Caesarean Section and Risk of Autism across Gestational Age: A Multi-national Cohort Study of 5 million births

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ABSTRACT

Background: The positive association between caesarean section (CS) and autism spectrum disorder (ASD) may be attributed to preterm delivery. However, due to lack of statistical power, no previous study thoroughly examined this association across gestational age. Moreover, most studies did not differentiate between emergency and planned CS.

Methods: Using population-based registries of four Nordic countries and Western Australia, our study population included 4987390 singletons surviving their first year of life, which included 671646 CS deliveries and 31073 ASD children. We used logistic regression to estimate odds ratios (OR) and their 95% confidence intervals (CI) for CS, adjusted for gestational age, site, maternal age and birth year. Stratified analyses were conducted by both gestational age subgroups and by week of gestation. We compared emergency versus planned CS to investigate their potential difference on the risk of ASD.

Results: Compared with vaginal delivery, the overall adjusted OR for ASD in CS delivery was 1.26 (95% CI 1.22-1.30). Stratified ORs were 1.25 (1.15-1.37), 1.16 (1.09-1.23), 1.34 (1.28-1.40) and 1.17 (1.04-1.30) for subgroups of gestational week 26-36, 37-38, 39-41 and 42-44, respectively. CS was significantly associated with risk of ASD for each week of gestation, from week 36 to 42, consistently across study sites (OR ranged 1.16-1.38). There was no statistically significant difference between emergency and planned CS on the risk of ASD.

Conclusion: Across the five countries, emergency or planned CS is consistently associated with a modest increased risk of ASD from gestational week 36 to 42 when compared to vaginal delivery.

Word count 249

Keywords: Autism; Emergency Caesarean section; Planned Caesarean section; Gestational age; Epidemiology; Population based

KEY MESSAGES

Caesarean section (CS) is associated with a modest increased risk of autism when compared to vaginal delivery.

Compared to vaginal delivery, CS is consistently associated with an increased risk of autism throughout all gestational weeks 36-42. Thus, the observed risk during this gestational period cannot be attributed to effects of preterm birth for children delivered by CS.

There were no differences in the risk of autism between planned and emergency CS.

INTRODUCTION

There has been a 20-fold increase in the diagnosis of autism spectrum disorder (ASD) since the 1980s [1–5] and it is believed that both genetic and environmental risk factors are important contributors [6,7]. Over a similar time period there has been an increasing trend in deliveries by Caesarean section (CS). In some populations close to half of all births are delivered by CSs [8,9]. In a US study, the population attributable fraction for ASD in relation to CS was estimated to be 7% for children born in 2000, higher than that for preterm birth and small for gestational age [10]. It is therefore of major etiological, clinical and public health interest to examine the relation between ASD and CS.

A positive association between CS and ASD has been shown in several earlier studies, but the results have varied in effect size [11–15]. A recent meta-analysis reported that delivery by CS was associated with a 23% increased risk of ASD when compared to vaginal delivery [16]. One possible explanation for the impact of CS on ASD is the fact that planned CS is normally scheduled weeks before the full 40-week gestation to avoid spontaneous labour [17]. It is also possible that the last few weeks before term are important for brain development [18]. Thus deliveries before the full-term might increase the risk of ASD, a hypothesis which is supported by previous studies of gestational age risk in relation to ASD [3,4,10,19–21]. Another aspect is the underlying indications leading to CS, which can vary by gestational ages [22]. Since the underlying maternal or fetal indications leading to emergency CS may be important independent risk factors of ASD, the magnitude of the independent risks of emergency CS on ASD may also vary by gestational ages [22]. We made a substantial search among the studies included in the most recent meta-analysis [16], and among all the studies cited in our study, to find any studies that investigated the association of CS and ASD stratified by gestational age. While many of the studies included gestational age [14,23–29] as a confounder, only one study reported the adjusted OR of emergency CS and planned CS among non-preterm births, in the appendix [11].

The rate of planned CS in singleton pregnancies has increased substantially in recent years [8,20,30–33].

As maternal and clinician preferences may play a greater role in planned than in emergency CS [34], outcomes due to the CS procedure itself in planned CS deliveries at or near term may be less confounded by underlying medical indication in comparison to emergency (non-planned) CS at similar gestational ages. However, none of the mentioned studies had sufficient sample size to assess the risk of ASD among children delivered by CS compared with children born after vaginal delivery across gestational age, and most of them did not differentiate between emergency and planned CS [3,4,10,19–21].

The aim of our study was to test the association between CS and ASD, overall and by type of CS, while taking gestational age into account and using the largest prospective, population based sample of ASD to date. We hypothesized that the relation between CS and ASD may vary between gestational ages and between planned and emergency CS, where CS conducted near or at full-term has lower risk than CS conducted at preterm, and planned CS has a lower risk than emergency CS.

METHODS

The study uses data from the International Collaboration for Autism Registry Epidemiology (iCARE) [35]. Access to this data is managed using the ViPAR software [36] that enables the pooled analysis of prospectively measured multi-national population-based data relating to ASD. Ethics committee approval, with waiver for informed consent, was obtained by each site.

Study population

The study population includes all singletons surviving their first year of living in Norway and Sweden 1984-2004; Denmark 1997-2004; Finland 1987-2004 and the non-Aboriginal population in Western Australia (WA) 1984-1999. Multiple births are highly correlated with ASD risk as well as with both preterm birth and CS which could introduce bias [37]. Excluding multiple births also reduces the risk of dependencies in the data which could affect the variance estimates. The final study sample size was not based on any statistical criteria but used all eligible national birth cohorts in the participating countries as made available by the ICARE collaboration, one of the largest

available databases for population based autism research.

ASD outcome, CS, gestational age and covariate information

Children were followed from birth to reported diagnosis of ASD or end of follow up which ever came first; through 2004 in WA, 2006 in Norway and 2009 in Denmark, Finland and Sweden. Denmark, Finland and Sweden provided ASD diagnoses from medical registries. ASD diagnoses from Norway and WA were derived from government-maintained service/benefits registries. Case identification, registry reporting procedures, details on the validity of case status and harmonization of diagnostic codes across sites have been described elsewhere [23]. Paternal and maternal age, gestational age, sex, birth year and mode of birth delivery-were obtained from birth or civil registries. For all countries in the study, throughout the births cohort in this study, mode of delivery was classified as vaginal or CS, with CS being further classified as planned, emergency, or unspecified. A planned CS is a planned before the onset of labour. Emergency CS is performed either before the onset of labour (due to medical or pregnancy complications) or complications in labour. Availability of data on mode of delivery and type of CS (planned, emergency, or unspecified) is presented in the online appendix (ST 1A).

Primarily, gestational age was estimated using ultrasound measurement throughout the study period for all studied countries. More specifically, gestational age in the Nordic countries was previously based on the date of last menstrual period (LMP), but since 1970s ultrasound measurements have been increasingly used to correct LMP. In Denmark, ultrasound-based pregnancy dating was used to determine gestational age for 93% of those born in 1995 and 2000 [38]. In Sweden, since 1990, early second trimester ultrasound examination is routinely offered, and more than 95% of women accepted this offer; otherwise the date of LMP is used [24]. In Finland, gestational age was estimated from the LMP, unless there was a discrepancy with the first-or second-trimester ultrasound examination of more than seven or 14 days, respectively, in which case the latter measurements were used [24]. In Norway, gestational age is estimated by ultrasound examination since 1967, with LMP as fall-back option. In WA, ultrasound was introduced in the 1980s [25].

Statistical analyses

Primary analyses

The primary analyses used a two-step approach. First, as commonly used in earlier studies, we estimated the risk of ASD comparing births following any type of CS with birth following vaginal delivery, which facilitates comparisons with previous findings. The odds ratio (OR) of ASD among births delivered by CS compared with vaginal delivery was obtained by fitting ordinary logistic regression models. We first estimated the OR for ASD overall, then in gestational age subgroups: weeks 26-36 (preterm), weeks 37-38 (early term), weeks 39-41 (term) and weeks 42-44 (post-term), and finally by week of gestation except for weeks 26-30, for which data were too sparse for week-by-week analysis. All models included sex, site, birth year (1984-89,1990-1994,1995-99, and 2000-2004), categorized maternal age (<25,25-29,30-34,35-39,≥40) and gestational age (by week or subgroup) as covariates, if not otherwise specified.

The medical indication for women undergoing either emergency or planned CS might be different. Thus, in the second step analysis we also estimated OR of ASD among births delivered by emergency CS compared with planned CS (unspecified CS were excluded from this analysis). Due to lack of data on CS type at some sites at different time periods (ST 1A) these analyses were restricted to birth cohorts 1997-2004 for Denmark, 1984-1999 for WA, 1988-2004 for Norway and Sweden, and 1990-2004 for Finland. For the analysis of emergency versus planned CS, we refitted the models adjusting for site, sex, birth year and categorized maternal age by week of gestation (weeks 26-30 as a single subgroup).

Site comparisons

Site-specific ORs of ASD among births delivered by CS compared with vaginal delivery were estimated by stratifying gestational age subgroup, and each week of gestation. Site heterogeneity and its influence on overall results were addressed by Cochran's Q test and by verifying pooled results through the use of leave-one-out approach, i.e., OR was estimated by removing the indicated

site being removed.

Subgroup and sensitivity analyses

ORs of ASD were estimated for CS in comparison with vaginal delivery in subgroups of gestational age (weeks 26-36, 37-38, 39-41 and 42-44) for male and female offspring respectively. We repeated these analyses for Autistic Disorder (AD) cases only. Due to low frequency in some subgroups, analyses by week of gestation were not feasible.

As the likelihood of CS is much higher in women who have had a CS in a previous pregnancy, we repeated the primary analyses restricting to first-born children only. Since information on paternal age was not available for Finnish births, the primary analyses did not include paternal age as a covariate. In a set of sensitivity analyses, having excluded Finland from the analysis dataset, we repeated the primary analyses by gestational age subgroups after adding paternal age (<25, 25-29, 30-34, 35-39 and ≥40 years) to the models together with the other model terms.

A logistic regression model assumes that all subjects have been observed for the same length of time (i.e., equal length of follow-up or risk time) or that the length of follow-up does not affect the risk. If this assumption is violated, bias may be introduced. For this purpose, birth year was included in all models. We also performed a sensitivity analysis on data from Denmark, Finland and Sweden, as these countries had information about the date of ASD diagnosis. For the sensitivity analyses, comparing between CS and vaginal delivery, we estimated the Hazard Ratios (HR) of ASD across subgroups of gestational age based on the hazard ratio obtained by fitting stratified Cox regression with age at diagnosis as the underlying time scale, adjusting for site, sex and maternal age group. To adjust for calendar effects, we fitted the Cox regression with different strata for each birth year group [26].

All statistical tests were performed using the two-sided 5% level of significance and corresponding two-sided 95% CI's. We did not adjust for multiplicity of statistical tests. It was not possible to identify siblings in the data and correct for possible sibling correlations in the analyses. Models' goodness of fit was addressed by calculating the Pearson chi-square test and the Hosmer-Lemeshow

test. All statistical analyses were performed using the R-software ver3.2.1.

RESULTS

The study cohort included a total of 5 250 034 births. After sequentially excluding 99 072 who were born very early (<26) or very late (>44) gestational age, 146 619 multiple births and 16 957 who died before age of one-year, 4 987 390 (95% of the total) remained in the final data for analysis (ST 1B). Sweden contributed 41% of the final data, Norway and Finland each contributed around 21%, and Denmark and WA each contributed less than 10%. Of the final data, a total 4 315 744 (86.5%) children were delivered by vaginal births, 243 749 (4.9%) by planned CS, 291 106 (5.9%) by emergency CS, and 136 791 (2.7%) by unspecified CS. There were 31 073 (0.6%) children with ASD of which 10 418 children were diagnosed with AD (0.2% of total). Study cohort distributions of births, ASD and AD cases, sex, birth year, parental age and sites are shown in Table 1 by mode of delivery.

[Table 1 here]

The vast majority of the deliveries (96.3%) were in gestational weeks 36-42 (ST 2). The most frequently occurring gestational age at delivery was 40 weeks for vaginal birth (30.4%), 38 weeks for planned CS (34.6%), 39 weeks for emergency CS (21.2%), and 38 weeks for unspecified CS (24.4%). The absolute risk of ASD declined with increased gestational age: the risk of ASD declined from 1.42% at gestational weeks 26-30 to 0.3% at gestational weeks 44 (ST 3). Overall, birth deliveries by CS had a higher risk to develop ASD than vaginal births throughout gestational weeks 26-44.

[Table 2 here]

Caesarean section vs vaginal delivery by gestational ages

The upper part of table 2 summarizes the results of the primary analysis. Compared to vaginal delivery, CS was associated with a statistically significant increased risk of ASD, with and without

adjustment of potential confounders (site, birth year, sex and maternal age): crude OR=1.33 (95% CI: 1.29 - 1.37) and adjusted OR=1.32 (95% CI: 1.28-1.36). Further adjustment by including gestational age as a covariate resulted in OR=1.26 (95% CI: 1.22-1.30). As shown in figure 1, the OR of ASD following CS was statistically significantly elevated across all gestational age subgroups (26-36, 37-38, 39-41 and 42-44 weeks gestation). When the OR of ASD was estimated by week of gestation, we found a statistically significant association between CS and ASD starting from week 36 through week 42 (Figure 2).

Emergency vs planned CS

The risk of ASD was similar for emergency and planned CS, both overall (OR=0.99, 95% CI: 0.92-1.06) and by gestational week (Figure 3). Broadly similar OR patterns were seen for ASD when comparing emergency CS with vaginal delivery and planned CS with vaginal delivery across gestational age (SF 1a, SF 1b, respectively).

Site comparison: Caesarean section vs vaginal delivery by gestational ages

Between sites, there were broadly similar OR patterns for ASD in children delivered by CS compared with children delivered by vaginal delivery by gestational age group (Figure 4 and ST 4). In the 37-38 weeks gestational age group, only Denmark had an OR point estimate less than 1. In the 39-41 weeks gestational age group, all ORs were greater than 1. Although Norway had a somewhat higher OR point estimate in the gestational age group 39-41 weeks, the direction was consistent with other sites. In the per week of gestation analysis, there was a statistically significant higher risk of ASD in children born by CS at 36-42 weeks gestation, which was consistent across sites (SF 2). The heterogeneity (Cochrane Q) test results indicated no site heterogeneity (all p-value > 0.9). We found no quantitative differences in the estimated ORs of ASD among children delivered by CS compared with vaginal delivery in the site influence analysis (SF 3).

Subgroup and sensitivity analyses

The OR of ASD among children delivered by CS compared with vaginal delivery was similar for

boys (OR=1.25, 95% CI: 1.20-1.29) and girls (OR=1.30, 95% CI: 1.22-1.38). The gestational age group 39-41 weeks had the highest point estimate of OR for both sexes (SF 4 and ST 5).

The results of the sensitivity analyses for ASD are given in the lower part of Table 2. Restricting to first-born children, the OR for ASD, CS versus vaginal delivery, was estimated to OR=1.22 (95% CI: 1.15-1.30). Excluding Finland and adding paternal age as a covariate, the OR for ASD, CS vs vaginal delivery, was OR=1.27 (95% CI: 1.22-1.31). The OR estimate only differed by the 4th decimal when we excluded paternal age in this restricted cohort. Using Cox regression models, the HR for ASD, CS vs vaginal delivery, was HR=1.27 (95% CI: 1.23–1.31). Restricted to the same date-of-diagnosis cohort (Denmark, Finland and Sweden), the OR for ASD, CS versus vaginal delivery, was OR=1.25 (95% CI: 1.21-1.29).

The results of AD are summarized in ST 6-7. Without adjustment for gestational age, the OR for AD from CS compared with vaginal delivery was 1.42 (95% CI: 1.35-1.49) and after including gestational age (26-36, 37-38, 39-41 and 42-44 weeks) the OR was estimated to 1.34 (95% CI: 1.27-1.41). The results of all the sensitivity analyses (first-born only, including paternal age [Finland excluded], and Cox regression analysis) were similar and the estimated ORs and HRs ranged from 1.31-1.35. CS was significantly associated with AD in all gestational age subgroups (26-36, 37-38, 39-41 and 42-44 weeks) (SF 5).

For all analyses (ASD, AD and subgroups), the goodness-of-fit analyses (Hosmer-Lemshow test), supported the assumption of data following a binomial distribution.

DISCUSSION

To date, this is the largest population-based study of association between Caesarean section and the risk of ASD. We included five million singletons in five countries (Norway, Sweden, Denmark, Finland and Western Australia) with more than 31 000 prospectively ascertained cases of ASD. Births delivered by CS had a moderately increased risk of ASD when compared with vaginal

delivery, consistently from 36 weeks to 42 weeks of gestation. On average, a 26% higher risk of ASD was presented in all births from week 36 to 42 representing 95% of the total number of births or 90% of all CS births. There was no evidence for differences in ASD risk between emergency CS and planned CS, overall or by week of gestation. Similar overall risk patterns were observed for males and females and for AD specifically, and the patterns remained robust across a variety of sensitivity analyses.

Different from previous studies [11–16,27,28,39–42], and made possible by the high statistical power, we were able to precisely estimate the association between CS and ASD in specific weeks of gestation, in subgroups of male and female offspring and for AD separately. Using a long study period, including sites with different type of diagnostic registries (medical registries vs government-maintained service/benefits registries), varying incidence of CS and ASD and different medical practice (planned CS without medical indication is more common in WA than Nordic counties) strengthens the generalizability of our results.

Our results did not support our original hypotheses that CS conducted at or near full-term has lower risk than CS conducted at preterm, or that emergency CS has a higher risk of ASD than planned CS. Although the precision of the risk estimates is lower for below 36 weeks of gestation compared to 36-42 weeks, the point estimates for ASD risk from CS below 36 weeks did not indicate any increased risk. This could mean that CS alone before 36 weeks does not add any additional risk for ASD, beyond the risks associated with the adverse medical indications leading to CS or with fetal prematurity. In contrast, CS may confer additional risk for ASD above risks associated with factors leading to CS between 36-42 weeks gestation. Also, since the risks from planned and emergency CS were very similar, even during weeks 36-42 gestation, it indicating there might by induced risk from CS per se at or near term. A previous meta-analysis was carried out to examine the association between CS and ASD [16]. When restricted to the 13 studies which had adjusted for other covariates (only nine of which were published after 1990 [12–15,27,28,39–41] and only one of which [39] had more cases than our smallest site), a pooled OR of 1.23 (95% CI: 1.07-1.40) was obtained [16]. The

pooled estimate is similar in magnitude to our overall estimate but with considerably lower precision.

In addition to the "before term" hypothesis, there are several other hypotheses regarding the potential relationship between delivery by CS and ASD. In one study of first born children, the risk of ASD was 52% higher in infants whose CS was performed under general anaesthesia than those who were delivered vaginally or born through CS under regional anaesthesia [42]. Neurotoxicity related to neonatal exposure to general anaesthesia might be a contributing factor. However, we found that the OR for ASD following emergency CS (more likely to be performed under general anaesthesia) was similar to the OR following planned CS. Exposure to skin microbiota due to the CS procedure [29] and the beneficial effect of being born vaginally (enhance the immune system) [43–45] are other potential hypotheses. Lastly, there are indications that CS may increase risk of immunological diseases since the child is not exposed to the stress following a vaginal delivery [46]. which normally would initiate positive changes in epigenetic expression.

Even though CS, planned or emergency, appears to be associated with of a complex mix of factors also associated with ASD risk [47], we did not see a difference in risk of ASD from planned versus emergency CS, even in near term and term deliveries. Similar to our results, a recent population-based study [11] reported that both elective (planned) CS and emergency CS were associated with increased risk of ASD also when adjusting for selected confounding factors. By restricting the population to families with at least two siblings they were able to further adjust for familial confounding. When adjusting for familial confounding neither emergency CS nor elective CS was associated with ASD, indicating the association may not be directly causal and implying that unknown familial factors might explain the increased risk of both CS and ASD. However, bias from residual confounding may still remain, e.g., confounding by calendar time arising from changes in obstetric practice over time. In addition, mode of delivery in one pregnancy influences mode of delivery in a subsequent pregnancy, which cannot be controlled for in a sibling design. Assuming the unknown familial factors truly exist and they are not being influenced by the aforementioned

potential biases, our results indicate that the ORs associated with these familiar factors increase the risk of CS and ASD consistently, across gestational week 36 to 42. A similar pattern also appeared consistently across study sites. Further work is needed to disentangle the contributions to ASD risk arising from the underlying indications versus the procedure itself, also in planned CS.

The major strength of this study is the large multi-national sample size with prospective follow-up. With approximately 5 million live born singletons and 31 073 ASD cases, our study is more than three times larger than the previous meta-analyses [16,48] and almost twice the size of the recent Swedish study, thus providing higher statistical power to conduct more detailed subgroup analyses. For instance, in the gestational week-by-week analyses conducted by each site alone (SF 2), all 95% CIs included 1.0 (with only exception at gestational week 44 for Norway) despite the consistent elevated ORs from gestational week 36-44 across sites. Our study also has limitations: the follow-up period from birth to reported diagnosis of ASD is too short for some of the birth years. For example, in Norway the follow-up was until 2006, but the last birth year included was 2004, i.e., the age of the child was two years old and unlikely to be diagnosed with ASD. Since logistic regression is not able to directly adjust for the differences in length of follow-up for different individuals due to censoring, a bias may have been introduced. For this reason we fitted Cox regression as a complementary model. Even though we did not have information for all types of censoring, e.g. date of emigration or date of death of a child, it should not effect the results. First, we only included children surviving their first year of life, removing the possibility of early censoring due to infant death, although the child death rates in the countries in the study are among the lowest in the world. Second, emigration out of the Nordic countries is very low. Third, bias could be introduced if participants are lost to follow-up due to reasons related to CS or gestational age. We see no reason to suspect differences in follow-up due to CS, although children born early or late term gestational age could be at slightly higher risk of censoring due to death or co-morbidity hiding symptoms of autism. Nevertheless, the ORs from logistic regression agreed very closely with the hazard ratios from Cox regression. Also noteworthy is the varying reported ASD

frequency between countries. For example, the prevalence of ASD is about one fifth in Norway compared to the other countries. One possible explanation is the diagnostic data from Norway were derived from government-maintained service/benefits registries, while other countries' diagnostic data were from medical registries (with WA as an exception). Results from the leave-one-out site influence analysis indicated that the different type of diagnostic data did not influence the estimated ORs. Our study also has similar limitations as previous studies in that we did not have access to the underlying indication for CS or the sufficient information to conduct sibling designed analysis. While several potential confounders, e.g., parental psychiatric information, maternal diabetes, pre-eclampsia and socioeconomic status are available in each site, iCARE data does not contain such information. Also, iCARE was purposely designed to focus only on ASD and does not contain information on other psychological developments, such as ADHD and intellectual disability, which have also been reported to be associated with CS as well [15,49,50]. As ultrasound dating of pregnancies was not universally used throughout the cohort, there may be some (but not) large inaccuracy in gestational age. However, the distribution of gestations periods at delivery (with the vast majority delivered at term) is reassuring in this regard. Due to the constraint of information available in the database, our classification of CS (planned and emergency) might be too simple. WHO recommended the 10-group classification (also known as Robson's classification) as a global standard for assessing, monitoring and comparing CS rates internationally based on simple obstetric parameters (parity, previous CS, gestational age, onset of labor, fetal presentation and number of fetuses). However, this classification still does not explicitly record the underlying indication for doing CS nor differentiation of elective (maternal request in the absence of maternal or fetal indications) and emergency CS. Finally, we could not take into account the date of death and the date of immigration in the Cox regression analysis since iCARE does not contain such information.

CONCLUSION

Across the five countries, birth delivery by CS, planned or emergency, is consistently associated with a modest increased risk of ASD from gestational week 36 to 42 when compared to vaginal

delivery. These findings may have important implications, especially given the rise in CS around term for non-medical indications.

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