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Maternal Smoking During Pregnancy and Autism Spectrum Disorder

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Maternal Smoking During Pregnancy and Autism Spectrum Disorder

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Huazhong Agricultural University
2015

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An abstract of
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Abstract

Maternal Smoking During Pregnancy
By Jingran Xiong

Maternal smoking during pregnancy may be a modifiable risk factor for Autism Spectrum Disorder (ASD). Existing research on this topic have reported inconsistent findings. We investigated whether maternal smoking during pregnancy was associated with an increased risk of ASD compared with children whose mothers did not smoke during pregnancy. We analyzed data on 766 ASD cases and 500 controls from the CHARGE study. Information on maternal smoking during pregnancy was collected through a questionnaire. ASD cases were identified from the California Department of Developmental Services which contracts regional centers, referrals from other research studies or health providers, as well as referrals from schools, friends, and families. We used multivariate logistic regressions to examine the association between maternal smoking during pregnancy and ASD. Maternal race/ethnicity, prenatal vitamin use, payment delivery method and matching variables child's birth year, gender, and regional center were controlled for as confounding factors. Maternal smoking during pregnancy was associated with an increased odds of ASD compared with children whose mothers did not smoke during pregnancy (OR=1.46, 95% CI: 0.89, 2.40). Similar associations were also observed for maternal smoking before pregnancy, in the child's first year, and in the child's second year and later. Maternal active or passive smoking during pregnancy was associated with an odds 1.62 times that of mothers were not exposed to any smoking during pregnancy (95% CI: 1.16, 2.27). Our results suggest that maternal smoking during pregnancy was associated with higher risk of ASD compared to children whose mothers did not use tobacco during pregnancy.

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CHAPTER 1

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by abnormalities in socialization and emotional development and by restricted, repetitive stereotyped behaviors. The etiology and risk factors for ASD are poorly understood, yet existing studies suggest multiple causes likely contribute to developing ASD, including several genes and environmental factors.[1] ASD is reported to occur in all race/ethnicities and across all socioeconomic classes.[2] In 2010, the Autism and Developmental Disabilities Monitoring (ADDM) Network at the CDC estimated the prevalence of ASD to be one in 68 of children aged 8 years old.[2]

Maternal smoking may affect neurodevelopment in the fetus and risk for ASD through mechanisms such as changing gene expression in the fetal brain[3] or reducing blood flow to the brain resulting in oxygen deprivation in the brain.[4] Based on the data obtained in 2011 from 24 states for the Pregnancy Risk Assessment and Monitoring System, about 10% of the pregnant women reported tobacco use during the third trimester.[5] The results from existing studies which examined the association between maternal smoking during pregnancy and ASD have been inconsistent.[1] Part of this lack of consistency may be due to exposure misclassification or failure to adjust for potential confounding factors.[6]

The purpose of this research is to evaluate whether maternal smoking during pregnancy

increases the risk of child ASD compared with children whose mothers did not smoke during pregnancy using participants in the Childhood Autism Risks from Genetics and the Environment (CHARGE) Study, a population-based case-control study.[7]

Autism Spectrum Disorder (ASD)

Epidemiology of ASD

Autism Spectrum Disorder is a serious developmental disorder which includes disabilities affecting socialization, emotional development, and verbal communication. People with ASD also commonly exhibit restricted or repetitive stereotyped behaviors.[8] ASD is hard to diagnose, and no medication or treatment is available to cure the disease.[9] However, early diagnosis and early intervention can improve developmental outcomes.[2] ASD symptoms are typically present at an early developmental period, and the median age at diagnosis of ASD is around 4.5 years old. In 2012, the Autism and Developmental Disabilities Monitoring (ADDM) Network at CDC estimated the prevalence of ASD to be 1 in 88 children or 1% to 2% among children aged 8.[2] The prevalence of ASD among boys (1 in 42) is reported to be about 4.5 times the prevalence among girls (1 in 189).[2] ASD occurs in all race/ethnicities and across all socioeconomic classes, but according to the ADDM Network data, the prevalence of ASD was higher in 2012 among white children (1 in 63) than among black children (1 in 81) or among Hispanic children (1 in 93).[2] Globally, the prevalence of ASD was estimated to be 62 per 10,000 in 2012, which is about 15 times the estimated prevalence in the late 1960s

and early 1970s (1 in 2,500).[10] The reported U.S. national prevalence in 2012 has also increased 23% compared to 2006 and 78% compared to 2002.[2] This rapid rise in prevalence seems to be caused by a mixture of increasing incidence and increasing diagnosis as a result of elevated public awareness, greater service availability, and changes in diagnostic criteria.[11]

Etiology of ASD

The etiology of autism spectrum disorder remained largely unknown, but it is commonly accepted that ASD arises from the interaction of genetic and environmental factors. The higher prevalence of ASD in children with autistic siblings compared to the general population[12] and a higher concordance rate among homozygous twins indicates that genetic factors are risk factors for ASD.[13] However, no specific gene has been identified as a cause of ASD.[14] Further, a twin study conducted by Hallmayer et al. recently suggested that 55% of the variance in ASD risk of twins is likely to be due to environmental factors, which supported the idea that environmental factors are correlated with and interact with genes in the etiology of autism.[15]

Advanced parental age was found to be associated with an increased risk of ASD in children in multiple studies.[16] The risk of having a child with ASD for fathers greater than 40 years of age was 5.75 times that of fathers younger than 30.[17] Likewise, a 2012 meta-analysis also suggested an association between maternal age and ASD.[18] Preterm

birth[19] and low birth weight[20] were also reported to be associated with higher risk of ASD. Other possible prenatal risk factors identified in a meta-analysis included maternal prenatal medication use, mother's original citizenship, gestational diabetes, and parity.[21]

In summary, the prevalence of ASD continues to rise globally, with a recent estimated prevalence of 0.62%,[10] but the causes for ASD are still largely unknown. Both environmental and genetic factors are believed to be risk factors for developing ASD. Considerable research has been conducted regarding the contribution of environmental factors to ASD. Maternal smoking during pregnancy is one of the maternal lifestyle factors that has been considered to be a potential risk factor for ASD, and information on cigarette smoking is accessible in medical records and birth certificates. Thus, the association between maternal smoking during pregnancy and ASD has been examined by several studies with inconsistent results.

Maternal Smoking

Tobacco use during pregnancy is a leading modifiable risk factor for adverse pregnancy outcomes and smoking-related long-term effects on child health.[22] Some of the adverse pregnancy outcomes and long-term effects reviewed may be along the causal path way between maternal smoking during pregnancy and ASD. Maternal smoking during pregnancy is reported to increase the risk of small-for-gestational-age (SGA) births,

low birth weight (LBW)[23] and preterm birth, with the risk of SGA, LBW, and preterm birth[24] increasing with the number of cigarettes smoked.[25]

Based on information from the U.S. standard birth certificates, the rate of smoking during pregnancy in 2014 was 8.4%, and 20.6% of those who reported smoking during pregnancy quit by the third trimester. Smoking during pregnancy was more prevalent in teenagers to 24 year-olds, non-Hispanic American Indian and Alaska Natives, non-Hispanic whites, unmarried women, and women of low socio-economic status assessed by educational attainment, payment method at delivery and receipt of benefits from WIC.[5] Overall, the smoking rate in pregnant women decreased from around 20% in 1989 to 8.4% in 2014.[22] Studies from Sweden also have shown a decrease in maternal smoking during pregnancy from 29% in 1997 to 16% in 2005. However, the prevalence of smoking in women younger than 20 years old at the time of delivery increased by 6% in Denmark.[26,27]

Because information on maternal cigarette smoking during pregnancy is commonly self-reported, it is a challenge to measure the true smoking prevalence in pregnant women. Validation studies using medical records, the questionnaire from the Pregnancy Risk Assessment Monitoring System, and cotinine biomarkers all indicated that the self-reported prevalence of maternal smoking during pregnancy is underestimated.[28-30] This under-reporting may be due to the social recognition of the harmful effects of smoking during pregnancy. Thus, misclassification of smoking status during pregnancy

is likely to be a source of bias in epidemiology research.

Maternal Smoking During Pregnancy and ASD

Eleven studies have examined the association between maternal tobacco use during pregnancy and ASD, but the results have been inconsistent (Table 1.1). Failure to adjust for confounding may contribute to the variability in reported results. Some of the earlier studies did not adjust for potential confounders such as socioeconomic status, maternal age, and maternal race/ethnicity,[31-34] while other studies[35,36] adjusted for factors that may be along the causal pathway.

Cohort Studies

Five cohort studies were conducted between 2010 and 2016. These studies were register-based except for the study conducted by Xiang et al..[34] Two of the reviewed studies were from Canada, two from Norway, and one from the U.S. The number of participants varied across studies with a range between 64,924 to 507,856. All of them used self-reported information on maternal smoking obtained from a register.

Burstyn et al.[31] and Dodds et al.[32] reported no association in the crude analysis of maternal smoking during pregnancy and ASD in Canada (RR:0.9 95% CI: 0.7, 1.0; OR:0.9 95% CI: 0.8, 1.1). ASD cases were identified thorough the specific diagnostic codes in the universal health insurance system in Brustyn's study and the administrative

database in Dodds's study.

Nilsen et al.[37] conducted two population-based cohort studies. One cohort was a nationwide population and another cohort was a sub-cohort from the Norwegian Mother and Child Cohort Study. ASD cases were identified from the Norwegian Patient Registry. A validation study was conducted using the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS). Maternal smoking had a weak association with ASD in the nationwide cohort (OR = 1.3, 95% CI: 1.2, 1.5) and in the sub-cohort (OR = 1.2, 95% CI: 1.0, 1.3). Year of birth, maternal age, paternal age, marital status, parity, and hospital size were adjusted for in the multivariate analysis.

Xiang et al. [34]evaluated a cohort of singleton children born in hospitals in California. ASD cases were diagnosed based on an evaluation form specialist. No association was found between maternal smoking and ASD (HR:0.8, 95% CI: 0.3, 2.1).

Case-Control Studies

Haglund et al.[38] conducted a case-control study in Sweden. Autism and Asperger syndrome cases were recruited based on diagnosis from the Malmoe Child Psychiatric Clinic. Information on maternal smoking was collected during all prenatal visits. They reported that a slightly protective association was found between maternal smoking and

autism (OR= 0.7, 95% CI:0.5, 1.0) and a slightly elevated association between maternal smoking and Asperger syndrome (OR=1.3, 95% CI:1.0, 1.7) after adjusted for maternal age, parity, mother's original citizenship, gender, gestational age, birth weight standard deviation score, and the presence of obstetrical risk factors.

Mrozek-Budzyn et al.[33] conducted a case-control study matched 1:2 on year of birth, sex and general practitioner. A total of 96 cases and 192 controls were included in the study. Cases were identified from medical records. A standardized questionnaire was used to obtain information on active smoking during pregnancy. They found that the risk of ASD was three times higher in children whose mothers smoked during pregnancy compared to children whose mothers did not smoke during pregnancy.

Maimburg et al.[35] conducted a population-based, matched case-control study, which consisted of 473 infantile autism cases who were diagnosed based on the International Classification of Diseases, 8 and 10 revision (ICD 8 and 10) and identified in a register. Cases and controls were matched 1:10 on sex, year of birth and county of birth. Information on maternal smoking during pregnancy was collected by midwives at the first prenatal visit at about 12 weeks gestation. They found no association between maternal smoking during pregnancy and infantile autism after adjusted for parental age, mothers' citizenship, birthweight, gestational age, Apgar Score, birth defects, and irregular fetal position. The strength of the study included that they matched cases on sex and birth year, and adjusted for maternal age and mothers' citizenship. However,

limitation included that they adjusted for variables that could be along causal pathway, and that they did not adjust for social-economic status. Also, reporting smoking status to midwives might lead to exposure misclassification, because women may be uncomfortable admitting smoking during pregnancy to their midwives.

Lee et al.[39] compared 3,958 ASD cases and 38,983 controls from the Stockholm Youth Cohort. ASD cases were identified by ICD 9, ICD 10 and Diagnostic and Statistical Manual, Fourth Edition (DSM-IV) codes and were ascertained through regional and national data registers. Each case was matched with 10 controls on sex and birth year. ASD was categorized into ASD without co-morbid intellectual disability and ASD with co-morbid intellectual disability. Information on maternal smoking was collected by midwives during the first prenatal visit. Maternal smoking was categorized as binary (smoking and non-smoking) and categorical (none, 1-9 cigarettes daily, and >= 10 cigarettes daily). They found that maternal smoking during pregnancy was not associated with ASD in a crude analysis and an analysis adjusted for maternal and paternal age and parity. After additionally adjusted for socioeconomic status, there was also no association. Limitations include excluding observations due to missing data, potential exposure misclassification due to self-report and outcome misclassification resulting from using administrative data. Meanwhile, reporting smoking status to midwives might lead to exposure misclassification, because women may be uncomfortable admitting smoking during pregnancy to their midwives. The strengths of the study included that all analyses were repeated stratified by calendar year and

associations were examined by degree of functioning in ASD. Moreover, a dose-response effect of maternal smoking was assessed. Also, the exposure data were validated against serum cotinine levels, and the data quality was considered high. The investigators used a multi-source case ascertainment system to capture most cases.

Kalkbrenner et al.[40] recruited 3,315 cases of autism identified through a population-based U.S. surveillance program. Children who were born in 1992, 1994, 1996, or 1998 and who resided in the ADDM surveillance regions were identified, and a total of 633,989 births who fulfilled the inclusion criteria were included in the study. Case subgroups were defined as: all ASD, Autistic disorder (AD), ASD- not otherwise specified (ASD-NOS), ASD with intellectual disability, and ASD without intellectual disability. Cases were identified using surveillance data if they met the criteria for ASD based on DSM-IV-TR. Exposure data were extracted from the birth certificate. They found no association between maternal smoking and ASD or AD and a modest association with ASD-NOS. Maternal race/ethnicity was examined as an effect measure modifier while maternal age, education, marital status, county population size, birth year, surveillance site and a cross product of surveillance site and birth year were included as confounders in the adjusted analysis. Strengths of the design included the sensitivity analyses conducted to correct for outcome misclassification, case subgroup examination, and adjustment for SES and other confounders. The inaccuracy of maternal smoking information on the birth certificate and the lack of information on dose and frequency of maternal smoking were limitations of the study.

Tran et al [36]. conducted a study nested in the Finnish Prenatal Study of Autism and ASD. The study included 4,019 ASD cases and 16,123 controls. Cases and controls were matched 1:4 on date of birth, sex, and whether they were a resident of Finland at the time of birth. ASD cases were diagnosed from the Finnish Hospital Discharge Register based on ICD 10 codes. Childhood autism, Asperger's syndrome, and pervasive developmental disorder unspecified (PDD) were examined separately as subtypes of ASD. Information on maternal smoking during pregnancy was recorded by nurses during routine prenatal visits and was obtained from the Finnish Medical Birth Register. Maternal smoking during pregnancy was defined as no smoking, quit smoking during the first trimester, smoked throughout the pregnancy, and unknown. No association was found between maternal smoking during pregnancy and ASD after adjusting for maternal age, social-economic status, birth weight adjusted for gestational age, and maternal psychiatric diagnosis. However, smoking throughout the whole pregnancy was found to be associated with PDD. Strengths included a simulation sensitivity analysis for missing data in maternal smoking, examining subtypes of ASD, and assessing timing of exposure. Limitations were potentially inaccurate self-reported exposure information and adjusting for birthweight which could be along the causal pathway.

Summary

Most studies found no association between maternal smoking during pregnancy and ASD. Some of the results[31,32,34] may be biased due to lack of adjustment for confounding; some[35,36,38] may be biased because of controlling for factors along the causal pathway. However, two studies found an elevated risk of ASD among mothers who smoked during pregnancy. One was a crude analysis and the other failed to control for socioeconomic status. Each of the reviewed studies had its limitation. Therefore, these inconsistent results could indicate the need for better studies to assess the association between maternal smoking during pregnancy and ASD.

The current study differs from the previous studies in several ways. First, ASD cases were confirmed by the UC Davis Medical Investigations of Neurodevelopmental Disorders Institute (MIND) using the ADI-R and the ADOS[7], which improves validity. Second, information on potential confounders such as maternal age, socioeconomic status, maternal race/ethnicity were collected. Thus, potential confounding should be well controlled. Finally, information on passive smoking during pregnancy was also collected, which enabled investigation of the association between passive and active smoking during pregnancy. In addition, information on maternal smoking before and after pregnancy were available for us to investigate the effect of maternal smoking on ASD in different time periods.

ASD is a serious developmental disorder which is hard to diagnose, and there is

currently no medication or best treatment available to cure the disease.[2] Moreover, the prevalence of ASD has increased greatly during the past half century globally.[10] Maternal smoking during pregnancy is a potential modifiable risk factor for ASD. Even though the prevalence of maternal smoking during pregnancy has experienced a decreased from 1984,[22] the rate of maternal smoking is still high according to studies from Sweden, the UK and the US.[26,27,41] Thus, it is important to further assess whether maternal smoking during pregnancy plays a role in ASD. If smoking does cause ASD, it would reinforce the importance of identifying strategies for maternal smoking prevention during pregnancy.

Table 1.1 A review of previously published studies of smoking during pregnancy and ASD in the offspring

Author	Year	Study Design	Exposure Ascertainment	Disease Ascertainment	OR (95%CI) (Maternal Smoking Yes V.S. No)	Covariates
Maimburg et al.	2006	Case-control Study; 473 cases, 4,720 controls matched on sex, year and county of birth	First prenatal visit, collected by midwives	Danish Psychiatric Central Register (ICD-8 and ICD-10)	0.9 (0.7-1.4)	Maternal age, paternal age, mothers' citizenship, birthweight, gestational age, Apgar, birth defect and irregular fetal position
Burstyn et al.	2010	Cohort Study; 1,122 cases, 215,220 singleton live births	Routinely clinical care at the admission to delivery	Physician Billing Record (ICD-9)	RR: 0.9 (0.7-1.0)	None
Dodds et al.	2011	Cohort Study; 924 ASD cases, 129,733 births	Perinatal Database	Administrative Health database (ICD-9 and ICD-10)	RR: 0.9 (0.8-1.1)	None
Haglund et al.	2011	Case-Control Study; 157 Autism, 93 Asperger syndrome, 68,714 controls	Swedish Medical Birth Registry; Antenatal clinic visit	Malmoe Child Psychiatric Clinic (DSM-IV, ICD-10)	Autism: 0.7 (0.5-1.0) Asperger syndrome: 1.3 (1.0-1.7)	Maternal age at delivery, parity, mother's original citizenship, gender, gestational age, SD-score, obstetrical risk factor
Lee et al.	2011	Case-control Study; 3,958 cases and 38,983 controls	Medical Birth Register	National and Regional Data Register (ICD-9, ICD-10, DSM-IV)	Crude:1.1 (1.0,1.2) Adjusted: no significant association	Maternal age, paternal age, parity, SES
Kalkbrenner et al.	2012	Case-control Study; 3,315	Birth Certificate	Autism and Developmental	PR: 0.9 (0.8-1.0)	Maternal age, education, marital

		cases, 630,674 controls		Disabilities Monitoring network (DSM-IV-TR)		status, County population size, birth year, surveillance site
Tran et al.	2013	Case-Control Study; 4,019 cases, 16,123 controls	Finnish Medical Birth Register	Finnish Hospital Discharge Register (ICD-10)	1.0 (0.9-1.2)	Maternal age at birth, SES, infant's weight for gestational age, maternal psychiatric diagnosis
Mrozek-Budzyn et al.	2013	Case-Control Study; 96 cases, 192 controls matched on year of birth, sex, and general practitioners	Questionnaire	Medical Record (ICD-10)	3.3 (1.1,9.8)	None
Nilsen et al.	2013	Cohort Study; 285 ASD cases, total 89,836 children	Medical Birth Registry of Norway; Beginning and end of pregnancy	Norwegian Patient Registry (ICD-10); Validation Assessment (DMS-IV)	1.3 (1.2,1.5)	Year of birth, maternal age, paternal age, marital status, parity, hospital size
		Cohort Study; 2,072 ASD cases, total 507,856 children	Medical Birth Registry of Norway; Beginning and end of pregnancy	Norwegian Patient Registry (ICD-10)	1.2 (1.0,1.3)	Year of birth, maternal age, paternal age, marital status, parity, hospital size
Xiang et al.	2015	Cohort Study; 643 ASD cases, 64,924 singleton children,	Medical Record and Birth Certificate	Diagnosed by pediatric developmental specialist	HR: 0.8 (0.3-2.1)	None

OR: Odds ratio; CI: Confidence interval.

CHAPTER 2

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder associated with abnormalities in socialization and emotional development and with restricted, repetitive stereotyped behaviors. Maternal smoking during pregnancy may affect neurodevelopment in the fetus and risk for ASD through mechanisms such as changing gene expression in the fetal brain[6] and reduced blood flow causing oxygen deprivation in the brain.[4] Maternal smoking during pregnancy remains a modifiable public health concern with 10% of infants exposed to maternal smoking during the third trimester in the US.[5]

Results from studies examining the association between maternal tobacco use during pregnancy and ASD have been inconsistent.[1] In reviewed studies, 8 studies found there was no association between maternal smoking during pregnancy and ASD[31,32,34-37,39,40], while 2 studies found an elevated association between maternal smoking during pregnancy and ASD[33,37], and one study reported that maternal smoking was protective for autism but increased the risk of Asperger Syndrome[38]. These studies varied in design, ASD definition and screening process, and exposure ascertainment. All the above factors may affect the study results.

In the present study, we evaluated whether maternal smoking during pregnancy increased the risk of child ASD compared with children whose mothers who did not

smoke during pregnancy using participants in the Childhood Autism Risks from Genetics and the Environment (CHARGE) Study, a population-based case-control study.[7] The current study differs from the previous studies in several ways. ASD cases were ascertained by trained personnel using the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedules (ADOS), which improves validity. In addition, information on passive smoking during pregnancy was collected, which enabled investigation of the association between passive as well as active smoking during pregnancy.

Methods

Study Population

Participants were identified in the CHARGE Study, which has been described elsewhere.[7] In brief, researchers recruited and collected data from children with full-syndrome autism and children selected from the general population regardless of their developmental status. Children with ASD were identified through the California Department of Developmental Services which contracts regional centers, referrals from other research studies or health providers, and referrals from schools, friends, and families. The typical development (TD) group were identified in state birth files frequency matched to autism cases by age, gender, and the regional center. All participating children were between 24 and 60 months at enrollment, born in California, living with at least one biologic English or Spanish speaking parent, and living within

the catchment areas of regional centers.

Diagnostic Classification

Child's diagnostic group was confirmed by trained study personnel using the ADI-R and the ADOS.[42] All participants were assessed for cognitive function and adaptive function using the Mullen Scales of Early Learning (MSEL) and the Vineland Adaptive Behavior Scales (VABS) respectively. ASD was defined as meeting established cut points on both the ADI-R and the ADOS as described in greater detail elsewhere.[43] The TD group were screened for sign of autism using the Social Communication Questionnaire (SCQ). Children who scored $\geq$ 15 were administered the ADI-R and ADOS and were reclassified to the ASD group if they met the criteria. The final TD group included children who obtained an MSEL score of $\geq$ 70 and a VABS score of $\geq$0 and scored $\leq$ 15 on SCQ.

Exposure

Maternal smoking during pregnancy information was obtained through questionnaires administered by trained interviewers over the phone or by self-administered questionnaires. Mothers were asked if they smoked before (three months before the conception), during, or after pregnancy (until the child was 4 years old). Further, they were asked if they lived with a person who smoked before, during, or after their pregnancy.

Covariates

A Directed Acyclic Graph (DAG) was created to select potential confounders based on existing research. Additional covariates based on the DAG were: maternal age, mother's birth place, maternal race/ethnicity, payment method, marital status, prenatal vitamin use, and highest education in the household. Information was obtained from questionnaires.

Statistical Analysis

All statistical analyses were performed using SAS 9.4. Univariate descriptive analyses were performed to check for outliers and to summarize demographic information. Correlations between maternal smoking during pregnancy and before and after pregnancy were evaluated to explore whether smoking behavior changed during these periods. Logistic regression was used to investigate the association between ASD and maternal smoking during pregnancy. In multivariate models, we included all potential confounders identified using the DAG and all the matching factors in the CHARGE study. Variables were then excluded using backward elimination and retained if excluding the variable caused greater than 10% change in the exposure estimate. All matching variables were included in the analysis regardless of their impact on the exposure estimate. The final model was also fit restricted to the subgroup of women who completed the questionnaire administered by interviewers. We also conducted

analyses exploring the effect of maternal smoking before or after pregnancy and analyses of passive maternal smoking during, before or during, or after pregnancy. Bias analyses were conducted to evaluate the potential effect of bias due to missing smoking status and of misclassification due to self-reported smoking information. Potential bias from missing smoking status was evaluated under four assumptions: 1. Considering all missing values as smoking; 2. Considering all missing values as no smoking; 3. Considering missing values for cases as smoking and missing values for controls as no smoking; 4. Considering missing values for cases as no smoking and missing values for controls as smoking. Potential exposure misclassification due to self-report was evaluated by conducting a bias analysis using sensitivity and specificity obtained from existing research comparing self-reported smoking status during pregnancy and cotinine levels in the blood. Also, the effect of smoking before or after pregnancy was evaluated among women who did not smoked during pregnancy.

Results

A total of 1,266 births were included in the analysis, with 766 ASD cases and 500 controls. The control group was frequency matched to the age, gender, and regional center distribution of the ASD cases. ASD cases were more likely to have a mother who reported other marital status, did not use prenatal vitamin, was born outside the USA, was Hispanic or non-Hispanic other than white, and had no insurance or was using a government sponsored insurance programs (Table 2.1).

In the case group, 9.1% of the mothers smoked during pregnancy, while in the control group, 5.7% of the mothers smoked during pregnancy. The percent smoking during pregnancy was greater for mothers who were younger, were unmarried, did not use prenatal vitamins and did not have insurance or were in a government program. Non-Hispanic white mothers who born in the US were more likely to smoke during pregnancy compared to Non-Hispanic other races or Hispanic mothers who born outside the US. Among mothers who smoked during pregnancy, 55% of the families had some college as the highest education level (Results not shown).

In the model adjusted only for matching variables, maternal smoking during pregnancy was associated with a higher odds of ASD compared with no maternal smoking during pregnancy (OR=1.64, 95% CI:1.01, 2 .66). The association remained after adjusted for maternal race/ethnicity, prenatal vitamin use, payment method, and the matching variables child's birth year, gender, and the regional center (OR=1.46, 95% CI: 0.89, 2.40). Among women who completed questionnaires administered by interviewers, there was also a weak association between maternal smoking during pregnancy and ASD (OR= 1.37, 95% CI: 0.77, 2.45). We also examined the association between ASD and maternal smoking before pregnancy, in child's first year, and after the child turned two adjusted for the same variables. Similar elevated associations were found for all time periods, although the association for smoking after the child turned two and ASD was slightly attenuated (Table 2.2).

Adjusted multivariate models were also fit to evaluate passive maternal smoking and ASD (Table 2.3). Passive maternal smoking during pregnancy, before or during pregnancy, and in child's first year were associated with an increased odds of ASD. The odds of maternal active or passive smoking was 1.62 times that of mothers who did not smoked and were not exposed to passive smoking.

Information on maternal smoking during pregnancy was missing for 137 individuals. Our sensitivity analysis of potential bias due to missing values for smoking suggested minimal bias (Table 2.4). Further, the associations between ASD and maternal smoking before pregnancy or in child's first year persisted even when we excluded women who reported smoking during pregnancy although the 95% CIs were wide. However, the elevated association between maternal smoking in child's second year and later and ASD became null after excluding women who smoked during pregnancy (Appendix Table 1). Finally, a bias analysis using the sensitivity and specificity smoking obtained from Mattsson et al.[44] showed a corrected crude odds ratio of 6.45, which suggested that there may be information bias due to inaccurate self-reported information has the potential to introduce substantial bias.

Discussion

This population based case-control study investigated the association between maternal

tobacco use during pregnancy and ASD. In the unadjusted analyses, there was an increased risk of ASD among children who were exposed to maternal smoking during pregnancy. After adjusted for maternal race/ethnicity, prenatal vitamin use, payment method, and the matching variables child's birth year, gender, and the regional center, women who smoked during pregnancy had a 46% increased odds of having a child with ASD compared to women who did not smoke during pregnancy. The association was stronger when both active smoking and passive smoking occurred during pregnancy compared to no active and passive smoking during pregnancy. Moreover, the elevated association was persistent for maternal smoking before, during, and after pregnancy and when we performed bias analyses to address missing data. The estimate was stronger when we performed a bias analysis to address misclassification of maternal smoking during pregnancy.

The elevated association is consistent with the studies conducted by Nilsen et al. (OR=1.29, 95% CI: 1.16, 1.45) and Mrozek-Budzyn et al (OR=3.32, 95% CI: 1.12, 9.82),[33,37] although, their studies failed to control for socioeconomic status. In contrast, several previous studies showed no association between maternal smoking during pregnancy and ASD.[31,32,34-36,38] Each of these studies had limitations. Some of the results may be biased due to lack of adjustment for confounding by socioeconomic status or maternal race/ethnicity,[31,32,34,38] while others may be biased from controlling for factors such as birth weight which may be along the causal pathway between maternal smoking and ASD.[35,36] Our study differs from the previous studies in that the

outcome ascertainment was performed by trained personnel after recruitment. In addition, we adjusted for potential confounding factors, including socioeconomic status as measured by highest education in the household and payment delivery method.

Despite the strengths of this study, several limitations should be noticed, and caution should be used when interpreting the study findings. Limitations include potential misclassification of maternal smoking during pregnancy. The exposure information was determined by self-report through a telephone interview or by self-administered questionnaires and some participants had missing values for smoking status. These potential sources of bias were addressed by conducting bias analyses. The results from our sensitivity analyses suggested that the impact of missing data may be small, but the bias from misclassification of smoking may be large and towards the null. However, the bias analysis of misclassification of smoking used sensitivity and specificity based on a Swedish study. The bias analysis results may be misleading if the bias parameters from the Swedish study do not apply to our study population. A subgroup analysis was performed among women who completed the questionnaires administered by interviewers, but a similar elevated association was found between maternal smoking and ASD, which suggested that the mode of questionnaire administration may not affect exposure reporting in this study.

Another limitation is that we did not have information regarding trimester-specific maternal smoking. Therefore, we were unable to evaluate whether there is a specific

critical exposure window. However, the results were robust for before and after pregnancy. Due to the strong correlation between smoking during pregnancy and smoking during other time periods, we had limited ability to assess whether smoking after pregnancy might be associated with ASD. However, there was a suggestion that maternal smoking during child's first year might be related to ASD, because the association persisted even when women who smoked during pregnancy were excluded. We observed a strong association between maternal smoking before pregnancy and ASD, even when women who smoked during pregnancy were excluded. Although there may be an effect of smoking before pregnancy on ASD, this association may be a result of some women who smoked during pregnancy only admitting to smoking before pregnancy. Alternatively, there could be residual confounding if there is a strong, unmeasured risk factor for ASD that is associated with smoking. Passive smoking during pregnancy was more strongly associated with ASD than active smoking in our study, which also could be an indication of residual confounding. Alternatively, this stronger association may be because women who smoked during pregnancy only reported exposure to environmental tobacco smoking but not to active smoking. We were also unable to evaluate whether there was a dose response relation between the frequency of maternal smoking and ASD.

Strengths of the present study included using a population base study design and having a large sample size. Also, ASD cases were confirmed by the UC Davis MIND Institute using the ADI-R and the ADOS, which improves validity. Furthermore, information on

passive smoking during pregnancy and postnatal smoking were collected, which enabled investigation of the association between passive and active smoking during pregnancy and the potential impact of postnatal smoking on ASD.

In conclusion, the present study suggests that maternal smoking during pregnancy may be related to higher risk of ASD compared to mothers who did not smoke during pregnancy. This risk may be related to passive as well as active smoking. Future studies may consider using objective markers of cigarette smoking such as serum cotinine levels to ascertain exposure. Also, the association between ASD and trimester-specific maternal smoking during pregnancy or the dose response effect of maternal smoking during pregnancy on ASD may be of value to investigate.

REFERENCES

1. Lyall K, Schmidt RJ, Hertz-Picciotto I. Maternal lifestyle and environmental risk factors for autism spectrum disorders. *Int J Epidemiol.* 2014;43(2):443-464.
2. Developmental Disabilities Monitoring Network Surveillance Year Principal I, Centers for Disease C, Prevention. Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *MMWR Surveill Summ.* 2014;63(2):1-21.
3. Luck W, Nau H, Hansen R, Steldinger R. Extent of nicotine and cotinine transfer to the human fetus, placenta and amniotic fluid of smoking mothers. *Dev Pharmacol Ther.* 1985;8(6):384-395.
4. Albuquerque CA, Smith KR, Johnson C, Chao R, Harding R. Influence of maternal tobacco smoking during pregnancy on uterine, umbilical and fetal cerebral artery blood flows. *Early Hum Dev.* 2004;80(1):31-42.
5. Curtin SC, Matthews TJ. Smoking Prevalence and Cessation Before and During Pregnancy: Data From the Birth Certificate, 2014. *Natl Vital Stat Rep.* 2016;65(1):1-14.
6. Bilder D, Pinborough-Zimmerman J, Miller J, McMahon W. Prenatal, perinatal, and neonatal factors associated with autism spectrum disorders. *Pediatrics.* 2009;123(5):1293-1300.
7. Hertz-Picciotto I, Croen LA, Hansen R, Jones CR, van de Water J, Pessah IN. The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism. *Environ Health Perspect.* 2006;114(7):1119-1125.
8. Association AP. *Diagnostic and statistical manual of mental disorders.* 5th ed. Arlington,VA: American Psychiatric Association; 2013.
9. Singh K, Connors SL, Macklin EA, et al. Sulforaphane treatment of autism spectrum disorder (ASD). *Proc Natl Acad Sci U S A.* 2014;111(43):15550-15555.
10. Elsabbagh M, Divan G, Koh YJ, et al. Global prevalence of autism and other pervasive developmental disorders. *Autism Res.* 2012;5(3):160-179.
11. Ornoy A, Weinstein-Fudim L, Ergaz Z. Prenatal factors associated with autism spectrum disorder (ASD). *Reprod Toxicol.* 2015;56:155-169.
12. Constantino JN, Zhang Y, Frazier T, Abbacchi AM, Law P. Sibling recurrence and the genetic epidemiology of autism. *Am J Psychiatry.* 2010;167(11):1349-1356.
13. Sandin S, Lichtenstein P, Kuja-Halkola R, Larsson H, Hultman CM, Reichenberg A. The familial risk of autism. *JAMA.* 2014;311(17):1770-1777.
14. Tchaconas A, Adesman A. Autism spectrum disorders: a pediatric overview and update. *Curr Opin Pediatr.* 2013;25(1):130-144.
15. Hallmayer J, Cleveland S, Torres A, et al. Genetic heritability and shared environmental factors among twin pairs with autism. *Arch Gen Psychiatry.* 2011;68(11):1095-1102.
16. Hultman CM, Sandin S, Levine SZ, Lichtenstein P, Reichenberg A. Advancing

paternal age and risk of autism: new evidence from a population-based study and a meta-analysis of epidemiological studies. *Mol Psychiatry.* 2011;16(12):1203-1212.

17. Reichenberg A, Gross R, Weiser M, et al. Advancing paternal age and autism. *Arch Gen Psychiatry.* 2006;63(9):1026-1032.
18. Sandin S, Hultman CM, Kolevzon A, Gross R, MacCabe JH, Reichenberg A. Advancing maternal age is associated with increasing risk for autism: a review and meta-analysis. *J Am Acad Child Adolesc Psychiatry.* 2012;51(5):477-486 e471.
19. Buchmayer S, Johansson S, Johansson A, Hultman CM, Sparen P, Cnattingius S. Can association between preterm birth and autism be explained by maternal or neonatal morbidity? *Pediatrics.* 2009;124(5):e817-825.
20. Gardener H, Spiegelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. *Pediatrics.* 2011;128(2):344-355.
21. Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. *Br J Psychiatry.* 2009;195(1):7-14.
22. Cnattingius S. The epidemiology of smoking during pregnancy: smoking prevalence, maternal characteristics, and pregnancy outcomes. *Nicotine Tob Res.* 2004;6 Suppl 2:S125-140.
23. Kramer MS. Intrauterine growth and gestational duration determinants. *Pediatrics.* 1987;80(4):502-511.
24. Cnattingius S, Granath F, Petersson G, Harlow BL. The influence of gestational age and smoking habits on the risk of subsequent preterm deliveries. *N Engl J Med.* 1999;341(13):943-948.
25. Harrod CS, Reynolds RM, Chasan-Taber L, et al. Quantity and timing of maternal prenatal smoking on neonatal body composition: the Healthy Start study. *J Pediatr.* 2014;165(4):707-712.
26. Cnattingius S, Haglund B. Decreasing smoking prevalence during pregnancy in Sweden: the effect on small-for-gestational-age births. *Am J Public Health.* 1997;87(3):410-413.
27. Egebjerg Jensen K, Jensen A, Nohr B, Kruger Kjaer S. Do pregnant women still smoke? A study of smoking patterns among 261,029 primiparous women in Denmark 1997-2005. *Acta Obstet Gynecol Scand.* 2008;87(7):760-767.
28. DiGiuseppe DL, Aron DC, Ranbom L, Harper DL, Rosenthal GE. Reliability of birth certificate data: a multi-hospital comparison to medical records information. *Matern Child Health J.* 2002;6(3):169-179.
29. Allen AM, Dietz PM, Tong VT, England L, Prince CB. Prenatal smoking prevalence ascertained from two population-based data sources: birth certificates and PRAMS questionnaires, 2004. *Public Health Rep.* 2008;123(5):586-592.
30. Klebanoff MA, Levine RJ, Clemens JD, DerSimonian R, Wilkins DG. Serum cotinine concentration and self-reported smoking during pregnancy. *Am J Epidemiol.* 1998;148(3):259-262.
31. Burstyn I, Sithole F, Zwaigenbaum L. Autism spectrum disorders, maternal characteristics and obstetric complications among singletons born in Alberta,

Canada. . *Chronic Disesase of Canada.* 2010;30(4):125-134.

32. Dodds L, Fell DB, Shea S, Armson BA, Allen AC, Bryson S. The role of prenatal, obstetric and neonatal factors in the development of autism. *J Autism Dev Disord.* 2011;41(7):891-902.
33. Mrozek-Budzyn D, Majewska R, Kieltyka A. Prenatal, perinatal and neonatal risk factors for autism- study in Poland. *Central European Journal of Medicine.* 2013;8(4):424-430.
34. Xiang AH, Wang X, Martinez MP, et al. Association of maternal diabetes with autism in offspring. *JAMA.* 2015;313(14):1425-1434.
35. Maimburg RD, Vaeth M. Perinatal risk factors and infantile autism. *Acta Psychiatr Scand.* 2006;114(4):257-264.
36. Tran PL, Lehti V, Lampi KM, et al. Smoking during pregnancy and risk of autism spectrum disorder in a Finnish National Birth Cohort. *Paediatr Perinat Epidemiol.* 2013;27(3):266-274.
37. Nilsen RM, Suren P, Gunnes N, et al. Analysis of self-selection bias in a population-based cohort study of autism spectrum disorders. *Paediatr Perinat Epidemiol.* 2013;27(6):553-563.
38. Haglund NGS, KALLEN KBM. Risk factors for autism and Asperger syndrome. *Autism.* 2011;15(2):163-183.
39. Lee BK, Gardner RM, Dal H, et al. Brief report: maternal smoking during pregnancy and autism spectrum disorders. *J Autism Dev Disord.* 2012;42(9):2000-2005.
40. Kalkbrenner AE, Braun JM, Durkin MS, et al. Maternal smoking during pregnancy and the prevalence of autism spectrum disorders, using data from the autism and developmental disabilities monitoring network. *Environ Health Perspect.* 2012;120(7):1042-1048.
41. Ward C, Lewis S, Coleman T. Prevalence of maternal smoking and environmental tobacco smoke exposure during pregnancy and impact on birth weight: retrospective study using Millennium Cohort. *BMC Public Health.* 2007;7:81.
42. Lord C, Risi S, Lambrecht L, et al. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord.* 2000;30(3):205-223.
43. Risi S, Lord C, Gotham K, et al. Combining information from multiple sources in the diagnosis of autism spectrum disorders. *J Am Acad Child Adolesc Psychiatry.* 2006;45(9):1094-1103.
44. Mattsson K, Kallen K, Rignell-Hydbom A, et al. Cotinine Validation of Self-Reported Smoking During Pregnancy in the Swedish Medical Birth Register. *Nicotine Tob Res.* 2016;18(1):79-83.

Table 2.1 Characteristics of the CHARGE cohort by disease status

Characteristic, N (%)	ASD (n=766)	TD (n=500)
Age at Assessment (month)		
23.4-36.0	153 (20.0)	138 (27.6)
36.1-48.0	259 (33.8)	181 (36.2)
48.1-53.0	143 (18.7)	80 (16.0)
53.1-65.2	211 (27.6)	101 (20.2)
Regional Center		
Alta, Far Northern, and Redwood Coast	301 (39.3)	235 (47.0)
North Bay	95 (12.4)	80 (16.0)
East Bay, San Andreas, and Golden Gate	124 (16.2)	77 (15.4)
Valley Mt, Central Valley, and Kern	148 (19.3)	86 (17.2)
All Los Angeles, Orange, San Diego, Tri-counties and Inland	98 (12.8)	22 (4.4)
Birth Weight		
Low Birth Weight (<=2500)	62 (8.1)	31 (6.2)
Not Low Birth Weight (>2500)	704 (91.9)	469 (93.8)
Gender		
Male	645 (84.2)	412 (82.4)
Female	121 (15.8)	88 (17.6)
Maternal Age		
16-20	33 (4.3)	24 (4.8)
21-25	129 (16.8)	68 (13.6)
26-30	215 (28.1)	42 (28.6)
31-35	239 (31.2)	172 (34.4)
36-40	127 (16.6)	74 (14.8)
41 or more	23 (3.0)	19 (3.8)
Payment Method		
Government Program/ No Insurance	151 (20.1)	77 (15.7)
Insurance	602 (79.9)	414 (84.3)
Missing	13	9
Marital Status		
Never Married	45 (6.4)	28 (5.7)
Married	572 (81.4)	422 (86.5)
Other	86 (12.2)	38 (7.8)
Missing	63	12
Prenatal Vitamin Use		
Yes	382 (56.2)	293 (63.8)
No	298 (42.8)	166 (36.2)
Missing	86	41
Birth Place of Mother		
Born in USA	569 (74.3)	414 (82.8)
Born in Mexico	62 (8.1)	28 (5.6)

Born outside USA or Mexico	135 (17.6)	58 (11.6)
Highest Education in household*		
High school diploma/GED or less	81 (10.6)	40 (8.0)
Some College	246 (32.2)	167 (33.4)
Bachelor degree	255 (33.3)	178 (35.6)
Graduate or higher degree	183 (23.9)	115 (23.0)
Maternal Race/Ethnicity		
White non-Hispanic	438 (57.2)	329 (65.8)
Other races non-Hispanic	147 (19.2)	73 (14.6)
Hispanic any race	181 (23.6)	98 (19.6)

Table 2.2 Adjusted* OR and 95% CI for ASD and maternal smoking during different periods

Main Exposure	Cases	Controls	OR	95% CI
During Pregnancy				
Yes	57	26	1.46	0.89, 2.40
No	595	422	1.00	Referent
Before or During Pregnancy				
Yes	92	43	1.47	0.99, 2.19
No	561	405	1.00	Referent
Before Pregnancy				
Yes	87	42	1.41	0.94, 2.12
No	567	406	1.00	Referent
In Child's First Year				
Yes	48	22	1.44	0.85, 2.45
No	604	426	1.00	Referent
In Child's 2 Second Year and After				
Yes	72	38	1.23	0.80, 1.89
No	580	410	1.00	Referent

OR: Odds ratio; CI: Confidence interval.

*Adjusted for maternal race/ethnicity, prenatal vitamin use, payment method, year of birth, child’s gender, and the regional center.

Table 2.3 Adjusted* OR and 95% CI for ASD and active and passive maternal smoking during different periods

Maternal Smoking	Cases	Controls	OR	95% CI
Passive				
During Pregnancy				
Yes	116	50	1.68	1.16, 2.43
No	537	396	1.00	Referent
Before or During Pregnancy				
Yes	122	51	1.77	1.23, 2.54
No	531	395	1.00	Referent
In Child's First Year				
Yes	111	45	1.74	1.18, 2.54
No	543	401	1.00	Referent
In Child's 2 Plus Year				
Yes	105	48	1.50	1.03, 2.19
No	549	398	1.00	Referent
During Pregnancy			**OR**	**95% CI**
Only Active Smoking	27	13	1.41	0.71, 2.83
Only Passive Smoking	83	37	1.65	1.09, 2.52
Both	30	13	1.73	0.87, 3.45
None	507	383	1.00	Referent
Either Active or Passive Smoking	140	63	1.62	1.16, 2.27
None	507	383	1.00	Referent
Before or During Pregnancy			**OR**	**95% CI**
Only Active Smoking	43	23	1.32	0.77, 2.27
Only Passive Smoking	71	31	1.74	1.10, 2.73
Both	49	20	1.88	1.08, 3.27
None	485	372	1.00	Referent
Either Active or Passive Smoking	163	74	1.64	1.19, 2.26
None	485	372	1.00	Referent

OR: Odds ratio; CI: Confidence interval.

*Adjusted for maternal race/ethnicity, prenatal vitamin use, payment method, year of birth, child’s gender and the regional center.

Table 2.4 Adjusted * OR and 95% CI for maternal smoking during pregnancy and ASD based on sensitivity analysis for missing values in maternal smoking status

Model	Case	Controls	OR	95% CI
All Missing Values as Smoking				
Yes	73	31	1.65	1.05, 2.60
No	595	422	1.00	Referent
All Missing Values as Nonsmoking				
Yes	57	26	1.40	0.85, 2.31
No	611	427	1.00	Referent
Missing Values for Case as Smoking, Missing Values for Control as Nonsmoking				
Yes	73	26	1.99	1.23, 3.23
No	595	427	1.00	Referent
Missing Values for Case as Nonsmoking, Missing Values for Control as Smoking				
Yes	57	31	1.17	0.73, 1.88
No	611	422	1.00	Referent

OR: Odds ratio; CI: Confidence interval.

*Adjusted for maternal race/ethnicity, prenatal vitamin use, payment method, year of birth, child's gender and the regional center.

APPENDIX

Table 1 Adjusted* OR and 95% CI for smoking before or after pregnancy among women who did not report smoking during pregnancy

Maternal Smoking	Cases	Controls	OR	95% CI
Only Before Pregnancy				
Yes	35	17	1.46	0.80, 2.68
No	562	405	1.00	Referent
Only In Child's First Year				
Yes	18	8	1.57	0.67, 3.70
No	579	414	1.00	Referent
Only In Child's Second Year and After				
Yes	31	22	0.90	0.50, 1.60
No	566	400	1.00	Referent

OR: Odds ratio; CI: Confidence interval.

a: Women who smoked during pregnancy were excluded when estimating odds ratio;

*Adjusted for maternal race/ethnicity, prenatal vitamin use, payment method, year of birth, child’s gender and the regional center.

Date: August 4th, 2016

Jingran Xiong
Principal Investigator

RE: **Exemption of Human Subjects Research**
IRB00090186
Maternal Smoking during Pregnancy and Autism Spectrum Disorder.

Dear Principal Investigator:

Thank you for submitting an application to the Emory IRB for the above-referenced project. Based on the information you have provided, we have determined on **August 4th, 2016** that although it is human subjects research, it is exempt from further IRB review and approval.

This determination is good indefinitely unless substantive revisions to the study design (e.g., population or type of data to be obtained) occur which alter our analysis. Please consult the Emory IRB for clarification in case of such a change. Exempt projects do not require continuing renewal applications.

This project meets the criteria for exemption under 45 CFR 46.101(b)(4). Specifically, you will be receiving de-identified data from another institution and only the other institution will have access to the link to identifiable information.

- Maternal Smoking during Pregnancy and Autism Disorder Spectrum Protocol

Please note that the Belmont Report principles apply to this research: respect for persons, beneficence, and justice. You should use the informed consent materials reviewed by the IRB unless a waiver of consent was granted. Similarly, if HIPAA applies to this project, you should use the HIPAA patient authorization and revocation materials reviewed by the IRB unless a waiver was granted. CITI certification is required of all personnel conducting this research.

Unanticipated problems involving risk to subjects or others or violations of the HIPAA Privacy Rule must be reported promptly to the Emory IRB and the sponsoring agency (if any).

In future correspondence about this matter, please refer to the study ID shown above. Thank you.

Sincerely,

Emilie Scheffer
IRB Analyst Assistant
This letter has been digitally signed