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ORIGINAL PAPER

Exposure to General Anesthesia May Contribute to the Association between Cesarean Delivery and Autism Spectrum Disorder

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Abstract

Cesarean section (CS) has been consistently associated with susceptibility to autism spectrum disorder (ASD), however, the underlying mechanism for this association remains vague. Here, we studied various pre-peri-and-neonatal factors among 347 children with ASD, 117 children with other developmental delays (DD), and 2226 age, sex and ethnicity matched controls. We found that CS is significantly associated with an increased risk of ASD but not DD ($p=0.019$ and $p=0.540$ respectively). Furthermore, we show that only CS performed with general anesthesia (GA) elevated the risk of ASD with no significant difference between indicated and non-indicated surgeries (aOR = 1.537; 95% CI 1.026–2.302, and aOR = 1.692; 95% CI 1.057–2.709, $p_{diff}=0.865$). We therefore suggest that exposure to GA during CS may explain the association between CS and ASD.

Keywords Autism spectrum disorder · Cesarean section · General anesthesia

Introduction

Autism spectrum disorder (ASD) is a lifelong neurodevelopmental condition that is characterized by impairment of social communication, along with restricted, repetitive patterns of behavior, interests, or activities (American Psychiatric Association 2013). In the past three decades, ASD has become a major public health concern, with a substantial increase in the prevalence of ASD worldwide (Elsabbagh et al. 2012). While the increase in ASD prevalence has largely been attributed to higher public awareness and changes in diagnostic criteria (Lord 2011; Maenner et al. 2014; Posserud et al. 2010), the contribution of environmental risk factors cannot be excluded.

The environmental causes of ASD are fiercely debated and extremely controversial (Matson et al. 2011; Newschaffer et al. 2012). Epidemiological studies that investigated the effect of prenatal and perinatal factors on the risk of ASD yielded variable and sometimes contradicting results. Meta-analyses of these data highlighted a number of consistent gestational and obstetric risk factors of ASD (Gardener et al. 2009, 2011; Kolevzon et al. 2007; Wang et al. 2017). Among these, the association between Cesarean deliveries and ASD is particularly interesting given the continuous increase in the implementation of this mode of delivery worldwide (Saleh et al. 2017).

Cesarean delivery, also known as C-section (CS), is a surgical procedure that is used to deliver a baby in cases where a vaginal delivery may risk the health of either the mother or the baby (Danforth 1985). Nevertheless, in the last couple of years, elective (planned) CSs, which are usually performed

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without any noticeable birth complication, are becoming popular (O'Donovan and O'Donovan 2018; Wiklund et al. 2012). CS can be performed with either general anesthesia (GA) or regional anesthesia (RA) which typically includes epidural and spinal sedation. RA is more commonly used during CS because of the potential maternal and neonatal risks associated with GA (Olutoye et al. 2018; Sumikura et al. 2016). Yet, some moderate adverse effects of RA during CSs have also been documented (Balki and Carvalho 2005). CS with GA will be usually preferred when certain pregnancy or birth complications are noted, even if RA was already administered.

Although CS is considered a relatively safe procedure, it has been associated with a wide range of short and long-term birth complications (Eyowas et al. 2016), which include, but are not limited to, neonatal respiratory outcome (Ramachandrappa and Jain Ramachandrappa 2008), the development of the offspring's immune system (Cho and Norman 2013), postpartum depression (Xu et al. 2017), and other maternal morbidities (Rossi and D'Addario 2008). Interestingly, CS has been consistently associated with an increased risk of ASD (Curran et al. 2015b; Polo-Kantola et al. 2014; Yip et al. 2017). Several factors have been suggested to underlie this association. These include biological mechanisms such as differences in gut microbiome composition due to the dearth of the mother's vaginal bacteria among children who were born with CS (Reardon 2014; Sharon et al. 2016). In addition, CSs are thought to induce aberrant short-term immune responses in infants which could lead to various childhood diseases, including ASD (Cho and Norman 2013). Exposure to general anesthesia (GA) during CS has also been suggested as a risk factor of ASD (Chien et al. 2015). Finally, the association between CS and ASD could be due to confounding by various birth complications\ and/or by genetic or environmental risk factors that are associated with both CS and ASD (Angelidou et al. 2012; Curran et al. 2015a; Smallwood et al. 2016).

The consistent association between CS and ASD across multiple studies and the mixed findings regarding the possible mechanisms underlying this association require further exploration. With this in mind, we designed a large nested case–control study in a hospital-based birth cohort that contains comprehensive clinical data on the pregnancy and birth of these children. The main goal of the study was to assess the risk of ASD associated with CS under the effect of various prenatal and perinatal confounders in this population.

Materials and Methods

Case–Control Ascertainment

This is a nested case–control study where both cases and controls ascertained from a cohort of all single live born children at the Soroka University Medical Center (SUMC) between the years 2009 and 2016. Cases included children who were referred to either the Child Development Institute (CDI) or to the Preschool Psychiatric Unit (PPU) at SUMC with suspected social communication difficulties and/or repetitive behaviors. Diagnoses of ASD or other types of developmental delay (DD) were determined by either a child psychiatrist or a pediatric neurologist according to DSM-5 criteria as described before (Meiri et al. 2017). Children with ASD were further assigned severity levels in social communication and in restrictive repetitive behavior according to DSM-5 criteria (i.e. 'requiring support', 'requiring substantial support', and 'requiring very substantial support') (American Psychiatric Association 2013). We used the severity levels in the social communication domain for our stratification analysis as described below. Controls were sampled from children who had no diagnosis of ASD or any other neurological or psychiatric disorder and matched to either children with ASD or children with DD by their gender, date of birth (± 3 months), and ethnicity (Jewish/Bedouin) at a 1:5 case–control ratio (supplementary Table S1). Separate analyses were conducted for children with ASD and children with other DD to identify factors that are explicitly associated with the risk of ASD.

Data Collection

Demographic, clinical and behavioral data of cases were obtained from the database of the Negev Autism Center (www.negevautism.org; (Meiri et al. 2017)). Prenatal, perinatal and neonatal variables for both cases and controls were obtained from the electronic database of the obstetrics and gynecology department (OGD) of SUMC. This database is used regularly for research purposes, and its accuracy is ensured through a standardized review of the data by a specialist medical secretary and a consulting obstetrician before it is coded (Amir et al. 2009). Variables with a population frequency of $< 1\%$ such as "Chorioamnionitis"; (0.04%), and "Abnormalities of chorion and the amnion"; (0.3%), were removed from our analysis, due to insufficient statistical power to detect their potential effect in our sample. Data from the OGD database were linked to subjects based on their unique national identification number. This study was approved by the ethic committee of SUMC (application number: 0222-14-SOR).

Statistical Analysis

The associations of CS and other prenatal, perinatal, and neonatal characteristics with risk of ASD or DD were evaluated using appropriate bivariate statistical tests. Specifically, case–control differences in continuous variables were evaluated using *t* test or Mann–Whitney test if normality

assumption was violated, and differences in nominal variables were evaluated using Chi square or fisher exact tests. Variables that were significantly associated with ASD (p-value < 0.05) in this study or in multiple other studies as depicted in a recent meta-analysis by Wang et al. (Wang et al. 2017) were included in multivariate conditional logistic regression models that were designed to assess the adjusted effect of CS on the risk of ASD (compared to vaginal deliveries). We also performed stratification analyses to assess the different CS types on the risk of ASD. Specifically, we stratified CS deliveries according to the type of anesthesia (only general, only regional, and both general and regional anesthesia), and according to the reason for the CS (indicated vs. non-indicated). In addition, we examined these associations in males and females separately. Regional anesthesia included epidural and spinal anesthesia. Indicated CS included CS deliveries that were performed according to the following indications: preeclampsia, umbilical cord prolapse, placental abruption, placenta previa, rupture of uterus, and non-reassuring monitor, and malpresentation of the fetus. All other CS deliveries that were performed without any known medical indication for CS were included in the 'non-indicated CS' group. All statistical analyses were two-sided, unless stated otherwise, and carried out using SPSS version 24.

Results

The association of eight prenatal, 22 perinatal, and 4 neonatal characteristics with the risk of ASD or DD are summarized in Table 1. CS was significantly associated with the risk of ASD (p-value = 0.019) however, no such association was observed with the risk of DD (p-value = 0.540). Other variables that were significantly associated with ASD were parity number (p-value = 0.01), amniocentesis (p-value = 0.014), GA (p-value = 0.004), and infant weight (p-value = 0.034). Variables that were significantly associated with the risk of DD included parity number (p-value = 0.006), past Cesarean section (p-value = 0.026), and non-reassuring monitor (p-value = 0.045).

Since the main goal of this study was to explore the reasons of the association between CS and ASD, we further assessed the risk of CS delivery that is associated with ASD in several multivariate conditional regression models as depicted in Fig. 1. The first model assessed the effect of CS delivery (vs. vaginal delivery) while adjusting for a range of prenatal, perinatal or neonatal variables that were significantly associated with ASD (p-value < 0.05) in this study or elsewhere (Table 2). In this model, the association between CS and ASD remained statistically significant (aOR = 1.371; 95% CI 1.004–1.872) (Table 2 and Fig. 1).

To further explore the reasons for the association between CS and ASD, we stratified our sample according to different anesthesia regimens and applied the same conditional logistic regression models to these subgroups. Stratification of the CS deliveries to those performed with only GA, those performed with regional anesthesia (RA), and those performed with both GA and RA revealed a trend whereby a two-fold increase risk of ASD was seen among surgeries where both GA and RA were used (aOR = 2.027; 95% CI 0.835–4.919; compared to vaginal deliveries), a 1.5 increased risk of ASD was seen among surgeries performed with only GA (aOR = 1.629; 95% CI 1.172–2.264; compared to vaginal deliveries), and no risk of ASD was seen among surgeries performed with RA only (aOR = 0.853; 95% CI 0.472–1.541; compared to vaginal deliveries) (Fig. 1).

Next, we combined all surgeries that were performed with GA (i.e. CS + GA and CS + GA + RA) and stratified them according to the surgery indication (i.e. indicated CS and non-indicated CS; see methods) (Fig. 1). This analysis revealed a statistically significant association of CS + GA with ASD in both of these groups (aOR = 1.537, 95% CI 1.026–2.302, aOR = 1.692, 95% CI 1.057–2.709 respectively), and these risk estimates were not statistically different (Breslow-Day test of homogeneity p-value = 0.865).

Finally, we used the same multivariate analysis to evaluate the effect of CS + GA (including both CS + GA and CS + GA + RA) on the risk of ASD among males and females separately, and with different DSM-5 severity levels of ASD (Fig. 2). Interestingly, the risk of ASD associated with exposure to GA during CS was twice higher among females than among males (aOR = 3.283, 95% CI 1.484–7.261 vs. aOR = 1.302, 95% CI 0.891–1.901 respectively). In addition, the association between CS + GA and ASD was statistically significant only among children with the most severe form of ASD (aOR = 2.522, 95% CI 1.488–4.275) suggesting that risk of ASD associated with exposure to GA during CS is mostly relevant to children with ASD that are 'requiring very substantial support' according to the DSM-5 criteria.

Discussion

Our findings suggest that the association between CS and ASD that has been reported in multiple other studies (Curran et al. 2015a, b; Polo-Kantola et al. 2014; Yip et al. 2017) is restricted to CSs that are performed under GA, without any significant difference between indicated and non-indicated surgeries. These results are consistent with findings of a population-based birth cohort study from Taiwan, which also showed that only children that were delivered by CS with GA are at risk of developing ASD (Chien et al. 2015). Another study that investigated the effect of exposure to GA

Table 1 Association between maternal, labor, and infant characteristics with ASD

	Cases (ASD) N = 347	Cases (DD) N = 117	Control N = 2226	P-value (ASD)	P-value (DD)
Maternal and prenatal characteristics, N (%)					
Mother's age at birth (Years), Mean (sd)	29.44 (5.77)	28.69 (5.73)	29.24 (5.67)	0.515[a]	0.309[a]
Gravity number, Median (IQR)	2 (2.4)	2 (2.3)	3 (2.4)	0.087[b]	0.113[b]
Parity number, Median (IQR)	2 (1.3)	2 (1.3)	2 (1.4)	**0.010[b]**	**0.006[b]**
Gestational diabetes	19 (5.5)	6 (5.1)	96 (4.3)	0.419[c]	0.605[d]
Polyhydramnios	6 (1.7)	3 (2.6)	44 (2.0)	0.856[c]	0.743[d]
Oligohydramnios	12 (3.5)	1 (0.9)	46 (2.1)	0.207[c]	1.000[d]
Amniocentesis	34 (9.8)	7 (6.0)	134 (6.0)	**0.014[c]**	0.872[c]
Assisted reproductive technology	11 (3.2)	7 (6.0)	66 (3.0)	0.883[c]	0.091[d]
Recurrent miscarriage	19 (5.5)	6 (5.1)	92 (4.1)	0.514[c]	0.361[d]
Past Cesarean section	60 (17.3)	9 (7.7)	325 (14.6)	0.148[c]	**0.026[c]**
Labor and perinatal characteristics, N (%)					
Pre-term delivery (< 37w)	34 (9.8)	7 (6.0)	168 (7.5)	0.108[c]	0.436[c]
Post term delivery (≥ 42w)	3 (0.9)	1 (0.9)	44 (2.0)	0.253[d]	0.484[d]
Malpresentation of fetus	13 (3.2)	9 (7.7)	85 (3.8)	0.756[c]	0.068[c]
Vacuum delivery	11 (3.2)	10 (8.5)	90 (4.0)	0.534[c]	0.077[c]
Caesarian section	83 (23.9)	25 (15.4)	413 (18.6)	**0.019[c]**	0.540[c]
Epidural anesthesia	95 (27.4)	34 (29.1)	671 (30.1)	0.358[c]	0.679[c]
Spinal anesthesia	14 (4.0)	5 (4.3)	72 (3.2)	0.509[c]	0.401[d]
General anesthesia	67 (19.3)	18 (15.4)	308 (13.8)	**0.006[c]**	0.852[c]
Pethidine	15 (4.3)	1 (0.9)	66 (3.0)	0.147[c]	0.226[d]
Labor induction	87 (25.1)	24 (20.5)	542 (24.3)	0.887[c]	0.870[c]
Oxytocin induction and/or augmentation	67 (19.3)	18 (15.4)	427 (19.2)	0.712[c]	0.809[c]
Premature rupture of membrane	51 (14.7)	14 (12)	295 (11.9)	0.192[c]	0.814[c]
Preeclampsia	17 (4.9)	5 (4.3)	89 (4.0)	0.578[c]	0.582[d]
Placental abruption	2 (0.6)	1 (0.9)	8)0.4)	0.659[d]	0.311[c]
Placenta Previa	1 (0.3)	0)0)	7 (0.3)	1.000[d]	1.000[d]
Umbilical cord prolapse	1 (0.3)	0 (0)	10 (0.4)	1.000[d]	1.000[d]
Umbilical cord around neck	51 (14.7)	25 (21.4)	381 (17.1)	0.578[c]	0.865[c]
Labor dystocia	11 (3.2)	3 (2.6)	54 (2.4)	0.377[c]	1.000[d]
Meconium	37 (10.7)	11 (9.4)	223 (10.0)	0.646[c]	0.720[c]
Non-reassuring monitor	28 (8.1)	16 (13.7)	150 (6.7)	0.241[c]	**0.045[c]**
Postpartum hemorrhage	5 (1.4)	0	24 (1.1)	0.578[d]	0.596[d]
Infant characteristics, N (%)					
Infant's weight at birth (kg), mean (sd)	3.15 (0.54)	3.20)0.52)	3.23 (0.53)	**0.034[c]**	0.287[a]
Birth weight for gestational age					
SGA	11 (3.6)	3 (2.6)	75 (3.4)	0.762[c]	0.950
AGA	311 (89.2)	107 (91.5)	1191 (89.4)		
LGA	21 (6.1)	7 (6.0%)	160 (7.2)		
1-min abnormal Apgar score (< 6)	16 (4.6)	7 (6.0)	102 (4.7)	0.960[c]	0.650[c]
5-min abnormal Apgar score (< 6)	2 (0.6)	1 (0.9)	13 (0.6)	0.835[d]	0.432[d]

Bold values are statistically significant associations (p-value < 0.05)

[a]T-test

[b]Mann–Whitney test

[c]Chi square test

[d]Fisher's exact test

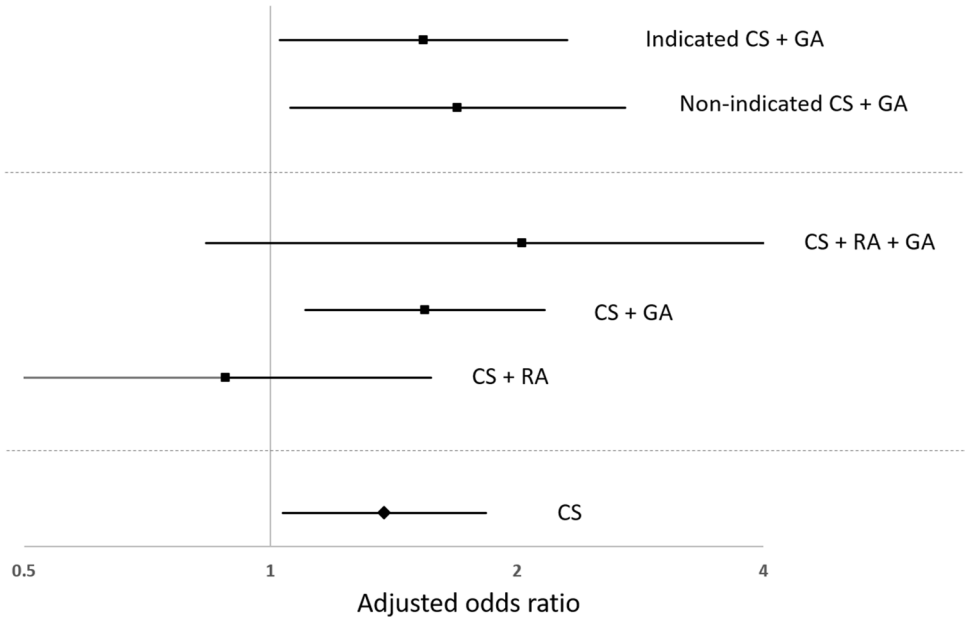

Fig. 1 A forest plot of the association between different types of Cesarean section (CS) and ASD. The adjusted odds ratios of having a child with ASD (Black squares) and their 95% confidence intervals (Black horizontal lines) are plotted for different modes of deliveries. Gray dashed horizontal lines separates the results of different stratification analyses. *CS* cesarean section, *GA* general anesthesia, *RA* regional anesthesia

Table 2 Multivariate analysis for the risk of ASD

Variable	Adjusted OR (CI 95%)	P-value
Amniocentesis	1.427 (0.933–2.181)	0.101
Infant weight (kg)	0.820 (0.639–1.052)	0.118
Parity number	**0.867 (0.803–0.936)**	**< 0.001**
Mother's age at birth	1.020 (0.995–1.045)	0.113
Gestational diabetes	1.126 (0.662–1.915)	0.661
Pre-term delivery (< 37w)	1.045 (0.647–1.687)	0.858
Malpresentation of fetus	1.510 (0.769–2.967)	0.232
Preeclampsia	0.931 (0.529–1.641)	0.806
Labor induction	1.102 (0.721–1.683)	0.653
Postpartum hemorrhage	**3.333 (1.062–10.465)**	**0.039**
CS	**1.371 (1.004–1.872)**	**0.047**

Bold values are statistically significant associations (p-value < 0.05)
CS cesarean section, *kg* kilograms

before, during and after delivery on the risk of ASD did not find any significant association between GA and ASD (Creagh et al. 2016). Nevertheless, the number of CS + GA deliveries in this study was remarkably low and hence lacked the statistical power to identify effect sizes that were found in our study and in the study by Chien et al. (Chien et al. 2015).

We show that the risk of ASD associated with CS + GA is even greater if both RA and GA are used (although this association was not statistically significant due to the low number of surgeries conducted under both RA and GA anesthesia). This finding could imply a dosage or interaction effects of anesthesia on the risk of ASD, or could be related to the fact that GA is mainly used in emergency CSs, or in surgical deliveries for women with clinical complications that could worsen during an epidural or spinal anesthesia or for women with certain pregnancy complications, such as preeclampsia (Shroff et al. 2004). Thus, one may suggest that the association between CS + GA deliveries and ASD that is observed in our study could be driven by such birth complications. Yet, we didn't see significant difference in the effect of CS + GA on the risk of ASD between indicated and non-indicated CS deliveries in our study, as well as in several other studies (Chien et al. 2015; Creagh et al. 2016; Curran et al. 2015a). Thus, the association between CS + GA is unlikely confounded by birth complications that are usually associated with this mode of delivery. Nevertheless, confounding by familial, or other pre-existing conditions cannot be excluded.

A unique aspect of our study was our ability to investigate the effect of CS + GA in groups of children with different severity levels of ASD. This analysis revealed that the association between CS + GA and ASD remained statistically significant only among the most severely affected children with ASD. This finding implies that exposure to GA during CS is probably not a risk factor for all cases of ASD, but only for children with the most severe form of the disorder. In this regard, the higher risk of ASD associated with CS + GA observed among females than among males in our study as well as in Chien et al. 2015, is in line with this observation, since females with ASD tend to have more severe symptoms than males with ASD (Lai et al. 2015; Mandy et al. 2012; Rynkiewicz et al. 2016; Werling and Geschwind 2013). This suggests that females may be more sensitive to the effect of GA during delivery that eventually increase the risk of developing severe ASD. Examining the effect of CS + GA on the manifestation of specific symptoms associated with severe ASD among males and females may help illuminating this possibility. However, such analysis

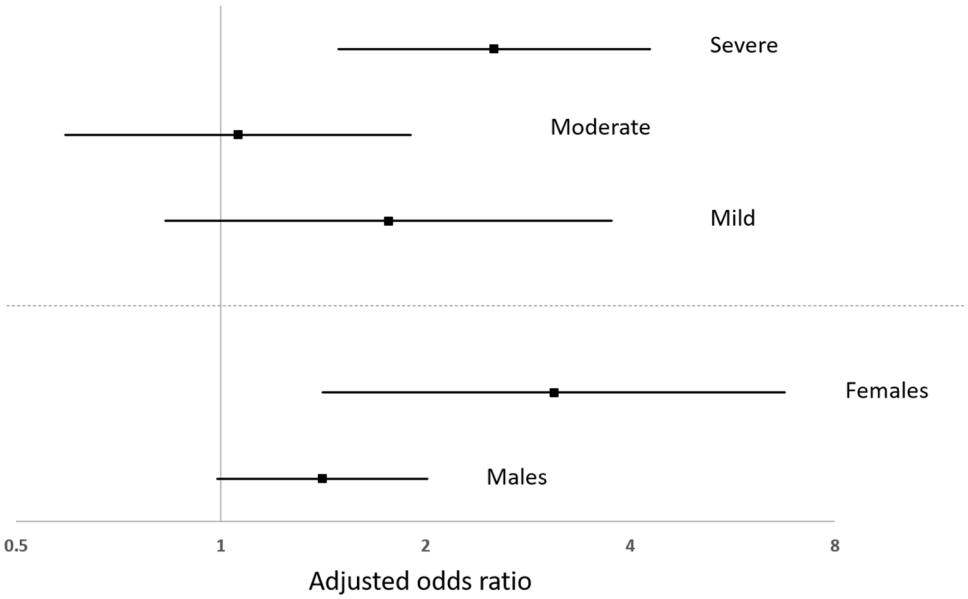

Fig. 2 A forest plot of the association between Cesarean section with general anesthesia (CS + GA) with sex and different ASD severity levels. The adjusted odds ratios (Black squares) and their 95% confidence intervals (Black horizontal lines) of the risks of ASD associated of Cesarean section conducted with general anesthesia are plotted for males and females as well as for different ASD severity levels (mild, moderate, and severe). Gray dashed horizontal lines separates the results of different stratification analyses

requires a much larger sample size than the one that was available in our study.

Recently, a number of animal and observational human studies raised concerns that early-life exposure to certain anesthetic agents that are used in GA may cause neurotoxic changes during brain development, leading to neurodevelopmental problems later in life (Castellheim et al. 2018; Lin et al. 2017; Rappaport et al. 2015; Sumikura et al. 2016). These finding led the US Food and Drug Administration to issue a warning regarding the use of GA among young children and its possible association with neurodevelopmental problems (FDA 2016). This warning led to the emergence of a wide-range of epidemiological studies aiming to explore the possible outcomes of fetal and neonatal exposure to GA in the human population. The results of our study may thus contribute valuable data to this global scientific effort.

Our results should be interpreted with caution since in observational studies like this one there is always a possibility that the observed association is confounded by other factors. For example, a recent large population-based sibling design study from Sweden (Curran et al. 2015a) suggested that the observed association between CS delivery and ASD is confounded by familial factors that increase the risk of both CS and ASD in these families. We attempted to evaluate the effect of such familial confounding in our data using the same sibling design described by Curran et al. (Curran et al. 2015a). However, there were only ten sibling pairs that were discordant on both ASD and CS in our sample, which is an insufficient sample size to assess the effect of CS on ASD within families.

We also found that parity number, amniocentesis, and infant weight are significantly associated with ASD in the bivariate analysis. However, the associations of infant weight and amniocentesis disappeared in the multivariate analysis suggesting they were a result of confounding by other prenatal or perinatal factors. In contrast, the association of parity number with a reduced risk of ASD remained significant even in the multivariate analysis. Low parity has been consistently associated with a reduced risk of ASD in multiple studies (Bilder et al. 2009; Grether et al. 2009; Haglund and Kallen 2011; Hultman et al. 2002; Wang et al. 2017) as well as with symptom variability among children with ASD (Martin and Horriat 2012; Reichenberg et al. 2007; Spiker et al. 2001; Tsai and Stewart 1983; Turner et al. 2011). However, since this was not the focus of this study, we did not examine the details of this effect further.

This study has some limitations. First, the sample size of the study has a limited power to detect ASD risk factors with small effect size. Consequently, a range of known prenatal and perinatal risk factors of ASD were not detected in our study. In addition, this sample size limited the number of strata used in our stratification analyses. Nevertheless, it was sufficient to explore most of our hypotheses except in the case of the sibling analysis. Second, the case–control design of the study did not allow us to calculate the exact risk of ASD associated with exposure to CS + GA. However, the calculated odds ratios should be good estimates of the relative risks in case of an outcome with low prevalence such as ASD, which is smaller than 1% in this population (Davidovitch et al. 2013; Raz et al. 2015). In addition, the effect of CS + GA that is observed in our study could be confounded by unmeasured covariates. Thus, further replication of our findings in other populations and study designs are warranted to affirm the contribution of GA during CS to the risk of ASD.

Conclusions

Our findings suggest that the reported associations between CS and ASD is likely due to the exposure to GA during CS. In addition, the observation that the effect of CS + GA was seen only among children with a severe form of ASD suggests that exposure to GA may contribute to a specific manifestation of ASD. These results resonate well with a recent FDA warning regarding the use of GA among young children or pregnant women and its potential effect on brain development.

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Author Contributions MHS helped to design the study, performed the statistical analyses, and drafted the manuscript. GM participated in the design and interpretation of the data. ID participated in the design and interpretation of the data and helped in drafting the manuscript. HF participated in the design and coordination of the study. AM participated in the design and coordination of the study. AB participated in the design and interpretation of the data and helped in drafting the manuscript. IM conceived the study, guided the statistical analyses, participated in the design and interpretation of the data, and drafted the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required. The study was approved by the SUMC institutional review board (IRB), IRB Approval Number: SOR 222-14.

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