(1.65)	1133	(1.91)
Born Prior to 1983	68493	(6.61)	515	(7.03)	2902	(5.57)	2734	(4.61)
Apgar Score (5 minutes)								
Low (0-3)	1484	(0.14)	241	(0.31)	141	(0.27)	417	(0.70)
Intermediate (4-6)	3375	(0.33)	1501	(1.91)	479	(0.92)	1836	(3.09)
High (7-10)	1004980	(96.97)	75464	(96.20)	50105	(96.16)	55775	(93.97)
Missing	26585	(2.57)	1235	(1.57)	1382	(2.65)	1329	(2.24)

Table 6-1 Continued

	Unassisted VD		Assisted VD		Elective CS		Emergency CS	
Gestational age								
<37 Weeks	39384	(3.80)	1971	(2.51)	5941	(11.40)	10820	(18.23)
37 Weeks	48430	(4.67)	2546	(3.25)	5923	(11.37)	4508	(7.59)
38 Weeks	122702	(11.84)	6707	(8.55)	25006	(47.99)	7487	(12.61)
39 Weeks	255027	(24.61)	15235	(19.42)	9990	(19.17)	8416	(14.18)
40 Weeks	311130	(30.02)	23094	(29.44)	2714	(5.21)	11056	(18.63)
>40 Weeks	257116	(24.81)	28678	(36.56)	2400	(4.61)	16892	(28.46)
Missing	2635	(0.25)	210	(0.27)	133	(0.26)	178	(0.30)
Maternal Depression								
Never Diagnosed	952661	(91.92)	71714	(91.42)	46936	(90.08)	53615	(90.33)
Diagnosed before birth	6051	(0.58)	457	(0.58)	584	(1.12)	540	(0.91)
Diagnosed after birth	77712	(7.50)	6270	(7.99)	4587	(8.80)	5202	(8.76)
Maternal Non-affective Disorder								
Never Diagnosed	1026908	(99.08)	77638	(99.98)	51460	(98.76)	58644	(98.82)
Diagnosed before birth	2392	(0.23)	227	(0.29)	209	(0.40)	201	(0.34)
Diagnosed after birth	7124	(0.69)	576	(0.73)	438	(0.84)	501	(0.84)
Maternal Bipolar Disorder								
Never Diagnosed	1026027	(99.00)	77649	(98.99)	51507	(98.85)	58702	(98.90)
Diagnosed before birth	818	(0.08)	82	(0.10)	80	(0.15)	61	(0.10)
Diagnosed after birth	9579	(0.92)	710	(0.91)	520	(1.00)	594	(1.00)
Paternal Depression								
Never Diagnosed	983156	(94.86)	74367	(94.81)	49255	(94.53)	56114	(94.54)
Diagnosed before birth	4697	(0.45)	309	(0.39)	309	(0.59)	325	(0.55)
Diagnosed after birth	48571	(4.69)	3765	(4.80)	2543	(4.88)	2918	(4.92)
Paternal Non-affective Disorder								
Never Diagnosed	1028187	(99.21)	77816	(99.20)	51705	(99.23)	58823	(99.10)
Diagnosed before birth	2360	(0.23)	171	(0.22)	134	(0.26)	167	(0.28)
Diagnosed after birth	5877	(0.57)	454	(0.58)	268	(0.51)	367	(0.62)
Paternal Bipolar Disorder								
Never Diagnosed	1029320	(99.31)	77915	(99.33)	51703	(99.22)	58919	(99.26)
Diagnosed before birth	805	(0.08)	65	(0.08)	68	(0.13)	43	(0.07)
Diagnosed after birth	6299	(0.61)	461	(0.59)	336	(0.63)	395	(0.67)

Table 6-1 Continued

	Unassisted VD		Assisted VD		Elective CS		Emergency CS	
Co-habitating with child's father								
Yes	894777	(86.33)	66362	(84.60)	45489	(87.30)	50855	(85.68)
No	47235	(4.56)	4655	(5.93)	2389	(4.58)	3464	(5.84)
Missing	94412	(9.11)	7424	(9.46)	4229	(8.12)	5038	(8.49)
Maternal Country of Birth								
Sweden	917862	(88.56)	69758	(88.93)	45638	(87.59)	51349	(86.51)
Other Nordic	41385	(3.99)	2935	(3.74)	2327	(4.47)	2526	(4.26)
Other	77170	(7.45)	5747	(7.33)	4141	(7.95)	5482	(9.24)
Missing	7	(0.00)	1	(0.00)	1	(0.00)	0	(0.00)
Paternal Country of Birth								
Sweden	905824	(87.40)	68936	(87.88)	45771	(87.84)	51084	(86.06)
Other Nordic	34953	(3.37)	2445	(3.12)	1910	(3.67)	2153	(3.63)
Other	90567	(8.74)	6485	(8.27)	4155	(7.97)	5713	(9.62)
Missing	5080	(0.49)	575	(0.70)	271	(0.52)	407	(0.69)

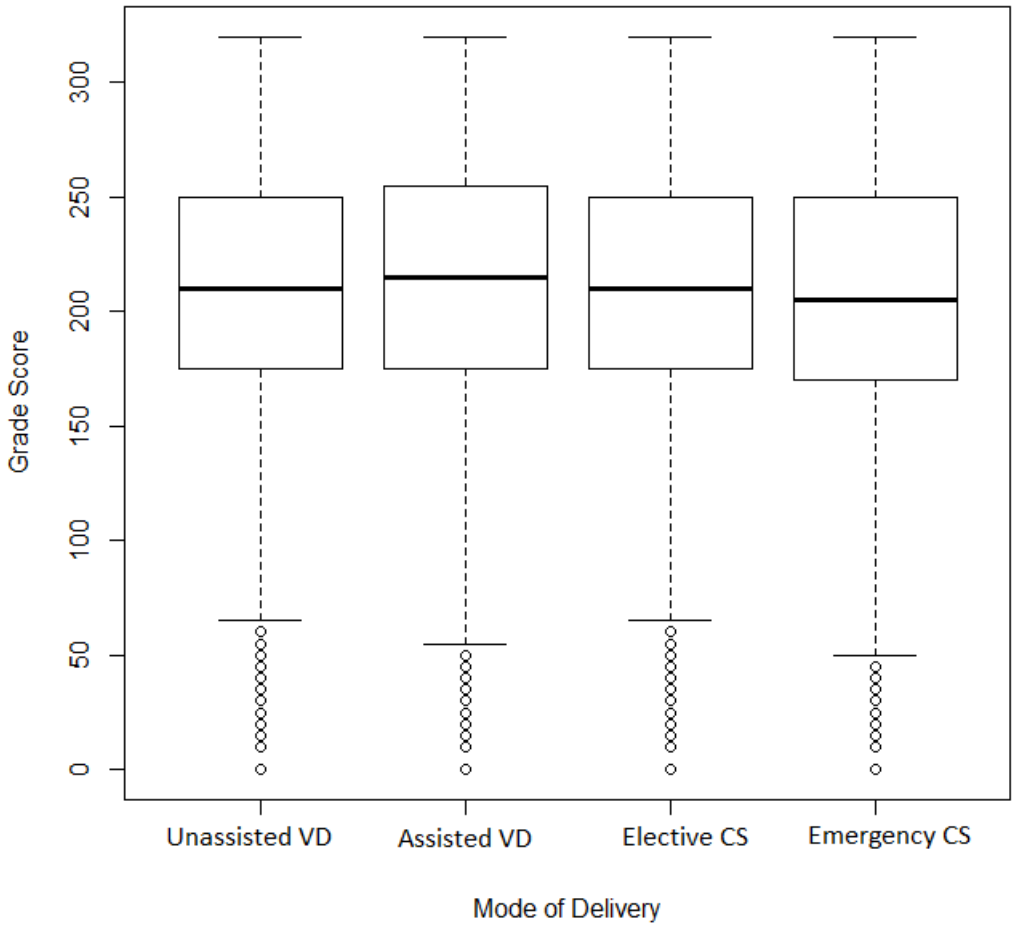
Abbreviations: VD: vaginal delivery, CS: Caesarean section

Table 6-2. Association between mode of delivery and poor school performance

	Exposed Cases	**Unadjusted OR (95% CI)**			**Adjusted OR (95% CI)**		
Unassisted VD	138202	Ref			Ref		
Assisted VD	8880	0.84	(0.82-	0.86)	1.06	(1.03-	1.08)
Elective CS	7064	1.05	(1.03-	1.08)	1.06	(1.03-	1.09)
Emergency CS	8203	1.05	(1.02-	1.07)	1.12	(1.09-	1.15)

Abbreviations: OR: odds ratio, VD: vaginal delivery, CS: Caesarean section

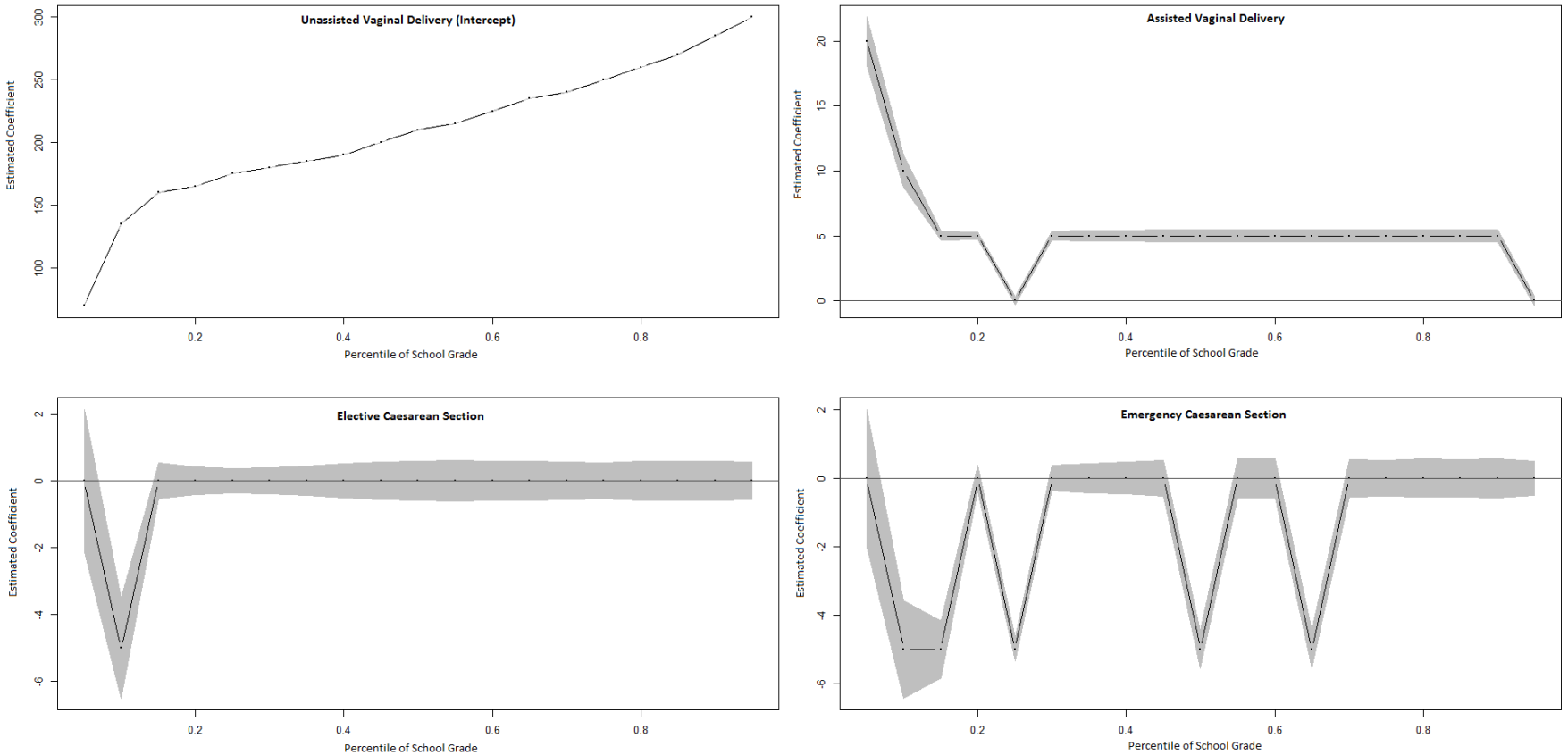
Figure 6-1. Distribution of school grades by mode of delivery*.



Abbreviations: OR: odds ratio, VD: vaginal delivery, CS: Caesarean section

*The bold lines represent the median (50th percentile), lines at the top and bottom of each box represent the 25th and 75th percentiles, respectively. Dotted lines extend to the most extreme data point that is within 1.5 times the inter-quartile range of the box. Circles represent outliers, or data points outside this range.

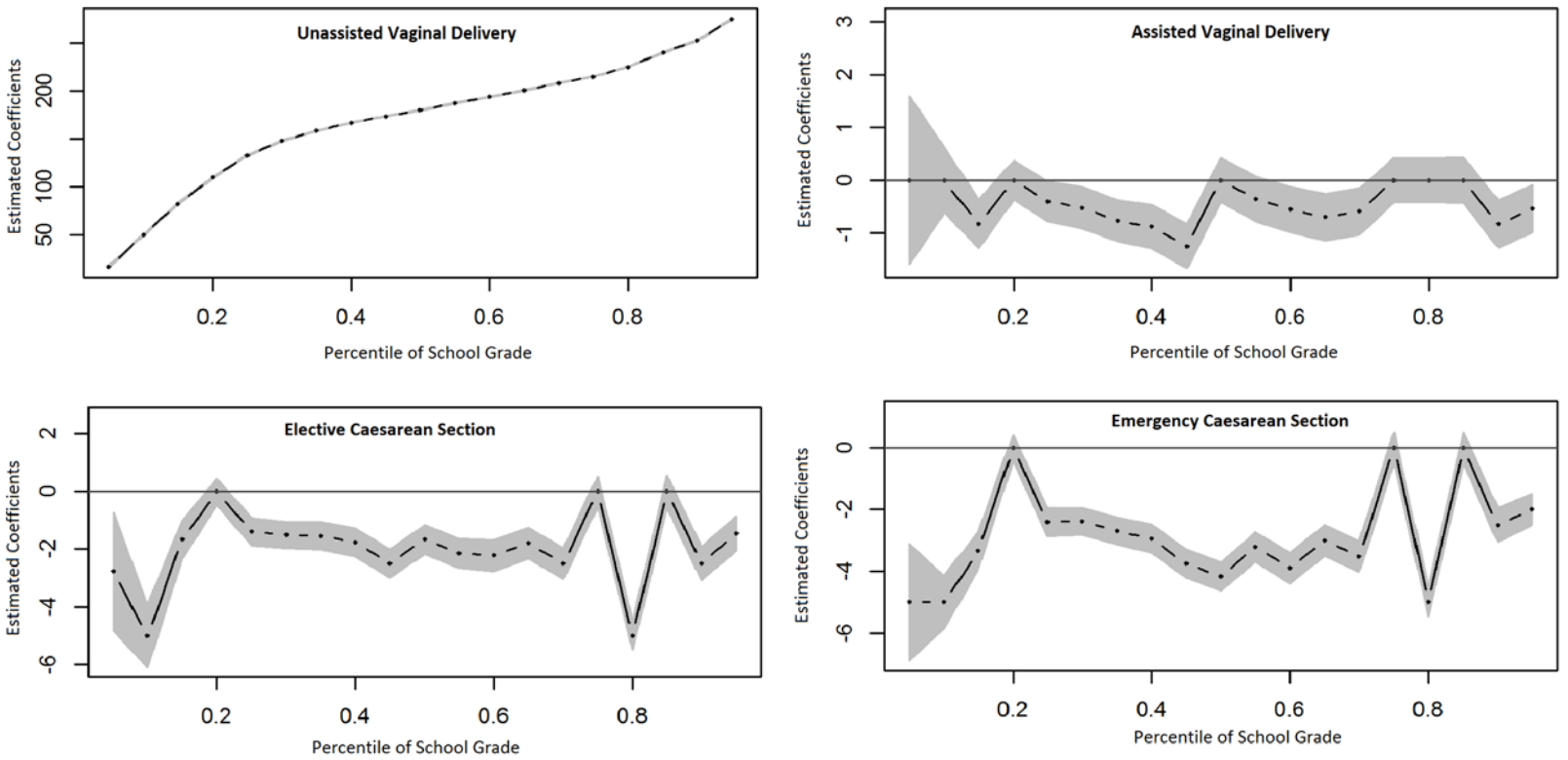
Figure 6-2. Unadjusted quantile regression modelling the association between mode of delivery and school performance.



*Interpretation: the coefficient values for unassisted vaginal delivery correspond to the school grade at that percentile. Estimated coefficients for other modes of delivery correspond to the estimated difference in score at each percentile. Grey area indicates 95% confidence intervals.

Note: Percentiles are recorded as proportions. For example, 0.2 corresponds to the 20th percentile etc.

Figure 6-3. Adjusted quantile regression modelling the association between mode of delivery and school performance.



*Interpretation: the coefficient values for unassisted vaginal delivery correspond to the predicted school grade at that percentile, given reference value for all co-variates. Estimated coefficients for other modes of delivery correspond to the estimated difference in score at each percentile. Grey area indicates 95% confidence intervals.

CHAPTER 7. UPDATED SYSTEMATIC REVIEW AND META-ANALYSIS

7.1 Updated review methods

An updated search utilizing the methods outlined in Chapter 2 was conducted on 12th January, 2016. This resulted in 3,300 new titles to search. Of these, four new studies (including the ones presented in Chapters 3[207] and 4[152]) were included investigating birth by Caesarean section and ASD[152,207,227,228]. One study only had a crude estimate[228], and thus was not included in adjusted analysis. One of the studies included in the original review was based on data from the Swedish National Registers[133]. To avoid using the same population twice, this study was excluded in the updated analysis.

There was one new study on ADHD[229], which reported a crude association. However, the actual association itself was not reported and the authors could not be reached by email. Thus, the updated ADHD results included only the results presented in Chapters 3[207] and 5.

Updated meta-analyses were conducted for both crude and adjusted estimates, as well as elective and emergency Caesarean section. Chapters 4 and 5 include two methods of adjustment; traditional statistical adjustment and sibling-matched analysis (both unadjusted and adjusted). For these studies, both adjusted estimates were included individually, but the adjusted sibling-matched results were considered the "fully adjusted" results for final analysis.

7.2 Updated meta-analysis results

Autism spectrum disorder

The combined crude estimate of the association between birth by Caesarean section and ASD, compared to vaginal delivery, including the studies from the updated search was 1.32 [95% CI: 1.29-1.36] (**Figure 7-1**). This was slightly lower

(though not significantly different) from the previous crude estimate of 1.44 [95% CI: 1.27-1.63]. The updated adjusted estimate was 1.13 [95% CI: 1.01-1.27] (**Figure 7-2**). Notably, this was not different from the estimate using the statistical adjustment, rather than the adjusted sibling design (1.16; [95% CI: 1.05-1.28]). When elective and emergency Caesarean section were separated, the adjusted estimate was not significant for either elective (1.04; [95% CI: 0.91-1.19]) (**Figure 7-3**) or emergency Caesarean section (1.16; 95% CI: 0.98-1.36) (**Figure 7-4**).

Attention-deficit/hyperactivity disorder

In the crude analysis, the combined estimate for the effect of birth by Caesarean section, compared to vaginal delivery, and ADHD was 1.21 [95% CI: 1.08-1.34] (**Figure 7-5**). In the adjusted analysis including traditional statistical adjustment, the combined estimate was 1.16 [95% CI: 1.13-1.19] (**Figure 7-6**). When the sibling design was included, the combined estimate was 1.09 [95% CI: 1.01-1.17] (**Figure 7-7**). Notably, the study from Chapter 5 including the Swedish National Registers was the largest study, and was responsible for the vast majority of the adjusted estimates (weighted 98.3% when using statistical adjustment and 87.3% when using sibling design). Unfortunately, prior to the work included in the current thesis, no studies reported separate adjusted estimates for elective and emergency Caesarean section, thus meta-analysis was only performed on overall birth by Caesarean section.

Figure 7-1. Crude estimate of the association of the association between birth by Caesarean section and autism spectrum disorder.

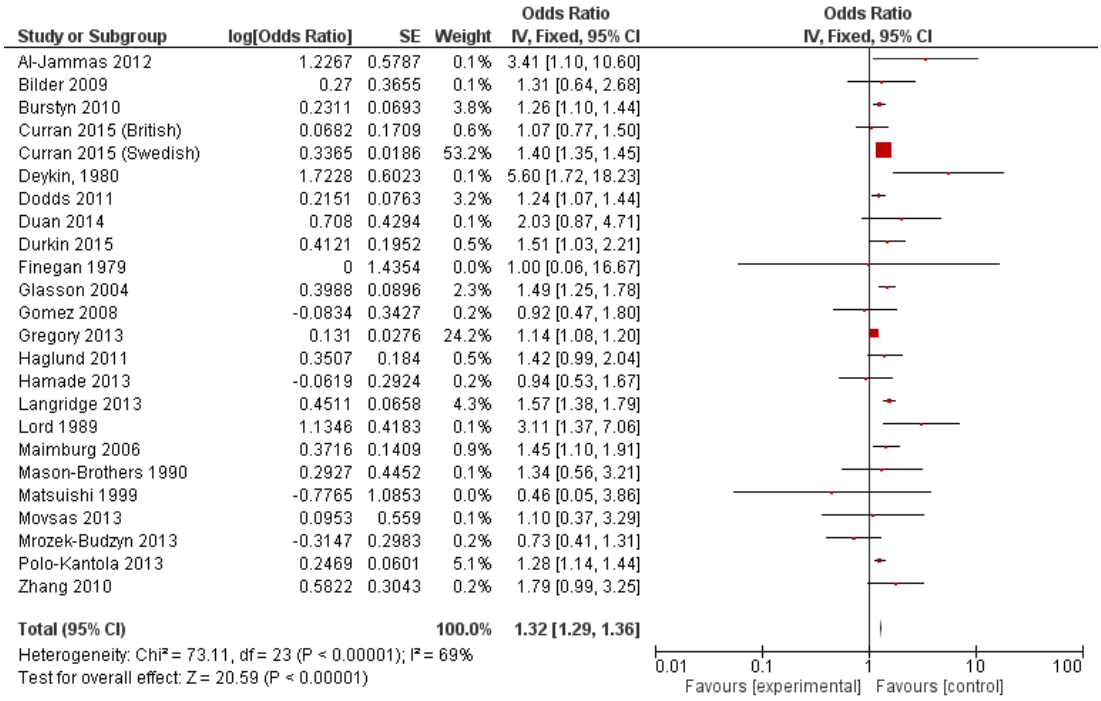


Figure 7-2. The adjusted estimate of the association between birth by Caesarean section and autism spectrum disorder.

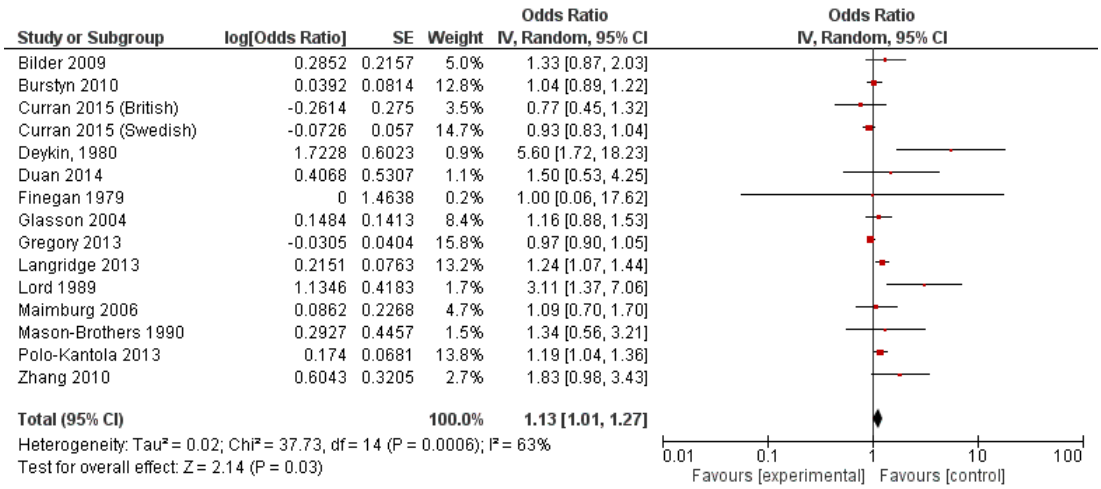


Figure 7-3. The adjusted estimate of the association between birth by elective Caesarean section and autism spectrum disorder.

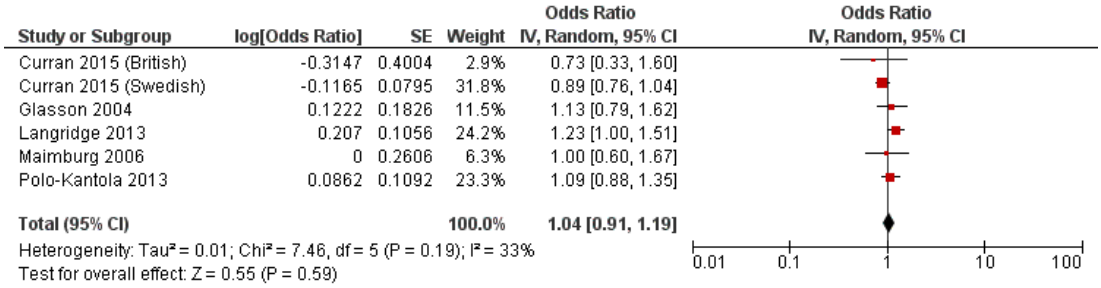


Figure 7-4. The adjusted estimate of the association between birth by emergency Caesarean section and autism spectrum disorder.

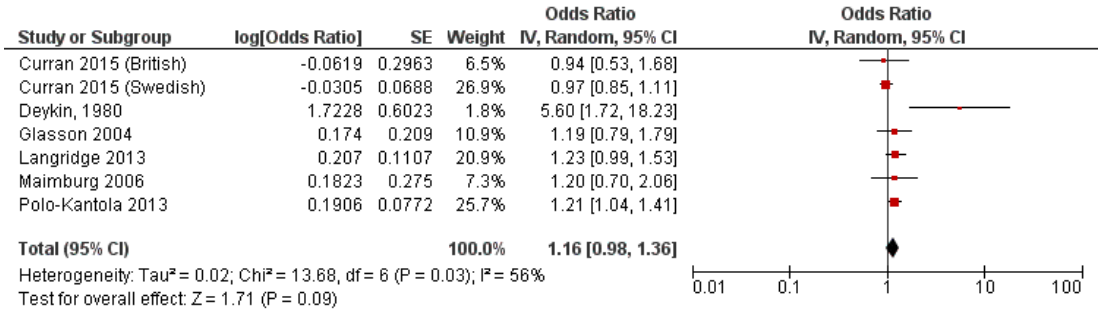


Figure 7-5. The crude combined estimate of the association between birth by Caesarean section and attention-deficit/hyperactivity disorder.

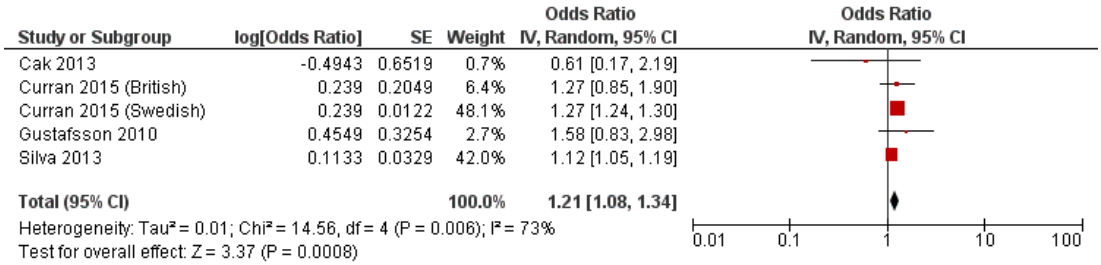


Figure 7-6. Adjusted estimate of the association between birth by Caesarean section and attention-deficit/hyperactivity disorder.

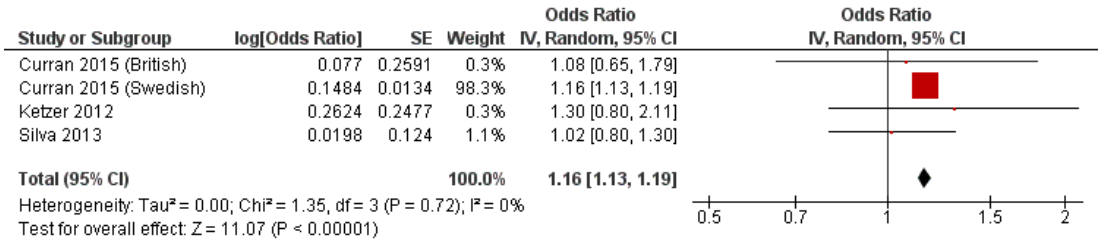
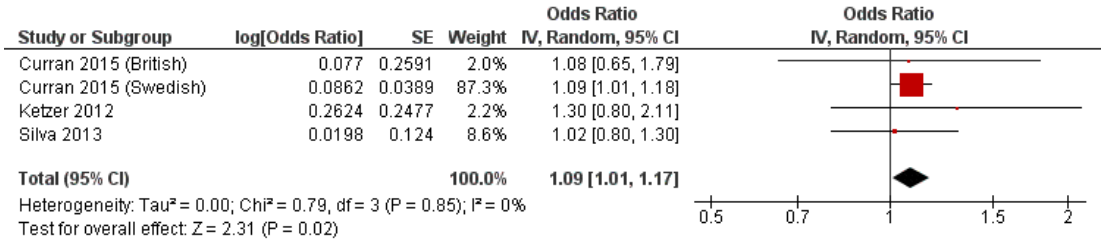


Figure 7-7. Adjusted estimate, included updated sibling design, of the association between birth by Caesarean section and attention-deficit/hyperactivity disorder.



CHAPTER 8. DISCUSSION

8.1 Summary of main findings

The aim of this thesis was to rigorously investigate the impact of birth by Caesarean section on psychological development using large datasets and a range of analytic approaches. Specifically, this work includes a systematic review of current literature, as well as several original investigations on the impact of birth by Caesarean section on autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), behavioural difficulties, and school performance. Chapter eight reviews the findings of these analyses, and discusses study methods, strengths and limitations, implications and overall conclusions.

8.1.1 Findings from systematic review

A systematic review and meta-analysis was conducted to identify and compile existing research on the association between birth by Caesarean section and autism spectrum disorder and ADHD. Our findings indicate that children born by Caesarean section are 44% more likely to be subsequently diagnosed with ASD. After controlling for known confounders, including maternal age, this was reduced to 23%, indicating this association is highly confounded. When results from the updated search, and Chapters 3 and 4 were included, the adjusted estimate indicated that children born through Caesarean section were 13% [95% CI: 1.01-1.27] more likely to be diagnosed with autism spectrum disorder.

For ADHD, there was limited previous research. There were only three studies with a crude estimate of the association, leading to an estimate of 12% [95% CI: 1.06-1.18] increased risk. After adjusting for known confounders, this was reduced to 7% [95% CI: 0.86-1.33] and was no longer significant. When the results from Chapters 3 and 5 were included, this was not significantly changed. Children

born by Caesarean section were 9% [95% CI: 1.13-1.19] more likely to be diagnosed with ADHD after controlling for confounding.

Results from the systematic review highlighted the importance of the results in this thesis due to the limitations of prior studies. Few studies differentiated between emergency and elective Caesarean section. As theories on the potential effect of birth by Caesarean section on psychological development apply more to elective Caesarean section, this is an important distinction to make. In addition, many of the studies included older birth cohorts and may not have been subject to more recent definitions of ASD. Therefore, though we could conclude that children born by Caesarean section were at increased risk of ASD, we could not conclude what was driving the association. In other words, we could not determine if it was a causal association, or if it was primarily driven by other factors such as selection or information bias, or unmeasured confounding. Similarly, with regards to ADHD, previous studies were limited and conflicting. No previous studies reported separate adjusted estimates for elective and emergency Caesarean section. Because of these limitations, we could not conclude if there was an association between birth by Caesarean section and ADHD.

8.1.2 Findings on autism spectrum disorder

The effect of birth by Caesarean section on ASD was assessed in Chapters 3 and 4 using two cohorts: the UK Millennium Cohort Study and the Swedish National Registers. In the UK Millennium Cohort Study, no association was found between mode of delivery, or birth by Caesarean section, and ASD. In the study using the Swedish National Registers, traditional cohort analysis provided similar results to the systematic review. After controlling for known confounders, children born through

Caesarean section were about 20% more likely to be diagnosed with ASD. However, in the sibling design study there was no association between birth by Caesarean section and ASD. This indicates that the association that has been reported in previous studies is most likely due to unmeasured confounding by genetics or family environment.

8.1.3 Findings on attention-deficit/hyperactivity disorder

The effect of birth by Caesarean section on ADHD was also assessed in both the UK Millennium Cohort Study (Chapter 3) and the Swedish National Registers (Chapter 5). In the UK Millennium Cohort Study, there was no association between mode of delivery and ADHD. In the Swedish National Register study, birth by Caesarean section was associated with a 15 and 16% increased likelihood of ADHD diagnosis (for elective and emergency Caesarean section, respectively). In the sibling design study, elective Caesarean section was no longer associated with ADHD, though the association with emergency Caesarean section remained. Even when compared with siblings, children born by emergency Caesarean section were 13% more likely to be diagnosed with ADHD. However, the proposed biological mechanisms for an association between birth by Caesarean section and psychological development apply more to elective rather than emergency Caesarean section. Therefore, if this were a causal relationship, it is improbable the association would remain with only emergency Caesarean section. A much more probable explanation is that the indications for the emergency Caesarean section are responsible for this increased risk of ADHD. Therefore, the analysis suggests that the association between birth by Caesarean section and ADHD is probably driven by unmeasured confounders. Notably, the results from the analysis on autism spectrum disorder and

ADHD were similar to an additional study on psychosis[230]. Though not included in the main thesis, the paper can be found in **Appendix 42**.

8.1.4 Findings on behaviour

The effect of mode of delivery on behaviour was assessed in Chapter 3 using the UK Millennium Cohort Study. The results indicated that birth by Caesarean section had no association with parent-rated behavioural difficulties. There was a small but statistically significant association with induction of labour, however this did not persist after adjustment for measured co-variates relating to pregnancy and delivery, demographics and socio-economics and maternal health and mental health, and was likely due to confounding. Therefore, the conclusion is that mode of delivery had no significant impact on behavioural development in this population.

8.1.5 Findings on school performance

As shown in Chapter 6, there was little evidence of an association between birth by elective Caesarean section and poor school performance. When grades were analysed in continuous form, children born by elective Caesarean section had scores that were 1-3 points (out of 320) lower than children born vaginally, and children born by emergency Caesarean had scores that were 2-5 points lower. This translates to a 0.3-1.6% decrease in overall score. Though these associations were statistically significant, it is possible that these results were driven by confounding by indication or residual confounding. Similarly, it is possible that such small associations are not clinically relevant and are only statistically significant due to the large population size.

8.2 Strengths and limitations of thesis

8.2.1 Strengths

The studies included in this thesis were not only the largest to date on ASD and ADHD, but were larger than all previous studies combined. In addition, the current studies were population-based, representative cohorts, providing evidence for the external validity of the results. In addition, both cohorts have been previously validated with regards to mode of delivery, and the Swedish National Registers have been additionally validated for psychiatric outcomes. Another strength lies in the years covered by the cohorts. Much of the previous work on ASD included older birth years, and children were not always diagnosed using more recent criteria. Only one cohort study examined the effect on children born specifically in the late 1990s and early 2000s, and it did not differentiate between emergency and elective Caesarean section[61].The UK Millennium Cohort Study included only children born in 2000 and 2001, meaning they all would have been subject to the most recent definitions of ASD and ADHD. Also, the Swedish National Registers include a wide range of years, and we were able to do sensitivity analyses by birth cohort to assess the potential impact of changing definitions of ASD and ADHD. In the UK MCS, behavioural difficulties and maternal attachment were measured using previously validated psychological scales. Both cohorts included detailed information on demographics and socio-economic status. The Swedish National Registers additionally included detailed information on parental psychiatric health. In addition, The Swedish National Registers provided the opportunity to use both traditional statistical adjustment as well as sibling designs, thus controlling for unmeasured confounders which may have affected previous work.

To my knowledge the current thesis includes the first study on birth by Caesarean section and school performance. This has many of the same strengths as the ASD and ADHD studies conducted with the Swedish National Registers. We were able to include the vast majority of people born in Sweden through the use of a population-based register, and were able to include a wide range of variables and sensitivity analyses to investigate the potential association. Additionally, as a result of the way grades are recorded in Sweden, we were able to do two types of analyses, one with a failing grade as an outcome, and another on grade as a continuous variable to determine if there was an effect on just the highest or lowest performers.

8.2.2 Limitations

There were several limitations to the work presented here. Firstly, we did not have access to the indications for Caesarean section in either the UK Millennium Cohort Study or the Swedish National Registers. Because of this, we were only able to categorise Caesarean section into "planned/unplanned" in the MCS (based on self-report) and "elective/emergency" in the Swedish National Registers (based on timing of labour). It is possible that results may have been different had we been able to define mode of delivery more specifically based on the indication for the Caesarean section. For example, pre-labour Caesarean section could have been further divided into "medically-indicated" and "non-medically-indicated" pre-labour Caesarean section. Therefore, we were not able to determine the effect indication may have had on this association, and cannot rule out confounding by indication. However, it's worth noting that we conducted many sensitivity analyses on variables associated with risk of Caesarean section (e.g. maternal age, birthweight etc.) which did not

appear to have an effect on the results, we cannot determine the effect indication may have had on this relationship.

UK Millennium Cohort Study

As the UK Millennium Cohort Survey requested data retrospectively, it is possible that there was recall bias, where people mis-remembered certain events. In particular, the first survey which collected data on pregnancy and delivery, was conducted 9 months after birth. There is also the possibility of mis-classification bias. Mode of delivery has been previously validated in this dataset[179], but as we included parent-reported diagnosis of ASD or ADHD, rather than original diagnosis, it is possible that diagnoses were mis-reported. For example, it has been theorised that the wording of the questions on ASD and ADHD diagnosis was overly broad and could have led to over-reporting[161]. In addition, the outcomes assessed in this study may have been subject to social desirability bias[231], as ASD, ADHD, and behavioural difficulties may have been viewed as "undesirable" by some parents and thus intentionally mis-reported. However, it is unlikely that any of these would have led to differential mis-reporting by mode of delivery and so most likely led to bias towards the null.

Swedish National Registers

Unlike the UK Millennium Cohort Study, the Swedish National Registers were less subject to recall bias or social desirability bias, as they included data from original medical records. However, there were several other potential sources of information bias. First, as outpatient data was only available from 2001, there was probably an over-representation of more severe cases, and less severe cases were recorded as controls. However, it is unlikely that this mis-classification was

differential based on mode of delivery and thus would have likely led to bias towards the null. In addition, sensitivity analyses indicated this did not appear to have a large impact on results.

With regards to bias in the sibling design studies, it is worth noting that birth by Caesarean section is correlated within families, leading to potential questions regarding the appropriateness of sibling designs in this case[232]. However, sensitivity analyses by birth order and Caesarean section order within the sibling design had no effect on results, indicating that this clustering had a minimal impact on results. On the other hand, potential unmeasured confounders such as maternal autism spectrum disorder, would have been shared by all siblings, strengthening the argument for the appropriateness of sibling design and subsequent interpretation of results[233].

For the school grade data, children who dropped out of school were recorded as receiving a "0." Therefore, while we could look at the risk of a failing grade, we could not predict what grade these children would have received had they continued in their schooling. In addition, as many factors affect school performance, we cannot rule out an impact of Caesarean section on any more specific outcomes which contribute to academic achievement.

8.3 Clinical and public health implications

The findings presented in here are important for two primary reasons. First, results will be of interest to parents, clinicians and public health policy makers. Birth by Caesarean section as well as autism spectrum disorder and ADHD are becoming increasingly prevalent. With previous results in animal and human populations suggesting that changes caused by birth by elective Caesarean section, such as altered microbiota, may be associated with psychological development and neurodevelopmental disorders[51,113], it is becoming increasingly important to

understand this association better. Unlike other outcomes associated with birth by Caesarean section such as asthma[17], obesity[17], and type 1 diabetes[19], which have been previously rigorously investigated in human populations, work on the connection between Caesarean section and mental health has been limited. Our results inform future parents and clinicians that risk of neurodevelopmental disorders is likely not a concern with regards to Caesarean section. That being said, Caesarean section is a medical procedure and is never without risks to both mother and baby, and should not be undertaken lightly.

Second, the results of these studies will be of interest to those conducting or disseminating epidemiological research on similar topics in the future. With robust traditional statistical adjustment and sensitivity analyses, using large, validated, population-based cohorts and a wide range of variables, an association was still present for both ASD and ADHD. It was the additional sibling design analysis which indicated that the association was most likely being driven by unmeasured confounding. These results highlight the importance of conducting observational epidemiological research robustly, in an attempt to determine what may be driving any association that is observed.

8.4 Recommendations for future research

Though results from these studies indicate that birth by Caesarean section likely does not have a causal association with neurodevelopmental disorders, behaviour, or school performance, there is still work to be done in this area.

8.4.1 Future research on Caesarean section

It is possible that birth by Caesarean section has cognitive, rather than behavioural, effects. Though we included school performance, there are many factors in addition to cognition which can affect school performance[43,215,219]. Given the slight association between Caesarean section and school performance, it may be of interest to further investigate more specific cognitive outcomes. In addition, it is possible that birth by Caesarean section affects a specific outcome rather than the wide range of behaviours, such as is required for a diagnosis with ASD or ADHD, or for school performance. Therefore, it may be of interest for future research to investigate the potential impact of birth by Caesarean section on other psychological outcomes, such as depression or sub-clinical outcomes like anxiety, impulse control or stress sensitivity.

8.4.2 Future research on psychological development

There have been several perinatal risk factors that have been associated with ASD, ADHD, and school performance in the past. Future research may focus on understanding some of these better. For example, previous results indicate that the anaesthesia used in the caesarean section procedure may be associated with autism spectrum disorder[182] and learning disabilities[234,235]. Future work may investigate how the outcomes discussed in this thesis may have been impacted by the type of anaesthesia used during birth. Similarly, previous results indicate that it is possible that pre-eclampsia and other forms of hypertension during pregnancy is associated with autism spectrum disorder[236] or ADHD[237]. Future research could investigate this further, not only to confirm the association but also determine any potential biological mechanisms. In addition, early term birth has reported to be associated

with special education requirements[48]. It may be of interest to see how gestational age may be associated with school performance as measured in continuous form. Also, augmented or induced labour, specifically through the use of oxytocin, has been reported to be associated with a modest increased risk of ASD[116,238]. Future work could investigate this association further, such as the effect dosage might have on this relationship[238].

Though there does not appear to be a causal association between birth by Caesarean section and child psychological development based on the current results, this by no means negates the theory of the effect of microbiota on brain development. Future work may investigate other avenues through which microbiota colonisation could be altered and the effect this may have. For example, maternal irritable bowel syndrome[239], or maternal or infant antibiotic use[240,241] may all impact the development of microbiota. The effect these exposures may have on psychological development could be of interest for future research in this area.

8.5 Conclusion

The data presented in this thesis show through rigorous investigation including multiple statistical models, and adjustment for a wide variety of confounders in two large population-based cohorts that children that are born through Caesarean section are at an increased risk of ASD and ADHD. However, the lack of association with the elective Caesarean section in the sibling design studies indicates that this association is most probably due to unmeasured confounding. No association was found between birth by Caesarean section and behavioural difficulties. A small but significant association was found between birth by Caesarean section and school performance. However, given the complexities of the relationship between perinatal risk factors and psychological development, the effect

may have been due to residual confounding or confounding by indication and should be interpreted with caution. The overall conclusion is that birth by Caesarean section does not appear to have a causal relationship with the aspects of child psychological development investigated.

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APPENDICES

Appendix 1. Description of statistical methods used, including equations and assumptions.

Logistic regression

Logistic regression predicts the probability (p) of outcome Y=1 compared to Y=0 using a logit equation[1]:

$$logit(p)=ln(p/(1-p))$$

The probability *p* is modelled using a logistic function. The result of a logistic regression is used to determine the odds ratio (OR) of an association[1].

Assumptions of logistic regression

- The same probability is maintained across predictor values (can be assumed if observations are independent)[2]
- Large sample size (minimum sample size of 100 and minimum observation to predictor ratio of 10:1)[2]

Conditional logistic regression

Conditional logistic regression is a particular kind of logistic regression, is used when the degrees of freedom are large compared to the number of observations included in an analysis, such as when observations are matched[1]. What makes conditional logistic regression different is how probabilities are calculated. In traditional (unconditional) logistic regression, probabilities are calculated by maximizing the unconditional likelihood of the event[1]:

$$(\Pi\, p(x))(\Pi(1-p(x))$$

In conditional logistic regression, the conditional maximum likelihood is calculated, using the equation[1]:

$$((\Pi\, p(x))(\Pi(1-p(x))) / \sum(\Pi\, exp(\sum \beta_i X_i))$$

The difference between this and unconditional likelihood is that the probability of the event (numerator) is divided by the probability of all possible configurations. On the other hand, the unconditional likelihood is the numerator alone.

Mixed effects logistic regression

Mixed effects logistic regression is used when data are clustered by group and thus observations are not independent[3]. This method inserts a random intercept at the group-level indicated, allowing for differences in the underlying risk by group[3]. In this way, the effect of group is controlled for without the loss of degrees of freedom that would have occurred if group had been included in the model as a covariate[3].

Cox regression

Survival analysis is a method of measuring associations in longitudinal data including time to an event[4]. In survival analysis, individuals in a cohort can enter at different times, and their follow-up begins at a pre-determined time or event (such as a particular year or date of birth). Individuals are "followed" until either they have experienced the event of interest, or they are "censored," meaning they are removed from the study because of a reason other than the event of interest (such as death by another cause or loss to follow-up). The amount of time that passes between when follow-up begins and ends is considered an individual's "follow-up time."

Cox regression is a widely-used form of survival analysis[4]. Cox regression estimates what is known as the hazard (*h*), or instantaneous risk of the outcome at any given follow-up time (*t*). This is modelled using the equation:

$$Log(h(t))=Log(h_0(t))+B_1X_1\ldots+B_pX_p$$

where $h(t)$ is the hazard at time *t* and $h_0(t)$ is the hazard in the unexposed group at time *t*[4]. Similar to an OR, the hazard ratio (HR), or ratio of the hazard in the exposed compared to unexposed, approximates the RR and is interpreted in the same way.

Assumptions of Cox regression

- The ratio of hazards in exposed compared to unexposed group remains constant over time (also known as the proportional hazards assumption)
- Non-informative censoring. In other words, the reason individuals are being censored is not related to the exposure or outcome of interest[4]

Quantile regression

Quantile regression is similar to an ordinary least squares (OLS) model. An OLS (i.e. linear regression) model is an extension of a simple linear model:

$$y = B_0 + B_1X_1\ldots+B_pX_p + error$$

Quantile regression follows the same formula, however instead of quantile regression regresses on the median, or any quantile specified rather than the mean. The most thorough way to report quantile regression is by graphing the value of B_1 at each quantile (assuming X_1 is the exposure of interest). If the quantiles are not graphed across the distribution, by convention the 5th, 25th, 50th, 75th and 95th quantiles should

be reported if the dataset is large enough to provide reliable estimates at those extremes.

Directed Acyclic Graphs

In this thesis, DAGs were used to clarify the theorised causal structure behind the association between birth by Caesarean section and psychological development and identify potential confounders to include in statistical models.

Terminology of DAGs

- Node: variable included in the diagram
- Arcs: arrows drawn from one node to another to indicate a causal effect
- Ancestor: all nodes directly or indirectly that cause the node of interest
- Descendant: all nodes directly or indirectly that the node of interest causes
- Path: a series of arcs in which no node is passed more than once
- Collider: a node in a path in which two arcs are pointing in

Rules of using DAGs

- A direct causal relationship is assumed to occur in the direction in which the arc points
- Must be acyclic, i.e. if one were to follow the direction of the arcs it would be impossible to go through a node more than once
- If a variable is seen as a collider on the path from the exposure to outcome of interest, it should not be controlled for as that will lead to a biased estimate
- A descendant of the exposure of interest should not be controlled for, as it is considered to be on the causal pathway[5]

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Appendix 2. Results of systematic search of terms relating to ASD[a], ADHD[b] and mode of delivery

	(Feb 28, 2014 limit human)	Pubmed Results	Cinahl Results	Psycinfo Results	Embase Results	Web of Science Results
1	Caesarean	12006	1065	362	13846	32616
2	Cesarean	39941	3797	739	45062	32881
3	Caesarean section	40246	819	264	13241	25096
4	Caesarean sections	37704	133	264	1842	25297
5	Cesarean section	40246	3313	426	43577	25297
6	Cesarean sections	37704	180	426	3629	25297
7	Delivery, abdominal	39045	20	3	129830	3606
8	Deliveries, abdominal	36980	5	3	1243	3606
9	Abdominal delivery	39405	20	3	8312	3606
10	Abdominal deliveries	36980	5	3	1243	3606
11	C-section	36834	43	38	811	646
12	C-sections	36716	18	38	157	646
13	C section	36834	75	1154	112923	143624
14	C sections	36716	30	1154	26099	143624
15	Postcesarean section	36694	5	1	251	242
16	Postcaesarean section	19	0	0	21	12
17	Post-cesarean section	320	13	1	326	295
18	Post-caesarean section	134	8	1	207	292
19	Caesarean delivery	39158	247	113	12177	17885
20	Cesarean delivery	39140	1274	349	41895	17990
21	Delivery, Caesarean	1003	247	113	12968	17885
22	Delivery, Cesarean	36611	1274	349	43560	17990
23	Mode of delivery	6373	616	727	4112	10005
24	Perinatal risk factors	9840	327	284	9826	7015
25	Perinatal risk factor	10279	30	284	11823	7015
26	Obstetric delivery	64419	1697	84	72773	7798
27	Obstetric labor	57524	44	42	11445	4175
28	Labor	434498	7255	24080	15246	158532
29	Labour	434498	2541	24080	15246	158532
30	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29	491805	12774	29050	332263	339926
31	Article	11964871	29320	277609	5253317	791963
32	Cohort study	1339593	19933	13986	25	224850
33	Cross-sectional study	199657	16606	18598	17	129424
34	Case-control study	676707	6367	4953	12	71323

35	Systematic review	1597650	18551	10849	53788	70903
36	Review	1857650	84619	375421	1019742	1286125
37	Retrospective study	508059	15643	8532	11	174091
38	Prospective study	447703	27785	21335	48	279644
39	#31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38	1203309	171688	649022	6270058	2557280
40	Autism	21172	3634	42684	26862	29791
41	Autism spectrum	6468	1781	8864	9	13090
42	Autistic	16572	3871	11933	7610	10258
43	Autism spectrum disorders	19776	1029	8269	7	12686
44	Autism Spectrum Disorder	19649	833	8269	9	12686
45	Autistic spectrum disorders	3216	167	1328	3	3589
46	Autistic Spectrum Disorder	19451	158	1328	4	3589
47	Asperger	1739	472	3044	2950	4261
48	Asperger's	674	173	3672	Error	1057
49	Aspergers	7	2	3044	22	592
50	Asperger's Syndrome	1807	104	2468	Error	731
51	Autistic Spectrum	3957	319	1581	4	3762
52	Pervasive developmental disorder	19560	199	8411	2836	4503
53	Pervasive developmental disorders	19564	142	8411	2393	4503
54	attention deficit disorder	22342	2372	21223	3807	26914
55	ADD	27199	3032	22600	38620	445791
56	ADHD	21289	1398	16562	26817	17812
57	attention-deficit	25614	2421	22171	26817	23307
58	attention-deficit/hyperactivity disorder	5370	2335	16257	3136	19249
59	hyperactiv*	32155	2895	25927	29790	43757
60	overactive*	3983	262	397	7405	6323
61	inattent*	3651	514	5046	3978	5166
62	hyperkinet*	2250	56	1075	1969	2220
63	#40 OR #41 OR #42 OR #43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50 OR #51 OR #52 OR #53 OR #54 OR #55 OR #56 OR #57 OR #58 OR #59 OR # 60 OR #61 OR #62	91391	10492	88473	115108	540554
64	#30 AND #39 AND #63	4235	25	161	2729	1204

[a]Autism spectrum disorder
[b]Attention-deficit/hyperactivity disorder

Appendix 3. Levels of bias in studies included in systematic review of birth by Caesarean section and autism spectrum disorders and attention-deficit/hyperactivity disorder

Study	Selection Bias	Exposure Bias	Outcome Bias	Analytic Bias	Attrition Bias	Confounding	Overall bias
Al-Jammas (2012)	Moderate-Cases were diagnosed at one university, controls were from a pediatric clinic and suffered from delayed speech development	Moderate-Self-reported by parents at time of recruitment	Minimal-Cases were diagnosed at a Unit of Psychiatric Researchers using DSM-IV-TR	Moderate-Just reports that odds ratios and chi-squared tests were used. No adjustment	Minimal-All the data were collected at once and so there was no loss to follow-up	Moderate-Does not control for confounding, reports univariate analysis of risk factors	moderate
Bilder (2009)	Minimal-Records from registry	Minimal-Records from registry	Minimal-Record review and coding methodology developed by the Metropolitan Atlanta Developmental Disabilities surveillance Program	Moderate-Multivariate logistic regression analysis, but reference group for mode of delivery not clearly defined	Minimal-Records from registry	Low-Adjusted for maternal age, gestational length and parity but nothing else	low
Burstyn (2010)	Minimal-Records from population based registry previously shown to be reliable	Minimal-Records from population based registry previously shown to be reliable	Minimal-Records from population based registry previously shown to be reliable	Minimal-Adjusted log-binomial regression	Minimal-Population based registry	Minimal-Conducted multiple sensitivity analyses to explore confounding and adjusted final model accordingly	minimal

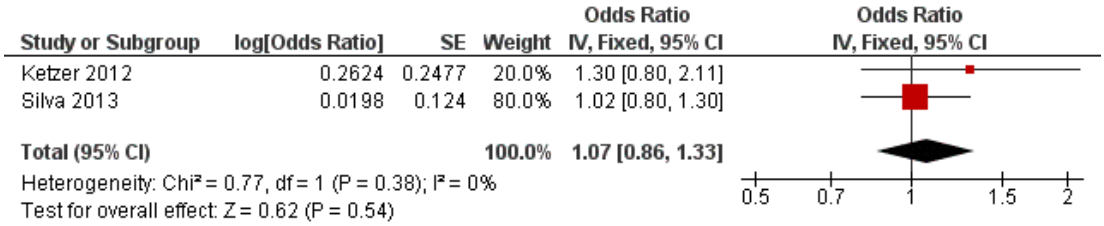
Study	Selection Bias	Exposure Bias	Outcome Bias	Analytic Bias	Attrition Bias	Confounding	Overall bias
Cak (2013)	Low-Included all children in the Neonatal Intensive Care Unit in 2003 born between 30 and 36 weeks gestation	Minimal-Records from medical records	Low-Interviews and DSM-IV criteria used for diagnosis	Moderate- T-tests for birth by CS	High- Of the 226 eligible, 118 were reached	Moderate- Does not adjust for confounders	moderate

Appendix 4. Characteristics of studies included in the systematic review.

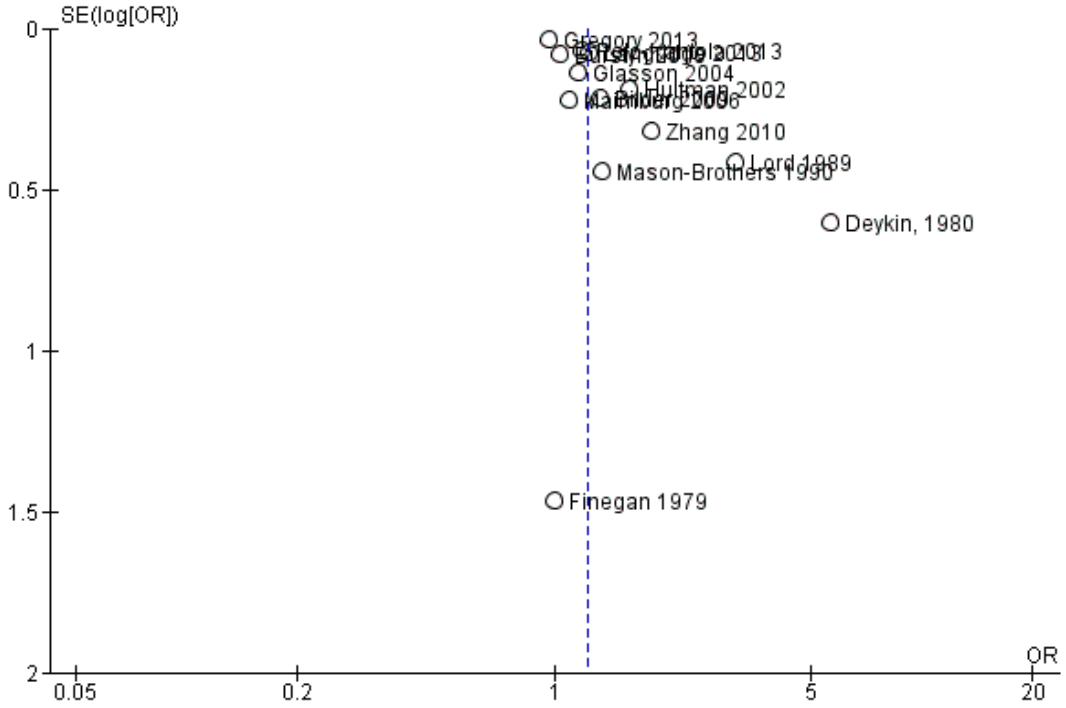
Study	Country/time period	Definition of outcome	Outcome	Study Design	Data Source	Cohort size	Confounders adjusted for	Reports emergency and elective separate?
Al-Jammas (2012)	Iraq, 2011-2012	DSM-IV-TR[a]	ASD[b]	Case-Control	Single institution	100	None	No
Bilder (2009)	USA, 2002	DSM-IV	ASD	Case-Control	Population-based registry	13,332	Maternal age, gestational length and parity	No
Burstyn (2010)	Canada, 1998-2008	ICD-9[c]	ASD	Cohort	Population-based registry	218,890	Maternal age, maternal weight, maternal height, pre-pregnancy diabetes, gestational diabetes, bleeding, cigarette smoking, poor weight gain, parity, socio-economic status of mother, pre-eclampsia, presentation, type of labour, gestational age, birth weight, Apgar, gender, birth year	No

Study	Country/time period	Definition of outcome	Outcome	Study Design	Data Source	Cohort size	Confounders adjusted for	Reports emergency and elective separate?
Deykin (1980)	USA, 1975-1977	Displaying symptoms in one of three areas by age six:impaired relatedness to the environment, stereopathy, and impaired language development	Autistic disorder	Case-Control	Interview or survey data	264	Birth Order, siblings comparison group	Only reports emergency
Dodds (2011)	Canada, 1990-2002	ICD-9 or ICD-10	Childhood autism, childhood disintegrative disorder and PDD-NOS[d]	Cohort	Population-based registry	129,733	None	No

Appendix 5. Meta-analysis of adjusted estimates of delivery by Caesarean section and attention-deficit/hyperactivity disorder.



Appendix 6. Funnel plot of studies published on delivery by Caesarean section and autism spectrum disorders.



Appendix 7. Description of the cohorts

The Millennium Cohort Study

The Millennium Cohort Study is the fourth British birth cohort study of its kind. Previous cohorts included the 1946 National Survey of Health and Development, the 1958 National Child Development Study, and the 1970 British Cohort Study[1]. The Millennium Cohort Study differs from previous cohorts in that it includes children born in the year 2000, children from the whole UK, and births from a 12 month (rather than one week) period to allow for research on seasonality of birth, and samples include an over-representation of ethnic and national minorities[1].

Aims and objectives

The Millennium Cohort Study was created to assess the impact of early life influences on development and outcomes throughout childhood and into adulthood[1]. To achieve this aim, the surveys in the cohort include a wide variety of variables to capture economic and socio-demographic information, as well as relationships within the family[1].

Sampling methods and surveys

The cohort was sampled using a stratified clustered sampling design. All live births in a 12 month period (starting 1 September, 2000 for England and Wales and 1 December, 2000 for Scotland and Northern Ireland) who remained living in the UK at the time of sampling were eligible for inclusion. The reason for the three month delay for Scotland and Northern Ireland births was to avoid overlap with the Department of Health survey on infant feeding practices, which occurred September-November of 2000. Children who were born outside the UK were included as long as they were residents at the time of sampling[1].

The population was first stratified by country within the UK (i.e. separate strata for Scotland, England, Wales and Northern Ireland). It was then further stratified by ward within the country. Countries other than England, and wards with a high percentage of ethnic minorities or low income workers were over-sampled to allow for sufficient numbers to study these populations[1]. Due to the complex sampling design, any analyses must be weighted to be considered representative[1]. There are several weights available including the original survey weights, as well as combined survey and response weights for each wave. Weights have all been standardized to have a mean of one, and there is no significant difference between them[2].

There have been five waves of the survey conducted so far: at ages 9 months and 3, 5, 6, and 11 years. The sixth wave (age 14) is currently being conducted, and the seventh wave (age 17) is planned for the year 2018[3]. The parents were interviewed at each wave, the children were additionally interviewed from wave 2 onwards. Older siblings were included in waves 2 and 3. Starting in wave 4, teachers were also included[1].

Data approval and extraction

Ethical approval for the Millennium Cohort Study was granted by the London Multicentre Research Ethics Committee[1]. Prior to being made available, all data are anonymised, so no further ethical approval is required. The planned use of data was registered with the UK data service[4], and data were obtained from the same website.

The Swedish National Registers

The Swedish registers have their root in the 17th century when the church began keeping information on its members[5]. At first the registers were used as a way

for the church and the Swedish state to keep track of the population for the purposes of enrolling soldiers in the army, but its use has since expanded. The registers now have data on education, medical history, family relations, and even taxes and it is used for a variety of purposes including research[5].

Personal identity numbers

Starting in 1947, permanent residents of Sweden were assigned an individual personal identity number (PIN)[5]. Each resident of Sweden is assigned this PIN upon registration of their birth, or immigration to the country (if they intend to stay for at least one year). The PINs consist of a six digit date of birth, a three digit birth number (which is odd for men and even for women) and an extra "check digit" which is calculated from the other digits[5]. The different registers in Sweden all use the same PIN, allowing data to be linked across registers[5].

The Medical Birth Register

The Medical Birth Register was founded in 1973, and includes data on over 98% of all births in Sweden[6]. An estimated 90% of all Swedish births are reported to Statistics Sweden within 10 days, and 98% are reported within 30 days. The Medical Birth register includes information on infant identification, as well as information on social factors, maternal history, pregnancy, delivery and infant characteristics. Three times, in 1976, 1988, and 2001, the quality of the register was assessed by comparing data from about 500 births to original medical records. Prior to the first quality review, data were input into the Medical Birth Register using summary documents completed by secretaries at obstetric clinics. However, as a result of the 1976 quality review, this was revised so that the Medical Birth Register includes data from three

medical records directly: delivery, infant examinations and antenatal care of the mother. This method has been followed since 1982[6].

The Multi-Generation Register

The Multi-Generation Register was first created in 2000, though prior to that (1994-1999) there was a similar register called the Second Generation register[7]. The Multi-Generation register includes data on people registered in Sweden from 1961 (born from 1932). The purpose of the register is to connect people with their biological parents, and it is a part of the Total Population Register, which receives data from the National Tax Board[7]. Starting in 2002, data were additionally received from an older national register, which includes information on people who died from 1947-1967 or were born from 1961-1967. For people included in the register who were born in Sweden, the Multi-Generation register includes data on biological mother 98% of the time, and biological father 95% of the time[7].

The National School Register

The Swedish National School Register includes data on children completing compulsory years of schooling, which is generally at age 16. The register includes public schools from 1988 and non-public schools from 1993[8]. Children with severe disabilities attend special schools, and are not recorded in the school register, whereas children who attend school but "drop out" before the time of grading are recorded as having a grade of 0[9]. The National School Register is regularly evaluated and considered to be of high enough quality that children who are not included in the cohort, and have not died or emigrated prior to school grading, can be assumed attend a special school[9].

The National Patient Register

The National Patient Register was founded in 1964[10,11]. It originally only collected data on inpatient somatic care in six Swedish counties, in 1970 it was expanded nationwide and in 1973 it additionally began collecting data on inpatient psychiatric care. By 1983 the patient register included 85% of all somatic and psychiatric discharges, and by 1987 it had almost complete national coverage (over 99%), including data on the roughly 1.5 million hospital discharges that happen each year in Sweden. For each discharge, information is recorded in several areas: patient-related data (PIN, age, sex etc.), hospital data, administrative data (admission and discharge dates, etc.), and medical data (diagnoses, procedures etc.)[10]. Inpatient data from the National Patient Register has been validated in a large number of studies, and a systematic review of these studies was conducted in 2010. The review concluded that the positive predictive values of inpatient diagnoses in the patient register ranged from 85-95% for most diagnoses[10].

In 2001, the National Patient Register began collecting data on outpatient visits, including psychiatric care, from both public and private healthcare providers (though this does not include primary care physicians). At the time of the review on the National Patient Register (2010) coverage of outpatient data was estimated to be about 80%[10]. Though there has not been a comprehensive validation and review of the outpatient data as there has with the inpatient data, quality control reviews on the National Patient Register as a whole are conducted internally and values that are obviously incorrect are corrected[11].

The National Drug Register

The National Drug Register was founded in 2005 to record data on prescription drugs in Sweden[12]. The register includes data on the dispensed item (substance, brand name, amount etc.), the patient (PIN, age, sex, place of residence), the prescriber (type of healthcare clinic and prescribers profession), and the date prescribed and dispensed. For drugs prescribed in ambulatory care, the register provides complete national coverage (data missing for 0.3% of items)[12]. The register does not include over the counter medication, drugs that are administered at the hospital, and is not complete with regards to vaccines or drugs used in nursing homes. The register also has no data on indication for the prescription, or information on drugs that are prescribed but not dispensed[12].

Data approval and extraction

Ethical approval for the use of these data was obtained from the research ethics committee at Karolinska Institutet. Informed consent was waived by the ethics committee. As the data were anonymised before use, no need for secondary ethics approval was required. Data were linked using PINs and required variables from each register were merged into one dataset for analysis.

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Appendix 8. Definitions of additional variables included in estimating the association between mode of delivery and induction of labour, and autism spectrum disorder, attention-deficit/hyperactivity disorder and behavioural difficulties.

Small for gestational age (SGA) was calculated using a customized centile calculator[1] accounting for maternal height and weight, ethnicity, birth order, gender, gestational age, and birthweight. A customized centile under 10 was considered SGA. Poverty was defined as having an income under the 60% of the national median equivalized income at the first survey, consistent with previous studies on the MCS cohort[2]. Respondents were asked if they suffered from depression. Those that responded "yes" were asked if they were currently being treated. From this, we created a three category variable for maternal depression: "not depressed," "depressed, not treated" and "depressed, treated." Maternal body mass index (BMI) was calculated using self-reported height and pre-pregnancy weight and categorized as "underweight" (BMI <18.5), "normal" (BMI 18.50-24.99), "overweight" (BMI 25-29.99), and "obese" (BMI >=30)[3]. Infant age when he/she came home from the hospital was categorized into "< 1 day," "1-7 days," "8-30 days," and ">30 days." Consistent with previous studies using the MCS data[4], breastfeeding was divided into "less than or equal to 4 months" and "greater than 4 months." Other variables were self-reported and are available in the MCS dataset.

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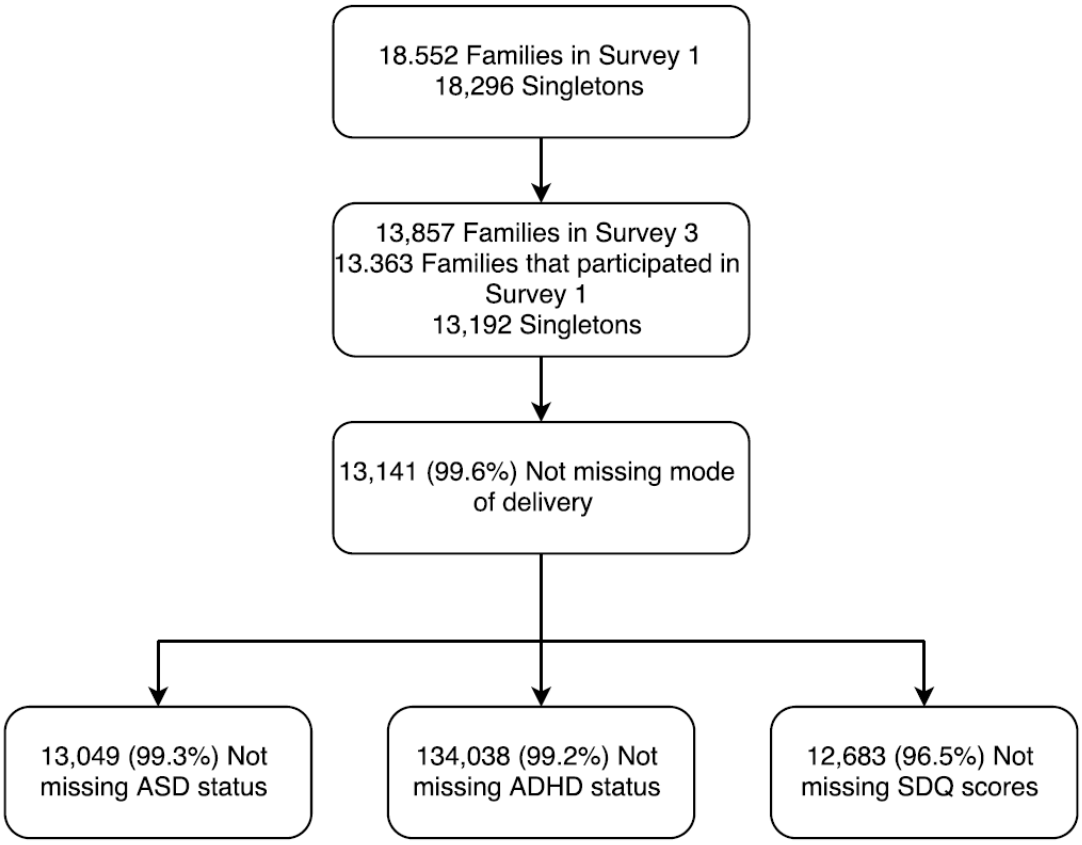
Appendix 9. Flow chart of UK Millennium Cohort Study participants included for analysis.



Appendix 10. The effect of maternal depression, gestational age, maternal irritable bowel syndrome and breast feeding on the association between mode of delivery and induction of labour, and autism spectrum disorders (ASD), and behavioural difficulties/attention-deficit/hyperactivity disorder (ADHD) at age 7 in the UK Millennium Cohort Study.

	Unadjusted model	Model adjusted for maternal depression	Model adjusted for gestational age	Model adjusted for maternal irritable bowel syndrome	Model adjusted for breast feeding
	OR(95%CI)	OR(95%CI)	OR(95%CI)	OR(95%CI)	OR(95%CI)
ASD					
Spontaneous vaginal delivery	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	1.06(0.56,2.01)	1.07(0.57,2.04)	1.13(0.58,2.17)	1.04(0.55,1.98)	1.05(0.56,2.00)
Emergency C-section	1.31(0.68,2.49)	1.31(0.69,2.50)	1.25(0.67,2.33)	1.29(0.68,2.46)	1.28(0.67,2.44)
Induced vaginal delivery	1.05(0.69,1.62)	1.03(0.67,1.58)	1.09(0.69,1.72)	1.05(0.68,1.61)	1.02(0.66,1.57)
Planned C-section	1.08(0.54,2.17)	1.06(0.53,2.11)	0.91(0.43,1.90)	1.06(0.53,1.13)	1.06(0.53,2.13)
Induced C-section	0.98(0.50,1.93)	0.97(0.79,1.92)	1.00(0.50,2.00)	0.98(0.50,1.93)	0.96(0.49,1.89)
ADHD					
Spontaneous vaginal delivery	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	0.88(0.45,1.71)	0.90(0.46,1.74)	0.91(0.46,1.80)	0.87(0.45,1.68)	0.88(0.45,1.70)
Emergency C-section	1.70(0.93,3.11)	1.70(0.93,3.12)	1.64(0.94,2.86)	1.68(0.92,3.07)	1.66(0.91,3.04)
Induced vaginal delivery	1.04(0.65,1.66)	1.00(0.63,1.59)	1.05(0.65,1.70)	1.04(0.65,1.65)	1.01(0.63,1.60)
Planned C-section	0.79(0.37,1.69)	0.77(0.36,1.64)	0.65(0.29,1.44)	0.78(0.37,1.65)	0.78(0.36,1.66)
Induced C-section	1.27(0.60,2.69)	1.27(0.60,2.69)	1.27(0.59,2.70)	1.27(0.60,2.69)	1.25(0.59,2.63)

	Unadjusted model **OR(95%CI)**	**Model adjusted for maternal depression** **OR(95%CI)**	**Model adjusted for gestational age** **OR(95%CI)**	**Model adjusted for maternal irritable bowel syndrome** **OR(95%CI)**	**Model adjusted for breast feeding** **OR(95%CI)**
Abnormal SDQ Score					
Spontaneous vaginal delivery	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	0.77(0.57,1.03)	0.78(0.58,1.05)	0.82(0.61,1.11)	0.76(0.56,1.02)	0.76(0.56,1.02)
Emergency C-section	0.88(0.63,1.23)	0.88(0.63,1.23)	0.80(0.57,1.12)	0.87(0.63,1.22)	0.84(0.60,1.18)
Induced vaginal delivery	**1.26(1.03,1.54)**	1.21(0.99,1.48)	**1.33(1.09,1.63)**	**1.26(1.03,1.53)**	1.20(0.98,1.46)
Planned C-section	0.83(0.60,1.17)	0.80(0.57,1.12)	0.75(0.53,1.05)	0.83(0.59,1.16)	0.80(0.57,1.12)
Induced C-section	0.83(0.59,1.17)	0.82(0.58,1.16)	0.85(0.60,1.21)	0.83(0.59,1.17)	0.80(0.56,1.13)

Appendix 11. The impact of mode of delivery and induction of labour on abnormal scores in sub-groups of the Strengths and Difficulties Questionnaire at age 7 in the UK Millennium Cohort Study.

	Exposed Cases	Unadjusted OR OR (95%CI)	Model 1 Pregnancy and delivery factors OR (95%CI)	Model 2 Maternal health/mental health OR (95%CI)	Model 3 Demographic /Socioeconomic factors OR (95%CI)	Model 4 Model 1 + 3 OR (95%CI)	Model 5 Model 1 + 2 + 3 OR (95%CI)
Emotional Problems							
Spontaneous vaginal delivery	441	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	97	1.05(0.80,1.39)	1.00(0.75,1.34)	1.19(0.89,1.43)	1.19(0.89,1.58)	1.11(0.82,1.51)	1.19(0.87,1.63)
Emergency C-section	68	1.03(0.75,1.42)	0.93(0.66,1.30)	1.06(0.76,1.57)	1.15(0.82,1.61)	1.04(0.74,1.48)	1.09(0.76,1.57)
Induced vaginal delivery	203	1.12(0.91,1.37)	1.09(0.88,1.35)	1.06(0.86,1.48)	1.05(0.84,1.30)	1.04(0.83,1.30)	1.00(0.79,1.26)
Planned C-section	74	0.89(0.65,1.23)	0.87(0.63,1.20)	0.91(0.65,1.32)	1.08(0.77,1.52)	1.03(0.73,1.46)	1.02(0.71,1.47)
Induced C-section	203	0.99(0.71,1.37)	0.93(0.65,1.31)	1.02(0.74,1.43)	1.18(0.84,1.64)	1.08(0.75,1.54)	1.10(0.77,1.58)
Conduct Problems							
Spontaneous vaginal delivery	604	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	103	0.78(0.60,1.01)	0.87(0.66,1.14)	0.84(0.65,1.10)	0.92(0.67,1.26)	1.04(0.74,1.45)	1.06(0.76,1.50)
Emergency C-section	70	0.72(0.53,0.98)	0.73(0.53,1.01)	0.73(0.53,1.01)	0.70(0.46,1.07)	0.72(0.47,1.11)	0.76(0.48,1.18)
Induced vaginal delivery	307	1.19(1.00,1.42)	**1.21(1.01,1.45)**	1.14(0.95,1.36)	1.18(0.94,1.49)	1.26(0.99,1.61)	1.24(0.97,1.59)
Planned C-section	98	0.90(0.69,1.18)	0.87(0.66,1.15)	0.84(0.63,1.11)	1.08(0.76,1.54)	0.97(0.67,1.40)	0.88(0.60,1.29)
Induced C-section	78	0.79(0.58,1.07)	0.88(0.64,1.21)	0.78(0.57,1.07)	0.89(0.60,1.30)	0.99(0.66,1.48)	0.98(0.65,1.48)
Hyperactivity							
Spontaneous vaginal delivery	799	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	149	0.79(0.63,1.00)	0.79(0.92,1.00)	0.86(0.68,1.08)	0.84(0.66,1.06)	0.84(0.66,1.07)	0.87(0.67,1.13)
Emergency C-section	124	1.18(0.93,1.51)	1.00(0.77,1.30)	1.22(0.95,1.57)	1.28(0.99,1.66)	1.10(0.84,1.44)	1.10(0.83,1.46)
Induced vaginal delivery	343	1.11(0.95,1.31)	1.07(0.90,1.26)	1.05(0.89,1.25)	1.07(0.91,1.27)	1.06(0.89,1.27)	1.02(0.85,1.23)
Planned C-section	118	0.84(0.65,1.08)	0.75(0.57,0.98)	0.83(0.64,1.08)	0.93(0.71,1.22)	0.80(0.60,1.07)	0.78(0.58,1.06)
Induced C-section	97	0.83(0.63,1.09)	0.74(0.55,1.00)	0.80(0.60,1.06)	0.91(0.69,1.22)	0.84(0.62,1.14)	0.77(0.56,1.06)

	Exposed Cases	Unadjusted OR OR (95%CI)	Model 1 Pregnancy and delivery factors OR (95%CI)	Model 2 Maternal health/mental health OR (95%CI)	Model 3 Demographic /Socioeconomic factors OR (95%CI)	Model 4 Model 1 + 3 OR (95%CI)	Model 5 Model 1 + 2 + 3 OR (95%CI)
Peer Problems							
Spontaneous vaginal delivery	543	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	124	1.22(0.95,1.56)	1.20(0.92,1.55)	**1.35(1.04,1.74)**	**1.44(1.07,1.95)**	1.39(1.00,1.92)	**1.42(1.01,2.00)**
Emergency C-section	80	1.10(0.82,1.48)	0.90(0.66,1.23)	1.11(0.81,1.51)	1.15(0.77,1.72)	0.89(0.59,1.36)	0.94(0.60,1.45)
Induced vaginal delivery	261	**1.29(1.07,1.55)**	**1.28(1.05,1.55)**	1.22(1.00,1.48)	**1.36(1.06,1.74)**	**1.37(1.05,1.78)**	**1.36(1.04,1.78)**
Planned C-section	74	0.77(0.57,1.05)	0.67(0.49,0.93)	0.74(0.54,1.02)	0.84(0.57,1.26)	0.69(0.45,1.04)	0.71(0.46,1.10)
Induced C-section	261	1.01(0.75,1.36)	0.90(0.65,1.25)	0.96(0.70,1.32)	1.11(0.75,1.65)	0.94(0.61,1.45)	0.89(0.57,1.39)
Prosocial							
Spontaneous vaginal delivery	119	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	19	0.70(0.37,1.35)	0.68(0.35,1.34)	0.75(0.38,1.48)	0.60(0.31,1.17)	0.52(0.26,1.04)	0.53(0.24,1.18)
Emergency C-section	17	0.83(0.45,1.53)	0.60(0.32,1.13)	0.87(0.46,1.65)	0.92(0.46,1.86)	0.56(0.27,1.15)	0.53(0.22,1.25)
Induced vaginal delivery	43	0.83(0.55,1.25)	0.83(0.54,1.26)	0.82(0.53,1.26)	0.96(0.59,1.55)	0.93(0.57,1.51)	0.94(0.54,1.64)
Planned C-section	14	0.57(0.29,1.13)	0.46(0.22,0.93)	0.58(0.29,1.19)	0.63(0.29,1.38)	0.42(0.18,0.98)	0.43(0.16,1.15)
Induced C-section	18	1.11(0.62,1.98)	0.94(0.50,1.76)	1.18(0.65,2.14)	1.45(0.77,2.73)	1.03(0.51,2.07)	1.15(0.53,2.50)

Pregnancy and delivery factors: SGA, gestational age, maternal high blood pressure/pre-eclampsia, maternal smoking during pregnancy, being the first born child, bleeding or threatened miscarriage during pregnancy, and infant age when he or she came home from the hospital

Demographic/socio-economic factors: poverty, ethnicity, maternal age, maternal education, urbanicity, single parent household at time of first survey[a], paternal age,[b] and paternal education[c].

Maternal health/mental health: maternal depression, maternal BMI, and if pregnancy was a surprise

[a] Included in hyperactivity analysis due to significance after adjustment

[b] Included in peer problems analysis due to significance in after adjustment

[c] Included in conduce problems analysis due to significant in after adjustment

Appendix 12. The effect of small for gestational age, pre-term birth, breech presentation, advanced maternal age, and gestational hypertension on the association between mode of delivery and induction of labour, and autism spectrum disorders (ASD), and behavioural difficulties/attention-deficit/hyperactivity disorder (ADHD) at age 7 in the UK Millennium Cohort Study.

	Exposed Cases	Adjusted OR in full cohort	Excluding small for gestational age OR(95%CI)	Excluding pre-term births OR(95%CI)	Excluding females OR(95%CI)	Excluding breech presentation OR(95%CI)	Excluding mothers over the age of 40 OR(95%CI)	Excluding mothers with gestational hypertension OR(95%CI)
ASD								
Spontaneous vaginal delivery	93	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	20	0.99(0.50,1.95)	0.96(0.45,2.03)	0.99(0.49,1.98)	0.83(0.37,1.85)	0.98(0.50,1.94)	0.96(0.48,1.94)	0.96(0.45,2.06)
Emergency C-section	27	0.94(0.52,1.68)	0.93(0.48,1.78)	0.80(0.42,1.55)	0.90(0.48,1.71)	0.99(0.55,1.81)	0.92(0.50,1.68)	0.87(0.46,1.68)
Induced vaginal delivery	40	0.90(0.54,1.49)	0.93(0.53,1.62)	0.92(0.55,1.54)	0.78(0.44,1.36)	0.90(0.55,1.49)	0.92(0.55,1.52)	0.89(0.51,1.54)
Planned C-section	18	0.73(0.34,1.60)	0.88(0.39,1.98)	0.79(0.36,1.75)	0.65(0.31,1.33)	0.84(0.38,1.84)	0.72(0.32,1.63)	0.74(0.31,1.75)
ADHD								
Spontaneous vaginal delivery	77	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	14	0.74(0.35,1.56)	0.77(0.34,1.74)	0.72(0.34,1.52)	0.84(0.39,1.82)	0.76(0.36,1.61)	0.71(0.32,1.54)	0.76(0.34,1.69)
Emergency C-section	25	1.28(0.72,2.28)	1.43(0.76,2.69)	0.89(0.43,1.86)	1.44(0.77,2.69)	1.29(0.70,2.37)	1.33(0.74,2.39)	1.42(0.77,2.62)
Induced vaginal delivery	35	0.85(0.51,1.41)	0.83(0.46,1.48)	0.83(0.50,1.38)	0.88(0.49,1.57)	0.84(0.50,1.39)	0.90(0.55,1.49)	0.87(0.51,1.50)
Planned C-section	16	0.68(0.29,1.58)	0.85(0.35,2.06)	0.71(0.30,1.67)	0.64(0.25,0.17)	0.59(0.23,1.52)	0.72(0.31,1.68)	0.73(0.29,1.79)
Abnormal SDQ Score								
Spontaneous vaginal delivery	448	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	83	0.95(0.68,1.34)	0.95(0.65,1.38)	0.90(0.63,1.29)	0.70(0.46,1.08)	0.95(0.67,1.34)	0.96(0.68,1.36)	0.95(0.66,1.37)
Emergency C-section	110	0.89(0.64,1.23)	0.94(0.65,1.34)	0.84(0.59,1.21)	0.83(0.56,1.22)	0.86(0.61,1.21)	0.86(0.62,1.20)	0.96(0.68,1.34)
Induced vaginal delivery	223	1.19(0.95,1.50)	1.21(0.94,1.56)	1.19(0.94,1.51)	1.13(0.84,1.53)	1.19(0.94,1.49)	1.22(0.96,1.53)	1.27(1.00,1.61)
Planned C-section	79	0.91(0.64,1.31)	0.90(0.61,1.33)	0.94(0.65,1.36)	0.70(0.43,1.13)	0.78(0.53,1.17)	0.93(0.64,1.34)	0.83(0.56,1.22)

Appendix 13. The effect of excluding children with other measure outcomes on the association between mode of delivery and induction of labour, and autism spectrum disorders (ASD), and behavioural difficulties/attention-deficit/hyperactivity disorder (ADHD) at age 7 in the UK Millennium Cohort Study.

	Exposed Cases	Adjusted model OR(95%CI)	Excluding children with other outcomes from control group OR(95%CI)
ASD			
Spontaneous vaginal delivery	93	Ref	Ref
Assisted vaginal delivery	20	0.99(0.50,1.95)	0.97(0.49,1.90)
Emergency C-section	27	0.94(0.52,1.68)	0.92(0.51,1.67)
Induced vaginal delivery	40	0.90(0.54,1.49)	0.93(0.56,1.54)
Planned C-section	18	0.73(0.34,1.60)	0.72(0.33,1.46)
ADHD			
Spontaneous vaginal delivery	77	Ref	Ref
Assisted vaginal delivery	14	0.74(0.35,1.56)	0.73(0.34,1.54)
Emergency C-section	25	1.28(0.72,2.28)	1.23(0.68,2.20)
Induced vaginal delivery	35	0.85(0.51,1.41)	0.88(0.53,1.45)
Planned C-section	16	0.68(0.29,1.58)	0.67(0.29,1.55)
Abnormal SDQ Score			
Spontaneous vaginal delivery	448	Ref	Ref
Assisted vaginal delivery	83	0.95(0.68,1.34)	0.95(0.68,1.34)
Emergency C-section	110	0.89(0.64,1.23)	0.89(0.64,1.23)
Induced vaginal delivery	223	1.19(0.95,1.50)	1.19(0.95,1.50)
Planned C-section	79	0.91(0.64,1.31)	0.91(0.64,1.31)

Appendix 14. The impact of induction of labour and mode of delivery on autism spectrum disorders (ASD), and behavioural difficulties/attention-deficit/hyperactivity disorder (ADHD) at age 7 in the UK Millennium Cohort Study.

	Exposed cases	Unadjusted OR OR(95%CI)	Model 1 Pregnancy and delivery factors OR(95%CI)	Model 2 Maternal health/mental health OR(95%CI)	Model 3 Demographic /Socioeconomic factors OR(95%CI)	Model 4 Model 1 + 3 OR(95%CI)	Model 5 Model 1 + 2 + 3 OR(95%CI)
Induction							
ASD	66	1.10(0.76,1.57)	1.03(0.69,1.55)	1.05(0.72,1.54)	1.07(0.73,1.59)	1.04(0.67,1.61)	0.97(0.61,1.52)
Behavioural Difficulties/ADHD	343	1.18(1.00,1.38)	**1.24(1.04,1.48)**	1.14(0.96,1.35)	1.13(0.95,1.35)	1.19(0.99,1.43)	1.16(0.96,1.41)
Mode of Delivery							
ASD							
Unassisted vaginal delivery	133	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	20	1.05(0.56,1.96)	1.03(0.54,1.96)	1.10(0.58,2.11)	0.86(0.46,1.61)	0.81(0.43,1.55)	0.84(0.43,1.64)
Emergency C-section	27	1.16(0.70,1.92)	0.86(0.49,1.51)	1.27(0.77,2.12)	1.16(0.67,2.00)	0.87(0.48,1.55)	0.95(0.53,1.71)
Planned C-section	18	1.05(0.56,1.97)	0.80(0.41,1.54)	1.14(0.60,2.16)	0.82(0.36,1.88)	0.56(0.23,1.36)	0.58(0.24,1.41)
Behavioural Difficulties/ADHD							
Unassisted vaginal delivery	707	Ref	Ref	Ref	Ref	Ref	Ref
Assisted vaginal delivery	92	0.78(0.60,1.03)	0.78(0.59,1.05)	0.90(0.68,1.20)	0.89(0.66,1.20)	0.93(0.66,1.32)	0.99(0.69,1.43)
Emergency C-section	121	0.86(0.68,1.10)	0.75(0.57,0.98)	0.91(0.71,1.18)	1.02(0.79,1.32)	0.89(0.64,1.24)	0.92(0.65,1.30)
Planned C-section	88	0.85(0.63,1.13)	0.77(0.57,1.04)	0.83(0.62,1.13)	1.06(0.77,1.44)	0.86(0.60,1.23)	0.84(0.57,1.23)

Pregnancy and delivery factors: SGA, gestational age, maternal high blood pressure/pre-eclampsia, maternal smoking during pregnancy, being the first born child, bleeding or threatened miscarriage during pregnancy, and infant age when he or she came home from the hospital

Demographic/socio-economic factors: poverty, ethnicity, maternal age, maternal education, urbanicity, single parent household at time of first survey[a], paternal age[b]

Maternal health/mental health: maternal depression, maternal BMI , maternal IBS[c], and if the pregnancy was a surprise[d]

[a] Included in behavioural difficulties/ADHD models due to significance after adjustment

[b] Included in ASD models due to significance in after adjustment

[c] Included in the induced ASD model due to significance in after adjustment

[d] Included in behavioural difficulties/ADHD models due to significance in after adjustment

Appendix 15. Definitions of potential confounders included in the Swedish National Register estimation of the association between mode of delivery and autism spectrum disorder.

We obtained data on small for gestational age (SGA), large for gestational age (LGA), infant sex, maternal age at birth, gestational age, birth order, 5 minute Apgar score, and maternal and paternal country of birth from the Medical Birth Register. For socio-economic factors we included variables on social welfare, disposable income, and parental highest education level. The variable on social welfare indicated whether either parent received social welfare the year the index child was born. The variable on disposable income indicated the disposable income of the household the year the child was born, and was divided into quintiles, ranging from "low income" to "high income". Both were available for the entire study period. Parental highest education level was available starting in 1990 and was divided into three categories: "pre-high school," "high school," and "post high school".

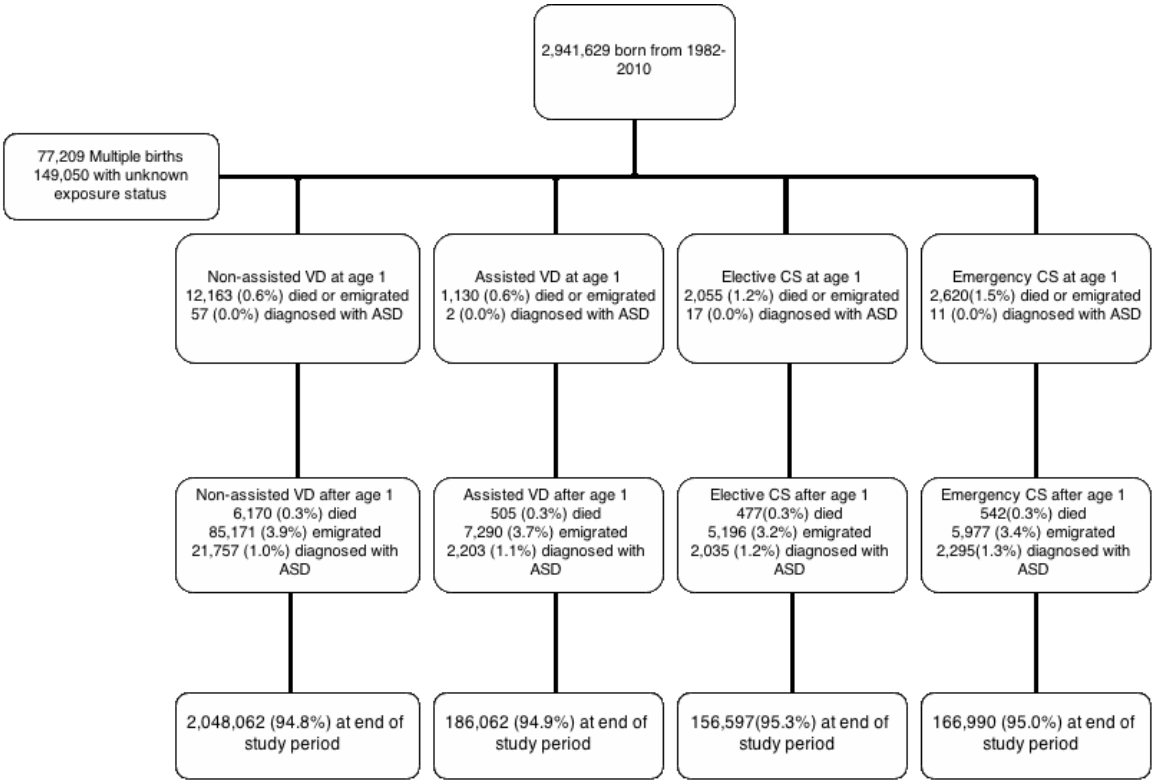
We obtained data on maternal and paternal depression (ICD-8: 29600, 3004; ICD-9: 296B, 311, 300E; ICD-10: F32, F33), bipolar disorder (ICD-8: 29610-29630; ICD-9: 296A,C-E; ICD-10: F30-31), and non-affective disorders (ICD-8: 295, 297, 298 [excluding 29800 and 29810], 29999; ICD-9: 295, 297, 298 [excluding 298A and 298B]; ICD-10: F20-29) from the National Patient Register, which has data on inpatient care from 1964 and outpatient care from 2001. Using date of child's birth and date of first parental diagnosis, maternal and paternal depression, bipolar disorder

and non-affective disorder were divided into three categories: never depressed, first diagnosis before birth and first diagnosis after birth.

Appendix 16. Sensitivity analyses performed to assess the association between mode of delivery and autism spectrum disorder.

We conducted sensitivity analyses by excluding children who were preterm (<37 weeks' gestation), female, SGA, LGA, born outside of Stockholm County (due to possible differences in diagnostic validity) and children whose mothers had ever been diagnosed with depression, non-affective disorder or bipolar disorder. We excluded children born before 1990, and before 2000 to examine the possible changes in risk over time as obstetric procedures and diagnostic criteria may have changed. We also restricted the analysis to the first child, second child, only child, or first two children for every mother in the cohort, to compare to the results from the sibling design. Data on induction of labour were available from 1990 onwards. To determine the effect of induction on the association between CS and ASD27, we divided "unassisted VD" into "induced VD" and "spontaneous, unassisted VD" and re-ran the analysis. We also conducted a sensitivity analysis, re-running the model including children diagnosed before 1 year of age to determine any impact on our results. We did not have data regarding specific indications for CS. To assess what impact this may have had on the association between mode of delivery and ASD, we conducted sensitivity analyses excluding mothers over the age of 40, obese mothers, mothers with pre-gestational diabetes or chronic hypertension, and babies who were LGA or SGA. We also examined the possible impact of breech presentation, as this is a possible indication for CS.

Appendix 17. Reasons for censoring study participants in the Sweden National Register autism spectrum disorder analysis by mode of delivery.



Appendix 18. The adjusted[a] association between mode of delivery and autism spectrum disorder among sub-populations in the Swedish National Registers.

	Exposed Cases	Among non-preterm babies HR (95% CI)	Exposed Cases	Among male babies HR (95% CI)	Exposed Cases	Among those with no maternal depression, bipolar disorder or non-affective disorder HR (95% CI)	Exposed Cases	Among those born in Stockholm County HR (95% CI)
Unassisted VD [b]	20,822	Ref	15,182	Ref	17,510	Ref	14,862	Ref
Assisted VD	2,147	1.03(0.98-1.08)	1,703	1.02(0.97-1.08)	1,746	1.02(0.97-1.08)	1,357	1.00(0.92-1.07)
Elective CS[c]	1,632	**1.20(1.14-1.27)**	1,440	**1.22(1.15-1.29)**	1,576	**1.22(1.16-1.30)**	1,296	**1.21(1.11-1.31)**
Emergency CS	1,923	**1.16(1.10-1.22)**	1,711	**1.15(1.09-1.21)**	1,832	**1.19(1.13-1.23)**	1,500	**1.09(1.01-1.17)**

[a] Adjusted for year of birth, infant gender, maternal age, gestational age, 5 minute Apgar score, maternal and paternal country of birth, small for gestational age, large for gestational age, first born, family income, maternal and paternal depression, bipolar disorder, and non-affective disorder
[b]Vaginal delivery
[c]Caesarean section

Appendix 19. The adjusted[a] association between mode of delivery and autism spectrum disorder as it relates to year of birth and age at diagnosis in the Swedish National Registers.

	Among those 2007 or earlier		Including those diagnosed before 1 year of age	
	Exposed Cases	**HR (95% CI)**	**Exposed Cases**	**HR (95% CI)**
Unassisted VD [a]	21,565	Ref	21,814	Ref
Assisted VD	2,180	1.03(0.98-1.08)	2,205	1.03(0.98-1.07)
Elective CS[b]	1,998	**1.20(1.14-1.27)**	2,052	**1.21(1.15-1.27)**
Emergency CS	2,269	**1.15(1.10-1.20)**	2,306	**1.15(1.10-1.20)**

[a] Adjusted for year of birth, infant gender, maternal age, gestational age, 5 minute Apgar score, maternal and paternal country of birth, small for gestational age, large for gestational age, first born, family income, maternal and paternal depression, bipolar disorder, and non-affective disorder
[b]Vaginal delivery
[c]Caesarean section

Appendix 20. The adjusted[a] association between mode of delivery and autism spectrum disorder as it relates to birth order in the Swedish National Registers.

	Exposed Cases	Among first born HR (95% CI)	Exposed Cases	Among first and second born HR (95% CI)	Exposed Cases	Among only children HR (95% CI)	Exposed Cases	Among families with 2 children HR (95% CI)	Exposed Cases	Among primary C-Section HR (95% CI)
Unassisted VD[b]	11760	Ref	18,805	Ref	3,862	Ref	9,379	Ref	22,015	Ref
Assisted VD	1932	1.02(0.97-1.07)	2,151	1.03(0.98-1.08)	588	0.96(0.87-1.05)	1,077	1.04(0.98-1.11)	22,36	1.04(0.99-1.09)
Elective CS[c]	1102	**1.24(1.16-1.32)**	1,755	**1.22(1.15-1.28)**	563	**1.22(1.10-1.34)**	881	**1.20(1.11-1.30)**	14,64	**1.25(1.18-1.32)**
Emergency CS	1674	**1.13(1.07-1.19)**	2,124	**1.15(1.10-1.20)**	652	1.06(0.97-1.16)	1,061	**1.17(1.10-1.26)**	2,069	**1.18(1.12-1.23)**

[a] Adjusted for year of birth, infant gender, maternal age, gestational age, 5 minute Apgar score, maternal and paternal country of birth, small for gestational age, large for gestational age, first born, family income, maternal and paternal depression, bipolar disorder, and non-affective disorder
[b]Vaginal delivery
[c]Caesarean section

Appendix 21. The association between mode of delivery, including induction of labour, and autism spectrum disorder in the Swedish National Registers.

	Exposed Cases	Partially Adjusted[a] HR (95% CI)	Adjusted[b] HR (95% CI)
Unassisted VD[c]	14,133	Ref	Ref
Induced VD	1,698	**1.40(1.33-1.48)**	**1.27(1.20-1.34)**
Assisted VD	1,696	**1.23(1.41-1.57)**	1.05(0.99-1.10)
Elective CS[d]	1,867	**1.49(1.37-1.51)**	**1.28(1.211.35)-**
Emergency CS	1,632	**1.44(1.33-1.48)**	**1.18(1.12-1.24)**

[a]Adjusted for year of birth
[b]Adjusted for year of birth, infant gender, maternal age, gestational age, 5 minute Apgar score, maternal and paternal country of birth, small for gestational age, large for gestational age, first born, family income, maternal and paternal depression, bipolar disorder, and non-affective disorder
[c]Vaginal delivery
[d]Caesarean section

Appendix 22. The adjusted[a] association between mode of delivery and autism spectrum disorder among sub-populations at lower risk for Caesarean section in the Swedish National Registers.

	Exposed Cases	Excluding women 40+ HR (95% CI)	Exposed Cases	Excluding obese women HR (95% CI)	Exposed Cases	Excluding women with pre-gestational diabetes HR (95% CI)	Exposed Cases	Excluding women with chronic hypertension HR (95% CI)	Exposed Cases	Excluding large and small for gestational age HR (95% CI)
Unassisted VD[b]	21,205	Ref	20,421	Ref	21,661	Ref	21,684	Ref	20,291	Ref
Assisted VD	2,144	1.03(0.99-1.08)	2,072	1.04(0.99-1.09)	2,185	1.03(0.99-1.08)	2,194	**1.04(0.99-1.08)**	2,041	1.04(0.99-1.09)
Elective CS[c]	1,893	**1.22(1.16-1.28)**	1,827	**1.21(1.15-1.28)**	1,997	**1.22(1.16-1.28)**	2,020	**1.22(1.16-1.28)**	1,648	**1.21(1.14-1.27)**
Emergency CS	2,184	**1.16(1.11-1.22)**	2,026	**1.14(1.09-1.20)**	2,265	**1.16(1.11-1.22)**	2,279	**1.16(1.11-1.22)**	1,921	**1.16(1.10-1.22)**

[a] Adjusted for year of birth, infant gender, maternal age, gestational age, 5 minute Apgar score, maternal and paternal country of birth, small for gestational age, large for gestational age, first born, family income, maternal and paternal depression, bipolar disorder, and non-affective disorder
[b]Vaginal delivery
[c]Caesarean section

Appendix 23. The adjusted[a] association between mode of delivery and autism spectrum disorder among breech and non-breech babies in the Swedish National Register.

	Exposed Cases	Including only babies with breech presentation HR (95% CI)			Exposed Cases	Excluding babies with breech presentation HR (95% CI)		
Unassisted VD [b]	198	Ref			21,559	Ref		
Assisted VD	7	1.52	(0.72-	3.24)	2,196	1.04	(0.99-	1.09)
Elective CS[c]	405	1.07	(0.89-	1.30)	1,630	**1.20**	**(1.14-**	**1.27)**
Emergency CS	239	0.96	(0.79-	1.17)	2,065	**1.17**	**(1.12-**	**1.23)**

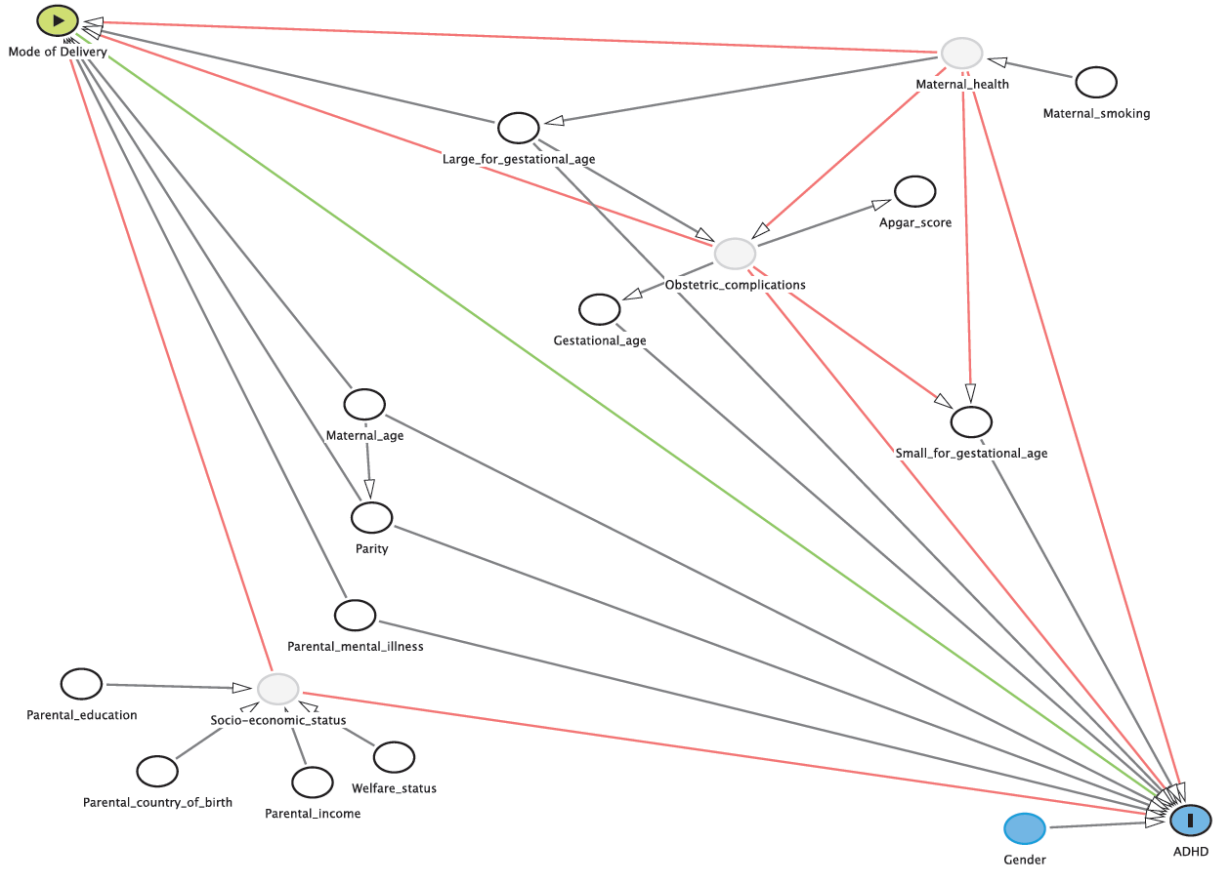
[a] Adjusted for year of birth, infant gender, maternal age, gestational age, 5 minute Apgar score, maternal and paternal country of birth, small for gestational age, large for gestational age, first born, family income, maternal and paternal depression, bipolar disorder, and non-affective disorder
[b]Vaginal delivery
[c]Caesarean section

Appendix 24. The association between mode of delivery, and breech presentation Caesarean sections and autism spectrum disorders in the Swedish National Registers.

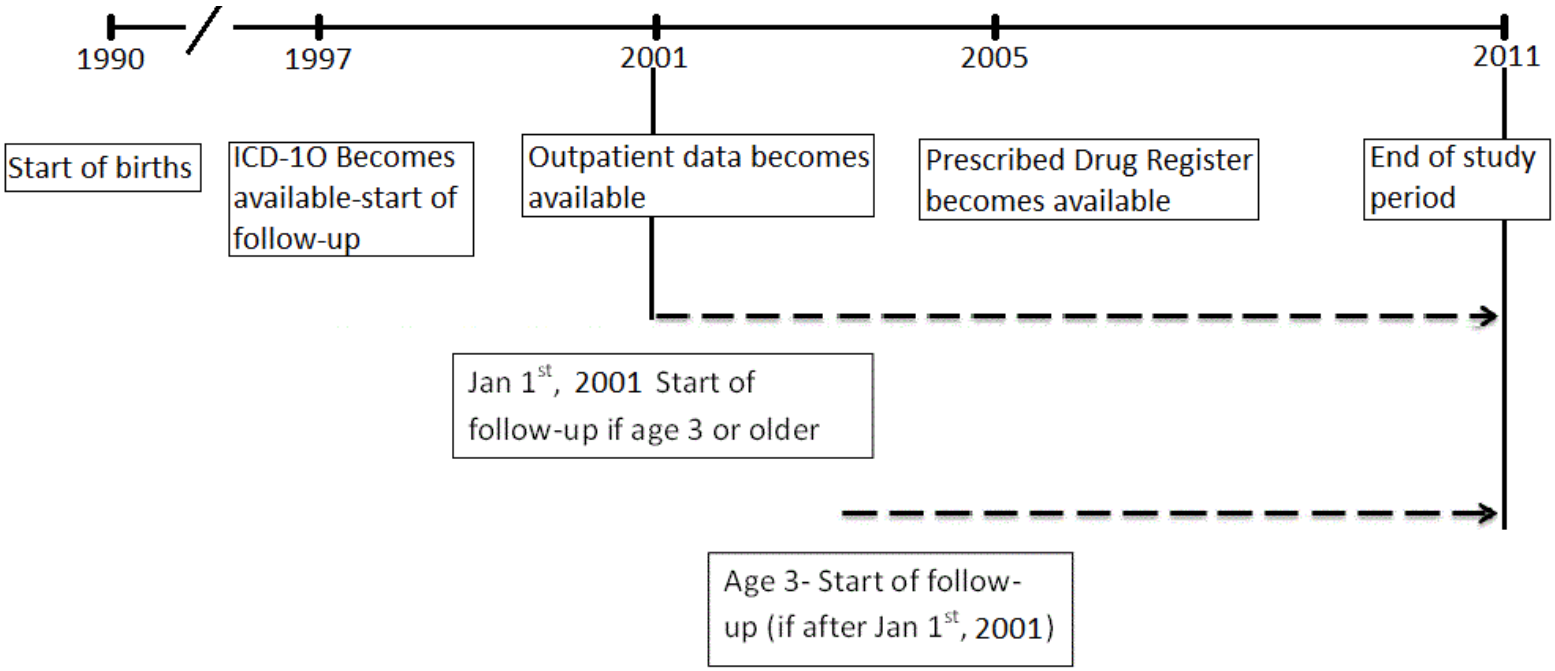
	Exposed Cases	Partially Adjusted[a] HR (95% CI)			Adjusted[b] HR (95% CI)		
Unassisted VD[c]	21,757	Ref			Ref		
Assisted VD	2,203	**1.18**	**(1.13-**	**1.23)**	1.04	(0.99-	1.08)
Elective CS[d]	1,630	**1.39**	**(1.32-**	**1.46)**	**1.20**	**(1.14-**	**1.26)**
Elective Breech	405	**1.38**	**(1.25-**	**1.52)**	**1.27**	**(1.15-**	**1.40)**
Emergency CS	2,056	**1.41**	**(1.34-**	**1.47)**	**1.17**	**(1.12-**	**1.23)**
Emergency Breech	238	**1.33**	**(1.17-**	**1.51)**	1.14	(1.00-	1.29)

[a]Adjusted for year of birth
[b]Adjusted for year of birth, infant gender, maternal age, gestational age, 5 minute Apgar score, maternal and paternal country of birth, small for gestational age, large for gestational age, first born, family income, maternal and paternal depression, bipolar disorder, and non-affective disorder
[c]Vaginal delivery
[d]Caesarean section

Appendix 25. Directed Acyclic Graph of the association between mode of delivery and attention-deficit/hyperactivity disorder



Appendix 26. Timeline of sensitivity analysis beginning in 2001 for the Swedish National Register study on attention-deficit/hyperactivity disorder.



Appendix 27. Description of methods for measuring association of mode of delivery and ADHD using sibling controls with conditional logistic regression in the Swedish National Registers.

For the conditional logistic regression sibling-control design, we included all sibling pairs in our population that included the first two children for each mother, where one was diagnosed with ADHD and one was not. We restricted the analysis to sibling pairs where follow-up time was longer for the control sibling as conditional logistic regression does not account for differential follow-up time. The case sibling could be either the first or second born, as long as they were diagnosed at a younger age than when the control sibling was censored from the study. We performed conditional logistic regression matched on maternal ID. Estimates for the association between mode of delivery and ADHD were obtained using pairs discordant on both ADHD and mode of delivery, although pairs concordant on mode of delivery were included in the analysis as they contribute to the covariate estimates.

Appendix 28. Sensitivity analyses performed to assess the association between mode of delivery and attention-deficit/hyperactivity disorder in the Swedish National Registers.

	Exposed Cases	Among non-preterm babies HR (95% CI)	Exposed Cases	Among male babies HR (95% CI)	Exposed Cases	Among those with no maternal depression, Bipolar Disorder or non-affective disorder HR (95% CI)	Exposed Cases	Among those born in Stockholm County HR (95% CI)	Exposed Cases	Including those diagnosed before 3 years of age HR (95% CI)
Unassisted VD	35,282	Ref	25,851	Ref	29,602	Ref	10,250	Ref	37,277	Ref
Assisted VD	3,319	1.02 (0.98-1.05)	2,642	1.02 (0.98-1.06)	2,745	1.02 (0.98-1.06)	1,172	0.99 (0.92-1.05)	3,470	1.01 (0.98-1.05)
Elective CS	2,603	**1.14 (1.09-1.19)**	2,393	**1.16 (1.11-1.21)**	2,522	**1.14 (1.09-1.19)**	956	1.06 (0.99-1.14)	3,384	**1.15 (1.11-1.20)**
Emergency CS	3,293	**1.16 (1.12-1.20)**	2,831	**1.14 (1.10-1.19)**	3,027	**1.16 (1.12-1.21)**	1,323	**1.17 (1.10-1.24)**	3,919	**1.16 (1.12-1.20)**

Abbreviations: HR-Hazard ratio; VD-vaginal delivery; CS-Caesarean section

Appendix 29. Sensitivity analyses relating to birth order and family size performed to assess the association between mode of delivery and attention-deficit/hyperactivity disorder in the Swedish National Registers.

	Exposed Cases	Primary CS HR (95% CI)	Exposed Cases	First Child HR (95% CI)	Exposed Cases	First and Second Child HR (95% CI)	Exposed Cases	Birth Order 3+ HR (95% CI)	Exposed Cases	One-child Families HR (95% CI)
Unassisted VD	37,099	Ref	22,551	Ref	34,259	Ref	2,840	Ref	9,213	Ref
Assisted VD	3,449	1.02 (0.98-1.06)	3,034	1.02 (0.98-1.06)	3,406	1.02 (0.98-1.05)	43	0.83 (0.61-1.12)	1,004	0.95 (0.88-1.02)
Elective CS	2,194	**1.19 (1.14-1.25)**	1,947	**1.15 (1.10-1.21)**	3,000	**1.14 (1.10-1.19)**	340	**1.18 (1.04-1.33)**	1,078	**1.22 (1.12-1.34)**
Emergency CS	3,315	**1.17 (1.12-1.21)**	2,971	**1.15 (1.11-1.20)**	3,720	**1.15 (1.11-1.20)**	170	**1.16 (0.98-1.36)**	1,307	**1.13 (1.05-1.22)**

Abbreviations: HR-Hazard ratio; VD-vaginal delivery; CS-Caesarean section

Appendix 30. The adjusted association between mode of delivery and attention-deficit/hyperactivity disorder using sibling controls, in first born cases and medicated cases in the Swedish National Registers.

	Exposed Cases	HR among first born cases			Exposed Cases	HR among medicated cases		
Unassisted VD	1,388	Ref			2404	Ref		
Assisted VD	1,330	1.27	(0.96-	1.69)	1195	1.02	(0.91-	1.15)
Elective CS	474	1.03	(0.74-	1.43)	914	1.05	(0.91-	1.20)
Emergency CS	1,004	1.26	(0.94-	1.69)	1097	1.14	(1.00-	1.29)

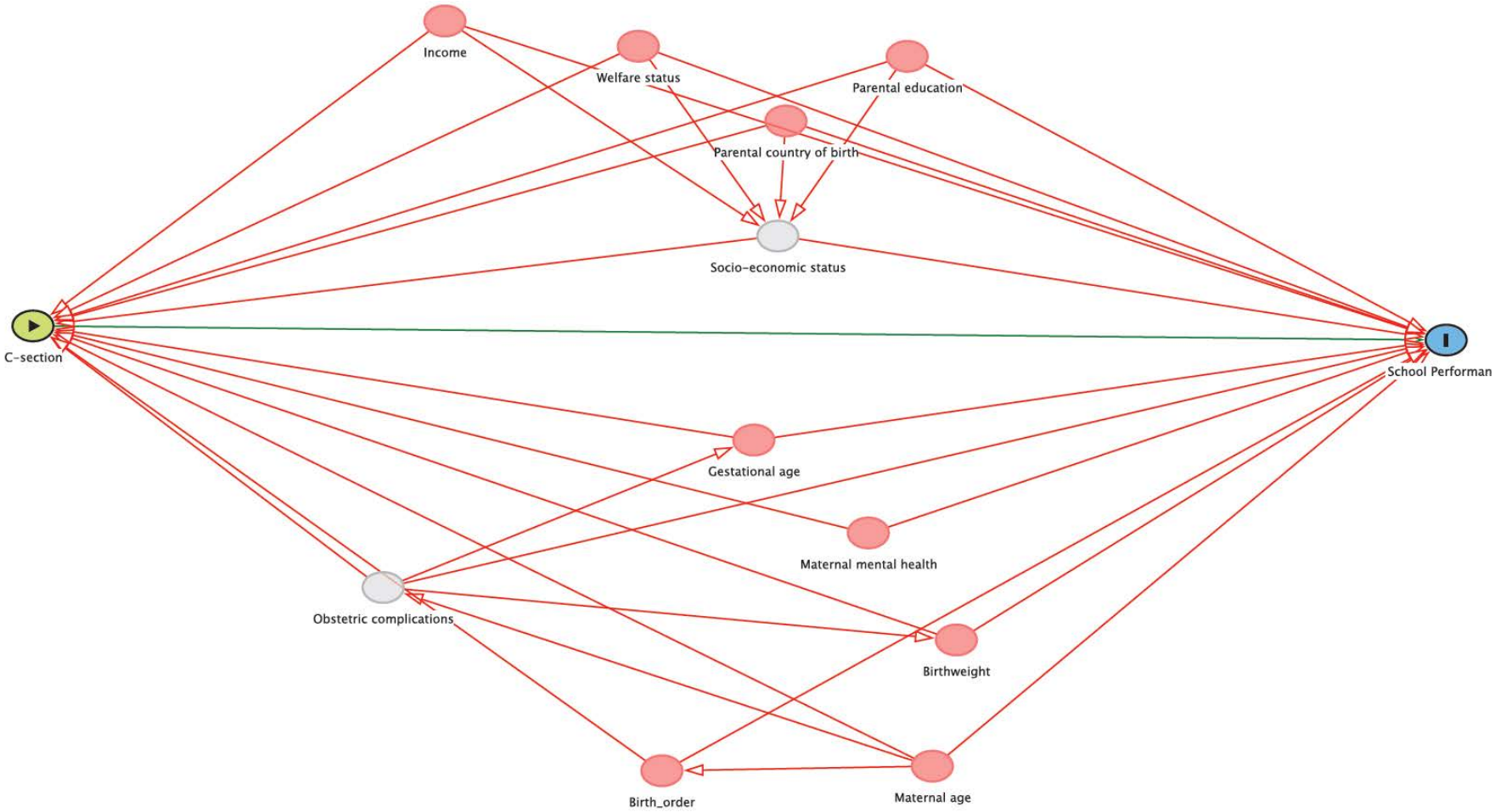
Abbreviations: HR-Hazard ratio; VD-vaginal delivery; CS-Caesarean section

Appendix 31. The association between mode of delivery and attention-deficit/hyperactivity disorder among sibling pairs in the Swedish National Registers.

	Exposed Cases	Partially Adjusted OR from 1997			Fully Adjusted OR from 1997			Exposed Cases	Partially Adjusted OR from 2001			Fully Adjusted OR from 2001		
Unassisted VD	15,621	Ref			Ref			14,501	Ref			Ref		
Assisted VD	1,186	1.11	(0.99-	1.24)	1.05	(0.93-	1.19)	1,058	1.11	(0.99-	1.25)	1.02	(0.89-	1.16)
Elective CS	1,206	1.08	(0.93-	1.24)	0.97	(0.82-	1.14)	1,146	1.04	(0.90-	1.21)	0.95	(0.80-	1.13)
Emergency CS	1,336	1.22	(1.07-	1.39)	1.09	(0.94-	1.26)	1,222	1.24	(1.08-	1.42)	1.07	(0.91-	1.25)

Abbreviations: OR-Odds ratio; VD-vaginal delivery; CS-Caesarean section

Appendix 32. Directed acyclic graph of the proposed association between birth by Caesarean section and school performance



Appendix 33. Effect of birth year and year of grading on the association between mode of delivery and poor school performance in the Swedish National Registers

	Unadjusted OR (95% CI)			Adjusted for birth year OR (95% CI)			Adjusted for year of grading OR (95% CI)		
Unassisted VD	Ref			Ref			Ref		
Assisted VD	0.84	(0.82-	0.86)	0.84	(0.82-	0.86)	0.84	(0.82-	0.86)
Elective CS	1.05	(1.03-	1.08)	1.05	(1.03-	1.08)	1.05	(1.03-	1.08)
Emergency CS	1.05	(1.02-	1.07)	1.05	(1.03-	1.08)	1.05	(1.03-	1.08)

Abbreviations: OR-Odds ratio; VD-vaginal delivery; CS-Caesarean section

Appendix 34. Effect of parental education on the association between mode of delivery and poor school performance among children born in 1990 or later in the Swedish National Registers.

	Unadjusted 1990 OR (95% CI)			Adjusted for parental education 1990 OR (95% CI)			Adjusted for co-variates except parental education 1990 OR (95% CI)			Adjusted including parental education 1990 OR (95% CI)		
Unassisted VD	Ref			Ref			Ref			Ref		
Assisted VD	0.82	(0.79-	0.85)	0.85	(0.81-	0.88)	1.05	(1.01-	1.09)	1.05	(1.01-	1.09)
Elective CS	1.11	(1.06-	1.15)	1.12	(1.08-	1.17)	1.09	(1.04-	0.13)	1.07	(1.02-	1.17)
Emergency CS	1.01	(0.97-	1.04)	1.00	(0.97-	1.04)	1.12	(1.08-	1.16)	1.09	(1.05-	1.13)

Abbreviations: OR-Odds ratio; VD-vaginal delivery; CS-Caesarean section

Appendix 35. Sensitivity analyses examining the effect of gender, county of birth, age at grading and Apgar score on the association between mode of delivery and poor school performance in the Swedish National Registers.

	Total Population OR (95% CI)			Among Male Babies OR (95% CI)			Stockholm County OR (95% CI)			16 Year Olds OR (95% CI)			No low Apgar Score OR (95% CI)		
Unassisted VD	Ref			Ref			Ref			Ref			Ref		
Assisted VD	1.06	(1.03-	1.08)	1.03	(1.00-	1.06)	1.07	(1.02-	1.13)	1.06	(1.03-	1.09)	1.06	(1.03-	1.08)
Elective CS	1.06	(1.03-	1.09)	1.06	(1.02-	1.09)	1.03	(0.96-	1.10)	1.05	(1.02-	1.08)	1.06	(1.03-	1.09)
Emergency CS	1.12	(1.09-	1.15)	1.11	(1.07-	1.14)	1.08	(1.02-	1.14)	1.10	(1.07-	1.13)	1.11	(1.08-	1.14)

Abbreviations: OR-Odds ratio; VD-vaginal delivery; CS-Caesarean section

Appendix 36. The association between mode of delivery and poor school performance with a random intercept for maternal ID in the Swedish National Registers.

	Unadjusted OR (95% CI)			Adjusted OR (95% CI)		
Unassisted VD	Ref			Ref		
Assisted VD	0.80	(0.75-	0.84)	1.06	(1.04-	1.09)
Elective CS	1.11	(1.04-	1.19)	1.05	(1.02-	1.08)
Emergency CS	1.03	(0.97-	1.10)	1.12	(1.09-	1.15)

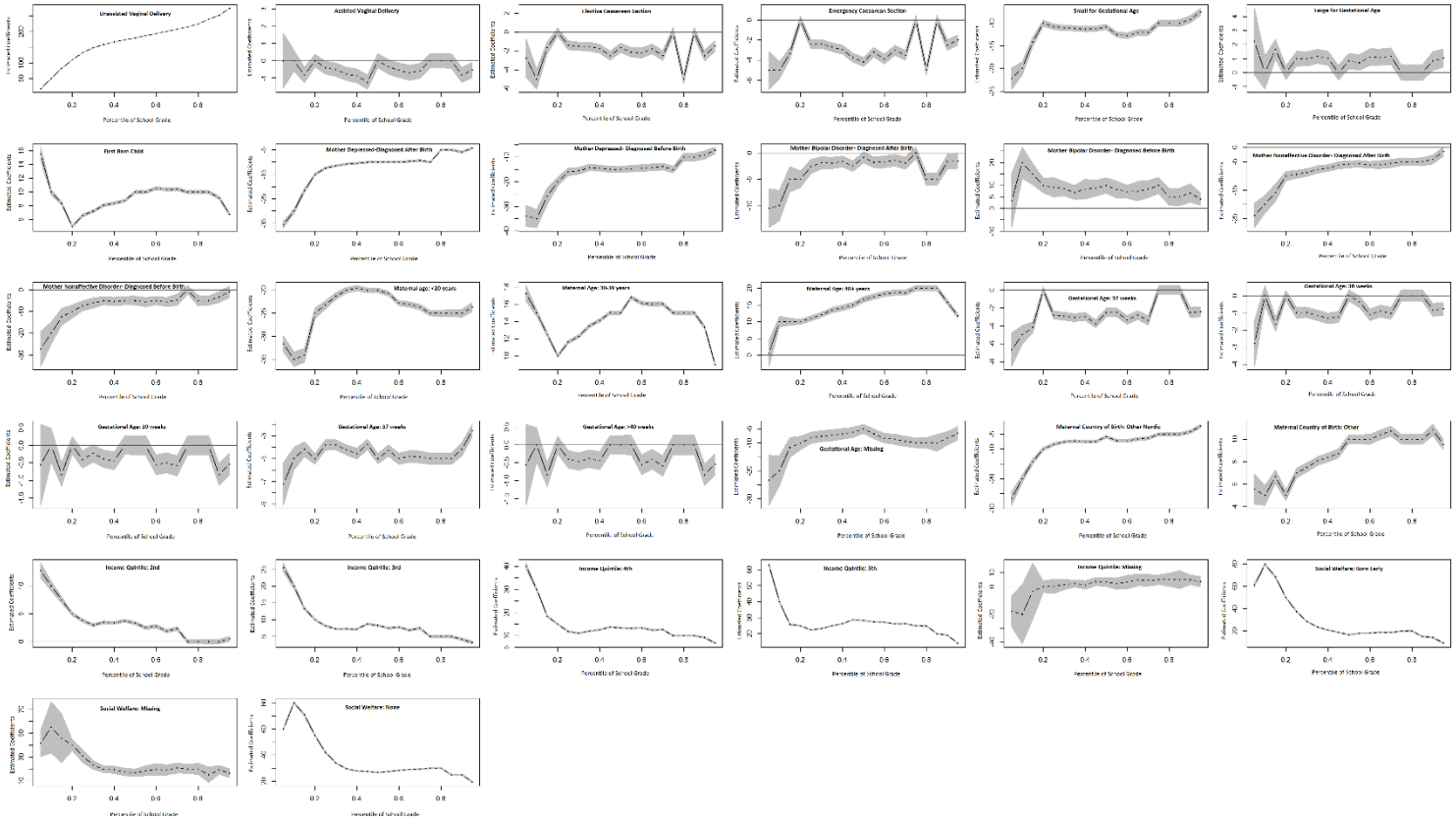
Abbreviations: OR-Odds ratio; VD-vaginal delivery; CS-Caesarean section

Appendix 37. Sensitivity analyses examining the effect of family size, birth order and Caesarean section order on the association between mode of delivery and poor school performance in the Swedish National Registers.

	One child families OR (95% CI)			First born children OR (95% CI)			Excluding primary CS OR (95% CI)			Excluding secondary CS OR (95% CI)		
Unassisted VD	Ref			Ref			Ref			Ref		
Assisted VD	1.03	(0.98-	1.08)	1.06	(1.03-	1.09)	1.06	(1.04-	1.09)	1.06	(1.04-	1.09)
Elective CS	1.06	(1.01-	1.11)	1.04	(1.01-	1.08)	1.07	(1.10-	1.12)	1.06	(1.03-	1.09)
Emergency CS	1.06	(1.10-	1.12)	1.11	(1.07-	1.14)	1.04	(0.96-	1.12)	1.13	(1.10-	1.16)

Abbreviations: OR-Odds ratio; VD-vaginal delivery; CS-Caesarean section

Appendix 38. Adjusted quantile regression, including co-variate estimates, of the association between mode of delivery and school performance in the Swedish National Registers.



Appendix 39. Unadjusted results of quantile regression modelling the association between mode of delivery and school performance including the 5th, 25th, 50th, 75th and 95th percentiles in the Swedish National Registers.

	Unassisted VD	Assisted VD	Elective CS	Emergency CS
5th	70	20	0	0
25th	175	0	0	5
50th	210	5	0	-5
75th	250	5	0	0
95th	300	0	0	0

*Coefficient for unassisted VD corresponds to the percentile of school performance in that group, coefficient values for other groups correspond to the difference between those children and children born through unassisted VD

Abbreviations: VD-vaginal delivery; CS-Caesarean section

Appendix 40. Adjusted results of quantile regression modelling the association between mode of delivery and school performance including the 5th, 25th, 50th, 75th and 95th percentiles in the Swedish National Registers.

	Unassisted VD	Assisted VD	Elective CS	Emergency CS
5th	17.22	0	-2.78	-5
25th	132.8	-0.4	-1.4	-2.4
50th	180	0	-1.66	-4.17
75th	215	0	0	0
95th	274.65	-0.54	-1.46	-2

Abbreviations: VD-vaginal delivery; CS-Caesarean section

Appendix 41. Unadjusted results of quantile regression modelling the association between mode of delivery and school performance including the 5th, 25th, 50th, 75th and 95th percentiles, excluding scores of 0 in the Swedish National Registers.

	Unassisted VD	Assisted VD	Elective CS	Emergency CS
5th	95	15	-5	-5
25th	175	5	0	0
50th	210	5	0	0
75th	250	5	0	0
95th	300	5	0	0

Abbreviations: VD-vaginal delivery; CS-Caesarean section

Appendix 42. PhD-related papers

Portable Document Format (PDF) versions of PhD-related papers can be found using the following DOI's:

1. DOI: 10.1111/jcpp.12351
2. DOI: 10.1007/s10803-015-2616-1
3. DOI: 10.1001/jamapsychiatry.2015.0846
4. DOI: 10.1001/jamapsychiatry.2015.2882
5. DOI: 10.1093/ije/dyw001
6. DOI: 10.1093/schbul/sbv152