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RESEARCH ARTICLE

A biomarker-based study of prenatal smoking exposure and autism in a Finnish national birth cohort

Keely Cheslack-Postava[1] | **Andre Sourander**[2,3,4] | **Susanna Hinkka-Yli-Salomäki**[2] | **Ian W. McKeague**[5] | **Heljä-Marja Surcel**[6,7] | **Alan S. Brown**[1,8]

[1]Department of Psychiatry, New York State Psychiatric Institute, Columbia University Irving Medical Center, New York, New York, USA

[2]Department of Child Psychiatry, Research Centre for Child Psychiatry, Institute of Clinical Medicine, Faculty of Medicine, University of Turku, Turku, Finland

[3]Department of Child Psychiatry, Turku University Hospital, Turku, Finland

[4]INVEST Research Flagship, University of Turku, Turku, Finland

[5]Department of Biostatistics, Columbia University Mailman School of Public Health, New York, New York, USA

[6]Faculty of Medicine, University of Oulu, Oulu, Finland

[7]Biobank Borealis of Northern Finland, Oulu University Hospital, Oulu, Finland

[8]Department of Epidemiology, Columbia University Mailman School of Public Health, New York, New York, USA

Correspondence
Keely Cheslack-Postava, Department of Psychiatry, New York State Psychiatric Institute, 1051 Riverside Drive Unit 23, New York, NY 10032, USA.
Email: kc2497@cumc.columbia.edu

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Abstract

Maternal exposure to tobacco smoke during pregnancy is a common and persistent exposure linked to adverse neurodevelopmental outcomes in the offspring. However, previous studies provide mixed evidence regarding the relationship between prenatal smoking and offspring autism. This study used cotinine level, a biomarker for nicotine, to investigate the relationship between prenatal smoking and autism. The authors conducted a population-based case–control study nested in a national cohort of all births in Finland from 1987 to 2005. Cases diagnosed with childhood autism (ICD-10/9 code F84.0/299.0) through 2007 were identified using data from linked national registers. Each case was matched with a control on date of birth (±30 days), sex, and place of birth ($N = 962$ pairs). Maternal serum cotinine levels were prospectively measured in first- to early second-trimester serum samples archived in a national biobank using a quantitative immunoassay. Data were analyzed using conditional logistic regression. Prenatal maternal levels of serum cotinine were not associated with the odds of autism, whether cotinine was classified continuously, by deciles, or using previously defined categories corresponding to probable maternal smoking status. After adjusting for maternal age, paternal age, previous births, and any history of parental psychiatric disorder, the odds ratio for categorical high versus low cotinine, using a 3-level exposure variable, was 0.98 (95% CI = 0.76, 1.26; $p = 0.88$). In conclusion, this national birth cohort-based study does not provide evidence for an association between maternal cotinine, a biomarker of maternal smoking, and risk of autism.

KEYWORDS
autism, autistic disorder, cotinine, prenatal exposure delayed effects, smoking

INTRODUCTION

Autism spectrum disorder (ASD) is a developmental condition involving impaired social interaction and communication, restricted interests, and repetitive behaviors, which is believed to result from a combination of genetic and prenatal environmental factors (Lyall et al., 2017). Maternal exposure to tobacco smoke during pregnancy may adversely affect the fetus through pathways including low-birth weight and reduced brain growth (Herrmann et al., 2008), and through epigenetic modifications (Joubert et al., 2016). Women can be exposed to tobacco smoke both directly, through maternal smoking, or indirectly, through environmental tobacco smoke (ETS). Despite decades of tobacco control policies, both remain common and persistent exposures (Ekblad et al., 2014; Öberg et al., 2011). In Finland, the prevalence of smoking during early pregnancy was around 15% in 2015, and 36% among women under age 25 (Rumrich et al., 2019).

Maternal prenatal smoking has been consistently linked to neurodevelopmental outcomes including

externalizing behaviors, attention deficit and hyperactivity disorder (ADHD), and cognitive performance (reviewed in [Herrmann et al., 2008]). Recent reports have also linked maternal pregnancy exposure to ETS with offspring neurodevelopment. Maternal self-reported exposure to ETS was associated with decreased mental development index (MDI) scores on the Bayley Scales of Infant Development and increased risk of developmental delay at 6 months (Lee et al., 2011). Cotinine is the primary metabolite of nicotine, and the most widely used biomarker for tobacco smoke exposure (Torres et al., 2018). When maternal urine cotinine concentrations were used to quantify pregnancy exposure to ETS, an inverse association was observed with the MDI at age 24 months (Lee et al., 2019). Another study measuring maternal saliva cotinine showed that decreased language function at age one, and language, cognitive, and motor function at age 2 (Polanska et al., 2017) were associated with higher cotinine levels.

A number of previous studies have examined the association between maternal prenatal smoking and ASD. Some of these investigations (Hultman et al., 2002; Larsson et al., 2009; Ronald et al., 2010; von Ehrenstein et al., 2020) provide evidence for an association between maternal smoking and increased risk of autism or autism related traits in the offspring, though others do not (Bilder et al., 2009; Burstyn et al., 2010; Dodds et al., 2011; Haglund & Källén, 2011; Kalkbrenner et al., 2012, 2020; Larsson et al., 2005; Lee et al., 2012; Maimburg & Væth, 2006; Nilsen et al., 2013; Visser et al., 2013). One previous study from Finland found an association of maternal self-reported smoking during pregnancy with PDD-NOS specifically but not with childhood autism or all ASD combined (Tran et al., 2013).

A shortcoming of these studies is potential exposure misclassification due to reliance on maternal self-report of smoking during pregnancy, which may misclassify over 25% of the mothers who use tobacco in pregnancy as non-users (Ashford et al., 2017). Additionally, self-reported smoking status does not account for ETS exposure or provide an accurate assessment of dose. While the overall estimates from meta-analyses have not shown significant associations between maternal smoking and ASD (Jung et al., 2017; Rosen et al., 2015; Tang et al., 2015), such summary measures may mask important heterogeneity, that is, by geographic population or study methodology (Jung et al., 2017). While one recent study that used Mendelian randomization and an epigenetic score as biological proxy measures for exposure to maternal smoking did not find sufficient evidence to support an association with ASD (Caramaschi et al., 2018), studies using direct biomarkers for maternal exposure to tobacco smoke are lacking. To address this gap, we examined the association between the levels of cotinine measured in prenatal maternal serum samples and diagnosed autism in a population-based sample of cases and matched controls in a national birth cohort from Finland.

METHODS AND MATERIALS

Identification of subjects

Subjects with autism and matched controls were identified through the Finnish Prenatal Study of Autism (FIPS-A), a nested case–control study based on a national birth cohort in Finland from 1987 to 2005, and described previously (Lampi et al., 2011). Childhood autism (International Classification of Diseases [ICD]-10/9 code F84.0/299.0) cases diagnosed through 2007 (age at the end of follow-up, range: 2–20) were identified through record linkage of the Finnish Medical Birth Registry (FMBR) with the Finnish Hospital and Outpatient Discharge Registry (FHDR). Finnish registry diagnoses of childhood autism have been validated using the Autism Diagnostic Interview-Revised, with high specificity (96%; Lampi et al., 2011). Controls from the FMBR without ASDs (ICD-10/9 codes F84/299) or severe/profound intellectual disability (ICD-10/9 codes F72, F73/318.1, 318.2) were matched 4:1 with the cases on the date of birth (±30 days), place of birth, and sex. One control per case was selected randomly for serologic analysis. Of 1131 cases initially identified with childhood autism, the present study included the 962 subjects with autism and 962 matched controls with sufficient archived serum available for the analysis of cotinine (see below).

Cotinine

Cotinine levels were measured from specimens of maternal serum drawn during the first and early second trimesters of pregnancy for prenatal screening purposes and subsequently stored at minus 25°C in a biorepository in Oulu, Finland (the Finnish Maternity Cohort, FMC). These samples are obtained from 98% of pregnant women in Finland beginning in 1983. The cotinine levels in the archived maternal serum samples from selected cases and controls were measured using a commercially available quantitative immunoassay kit (OraSure Technologies, Bethlehem, Pa.; sensitivity = 96–97%, specificity = 99–100%). Intra- and inter-assay variation are 3.5–6.2% and 6.0–9.6%, respectively. The limit of detection was 0.08 ng/ml, sufficiently sensitive to detect most levels falling within the range of environmental tobacco exposure (estimated at 0.015 to 14.6 ng/ml [Torres et al., 2018]). Researchers conducting cotinine measurements were blinded to case/control status.

Cotinine was examined as a continuous and as a categorical measure. Given the skewed distribution of cotinine levels, the continuous variable was natural log-transformed before analysis. Maternal cotinine was

categorized as a three-level variable: reference (<20 ng/ml), moderate exposure (20–50 ng/ml), and heavy exposure (>50 ng/ml). These cut-off points were recommended by the cotinine immunoassay kit and have also been used in previous studies based on the Finnish Maternity Cohort serum bank (Niemelä et al., 2016). Finally, to further explore potential non-linear relationships or evidence for association within the lower end of the range of exposure, maternal cotinine was categorized by deciles of the control distribution.

Covariates

Covariates were included in the analyses in order to address potential confounding, mediation, and to examine potential heterogeneity of associations. Information on maternal age, parity, occupational-based socioeconomic status, and self-reported smoking during pregnancy; paternal age; and infant gestational age and birth weight were obtained from the FMBR. Maternal and paternal history of psychiatric diagnoses were obtained from the FHDR and treated dichotomously, based on either parent being positive for any of the following according to the ICD codes listed in the register: schizophrenia/ schizotypal/delusional disorders, affective disorders, pervasive developmental disorders, neurotic/ personality disorders/other nonpsychotic disorders, or alcohol/drug addiction/abuse. Subject diagnoses of intellectual disability (ID) and ADHD were determined using the FHDR. ICD codes used for the classification of maternal and paternal history of psychiatric diagnoses and for subject diagnoses of ID and ADHD are given in Table 1. The gestational age at the blood draw was obtained from the FMC.

Statistical analysis

The frequencies or means and standard deviations of each characteristic shown in Table 1 were calculated for cases and controls and compared using chi-squared or *t*-tests; comparisons were also made by cotinine category among the controls using chi-squared tests or ANOVA. Conditional logistic regression of matched case–control pairs was used to determine the association of maternal cotinine levels with autism. Initial models were unadjusted. To address potential confounding, subsequent models were adjusted for covariates associated with autism and cotinine category ($p < 0.1$) and not in potential causal pathways (maternal age, paternal age, previous births, and any history of parental psychiatric disorder). A sensitivity analysis further adjusted for all covariates initially considered as potential confounders (those noted above, plus maternal socioeconomic status and gestational age at blood draw) among those observations with information available. Infant gestational age and birthweight were considered potential mediators and added to adjusted models separately.

Additional analyses were conducted to assess the sensitivity of the findings to specific analytic decisions. These included: (1) restricting analyses to observations with at least 5 years of follow-up (birth years 2002 and earlier); (2) using a lower cut-point (<10 ng/ml) to define the reference group for the categorical cotinine variable; (3) due to the high percentage of missing data for maternal socioeconomic status (SES), an analysis was conducted restricted to observations with information available for this variable; (4) an unconditional logistic regression model, additionally adjusting for subject sex, birth year, and hospital district (proxy for place of birth, which was not available in the analytic data set); (5) omitting the adjustment for parental psychiatric disorder as a basis for comparison with the parental psychiatric disorder adjusted model; (6) to incorporate information on maternal self-report of smoking during pregnancy, we examined the association of maternal self-reported (yes/no) smoking during pregnancy, and of a combined (high cotinine or maternal self-reported smoking vs. moderate cotinine and no maternal self-reported smoking vs. low cotinine and no maternal self-reported smoking) categorical variable, with autism case–control status; (7) to allow for different relationships between maternal cotinine level and autism within ranges typical of exposure through the environmental versus smoking, we fit an adjusted model including linear terms for the natural log of cotinine with a spline at 15 ng/ml (Torres et al., 2018). Values below the limit of detection were set to one-half the limit, and a dichotomous indicator was included to test the impact of falling below versus above this limit.

Covariate-adjusted models were also fit after stratifying by co-morbid intellectual disability and ADHD in the cases and by subject sex. The magnitudes of the odds ratios were then compared between strata using tests of interaction for estimates obtained from independent samples such as population subgroups (Altman & Bland, 2003). In order to obtain stratum-specific estimates of the association of autism with maternal cotinine levels by parental history of psychiatric disorder and tests of interaction between these two variables on the multiplicative scale, maternal cotinine X parental psychiatric history product terms were added to adjusted models, which included all observations. All analyses were conducted using SAS statistical software (SAS Version 9.4; SAS Institute, Inc., Cary, NC). The study was approved by the Ministry of Social Affairs and Health of Finland and by the Institutional Review Board of the New York State Psychiatric Institute.

RESULTS

The study sample was comprised of 79% males and 21% females. The characteristics of the autism cases and the

TABLE 1 Characteristics of subjects with childhood autism and matched controls, among Finnish births between 1987 and 2005

	Cases		Controls		
	$N = 962$		$n = 962$		
Characteristic	**Mean**	***SD***	**Mean**	***SD***	***p*-Value[a]**
Maternal age	30.16	(5.37)	29.20	(5.30)	<0.0001
Paternal age[b]	32.95	(6.46)	31.61	(6.06)	<0.0001
Gestational week of blood draw[b]	11.04	(3.61)	10.83	(3.37)	0.21
	n	%	*n*	%	*p*-Value[a]
Maternal parity > = 1[b]	637	(66.4)	552	(57.7)	<0.0001
Maternal smoking during pregnancy (self-report)[b]	160	(17.4)	155	(16.6)	0.66
Maternal SES[b]					
Upper white collar	110	(14.1)	117	(15.4)	0.90
Lower white collar	354	(45.4)	343	(45.3)	
Blue collar	169	(21.7)	161	(21.2)	
Others	146	(18.7)	137	(18.1)	
Parental history of psychiatric disorders[c]	246	(25.6)	187	(19.4)	0.001
Gestational age <37 weeks[b]	57	(6.0)	42	(4.4)	0.12
Birth weight <2500g[b]	45	(4.7)	22	(2.3)	0.004
Intellectual disability[d]	274	(28.5)	3	(0.3)	<0.0001
ADHD[e]	53	(5.5)	10	(1.0)	<0.0001

Abbreviation: ADHD, attention deficit and hyperactivity disorder.
[a]*p*-Values from chi-squared tests (categorical variables) or *t*-tests (continuous variables). Fisher's exact test was used for categorical variables with cell counts <5.
[b]Information was missing for paternal age ($n = 22$), gestational week of blood draw ($n = 103$), maternal parity ($n = 8$), maternal self-reported smoking during pregnancy ($n = 69$), maternal SES ($n = 387$), gestational age ($n = 10$), and birth weight ($n = 6$).
[c]ICD-10 codes F10-F19, F20-F25, F28-F29, F30-F34, F38-F39, F40-F45; F48; F50-53; F55; F59-66; F68-69, F84, F99.
[d]ICD-10/9 codes F70/317, F71/318.0, F72/318.1, F73/318.2, F78 (no ICD-9 code), and F79/319.
[e]ICD-10/9 codes F90/314.

matched controls are presented in Table 1. Relative to controls, subjects with autism were born to older mothers and fathers, were significantly more likely to be non-first-born, to have a parental history of psychiatric disorder, and to be born at low birthweight. Twenty-eight-point 5% of autism cases had co-morbid intellectual disability, and 5.5% had ADHD diagnoses versus 0.3 and 1.0% among controls, respectively. The mean gestational age at the blood draw was similar for cases (11.0 weeks, $SD = 3.6$) and for controls (10.8 weeks, $SD = 3.4$; $p = 0.21$).

Among the controls, parental history of psychiatric disorders, lower maternal SES, and gestational age <37 weeks were associated with maternal cotinine exposure (see Table S1). Mean maternal and paternal ages were highest among the reference group, and lowest among those with moderate maternal cotinine levels. Few (3.7%) mothers from the reference cotinine category reported smoking during pregnancy, while 65.2% of those from the moderate cotinine group, and 74.7% of those from the high cotinine group did so.

Comparisons of prenatal maternal cotinine levels between autism cases and controls are shown in Table 2. These levels were similar between cases and controls, whether cotinine was considered continuously, categorically, or by deciles. Neither unadjusted models nor models adjusted for potential confounders showed evidence for association between autism and maternal cotinine levels. Results of sensitivity analysis (see above) are shown in Table S2. Models also adjusted for maternal SES and gestational age at blood draw did not show any significant associations between maternal cotinine levels and autism, with the exception of a protective effect for log of cotinine (OR [95% CI] = 0.95 [0.90, 0.99]; $p = 0.03$); however, based on estimates for cotinine considered by deciles, there was no clear evidence for a dose–response relationship. Further adjusting for preterm birth and/or low birth weight had little impact on results (not shown). Results were consistent, and did not show evidence for an association between autism and maternal cotinine, when restricting to subjects with at least 5 years of follow-up, when excluding observations with missing information for maternal SES, when omitting adjustment for parental psychopathology, or when using unconditional logistic regression.

Results using alternative measures of exposure are shown in Table S3. Self-reported maternal smoking was not associated with autism in this sample (OR [95% CI] = 1.02 [0.79, 1.32]; $p = 0.88$), nor was a measure that combined evidence for maternal smoking based on both measured cotinine and maternal self-report. Cotinine exposure categories remained unassociated with autism

TABLE 2 Association of maternal cotinine and childhood autism among Finnish births between 1987 and 2005

Measure	Cases $N = 962$		Controls $N = 962$		Unadjusted			Adjusted[a]		
					OR	95% CI	*p*-Value	OR	95% CI	*p*-Value
	Median	IQR	Median	IQR						
Cotinine level (ng/ml, continuous)	1.23	0.15, 2.28	1.26	0.16, 2.26	1.00	(1.00, 1.00)	0.44	1.00	(1.00, 1.00)	0.89
ln(cotinine)	0.21	−1.93, 0.82	0.23	−1.83, 0.82	0.99	(0.96, 1.03)	0.68	0.98	(0.94, 1.02)	0.41
Categorical measure of cotinine	*n*	%	*n*	%						
Low (<20 ng/ml)	785	81.6%	785	81.6%	1.00	Ref	–	1.00	Ref	–
Moderate (20–50 ng/ml)	16	1.7%	23	2.4%	0.69	(0.36, 1.32)	0.26	0.73	(0.37, 1.45)	0.37
Heavy (>50 ng/ml)	161	16.7%	154	16.0%	1.05	(0.82, 1.33)	0.70	0.98	(0.76, 1.26)	0.88
Deciles of cotinine	*n*	%	*n*	%						
<10% (Ref: <0.07 ng/ml)	95	9.9%	96	10.0%	1.00	Ref	–	1.00	Ref	–
10–19% (0.07–0.11 ng/ml)	109	11.3%	96	10.0%	1.16	(0.75, 1.78)	0.46	1.25	(0.80, 1.95)	0.33
20–29% (0.11–0.64 ng/ml)	110	11.4%	96	10.0%	1.18	(0.76, 1.83)	0.59	1.23	(0.78, 1.94)	0.37
30–39% (0.64–1.11 ng/ml)	96	10.0%	97	10.1%	0.88	(0.54, 1.42)	0.55	0.84	(0.50, 1.39)	0.49
40–49% (1.11–1.26 ng/ml)	95	9.9%	96	10.0%	0.86	(0.53, 1.41)	0.25	0.88	(0.52, 1.47)	0.62
50–59% (1.26–1.45 ng/ml)	84	8.7%	96	10.0%	0.74	(0.45,1.23)	0.23	0.70	(0.41, 1.18)	0.18
60–59% (1.45–1.83 ng/ml)	85	8.8%	97	10.1%	0.74	(0.45, 1.21)	0.57	0.72	(0.43, 1.21)	0.21
70%–79% (1.83–8.82 ng/ml)	95	9.9%	95	9.9%	0.87	(0.53, 1.42)	0.39	0.89	(0.53, 1.50)	0.67
80%–89% (8.82–103 ng/ml)	82	8.5%	96	10.0%	0.83	(0.54, 1.28)	0.92	0.88	(0.56,1.39)	0.58
≥90% (>103 ng/ml)	111	11.5%	97	10.1%	1.02	(0.66, 1.60)	0.50	0.93	(0.58, 1.49)	0.77

[a]Adjusted for maternal age, paternal age, previous births, and any history of parental psychiatric disorder.

when using a lower cut-off of <10 ng/ml to define the reference category. Natural log of the cotinine level was not linearly associated with autism in either the range below (OR [95% CI] = 1.08 [0.74, 1.58]; $p = 0.71$) or above (OR [95% CI] = 0.91 [0.61, 1.34]; $p = 0.62$) 15 ng/ml. Stratifying by intellectual disability in the case, subject sex, or parental history of psychiatric disorder showed no statistical evidence for heterogeneity of the autism-cotinine association across strata, and did not show any associations of autism with cotinine in individual strata (Table 3). When we stratified by record of ADHD diagnosis in the case, there was a borderline significant association between heavy cotinine exposure and autism with ADHD (adjusted OR [95% CI] = 4.5 [0.9–24; $p = 0.07$]) but not autism without ADHD (adjusted OR = 0.93 [0.72, 1.20]; $p = 0.57$; *p*-value for interaction = 0.07).

DISCUSSION

In the largest study using prenatal cotinine levels to quantify maternal nicotine exposure in relation to autism in a population-based national sample, we did not observe evidence for an association. Prenatal maternal levels of serum cotinine were not associated with an increased odds of autism, whether cotinine was classified continuously (with or without the inclusion of a spline to differentiate ranges typical of active versus environmental tobacco exposure), by deciles, or using previously defined categories corresponding to probable maternal smoking status. We found marginal evidence for an association between high maternal cotinine and autism diagnosis in the offspring, when restricting the diagnosis to autism cases who had also been diagnosed with ADHD; however, this should be interpreted cautiously given the limited size of the stratum.

The finding that prenatal maternal cotinine was not associated with autism in the offspring is consistent with a number of prior studies which also did not support an association between maternal prenatal smoking and ASD. These included population-based cohorts (Burstyn et al., 2010; Dodds et al., 2011; Haglund & Källén, 2011; Kalkbrenner et al., 2012, 2020) and case–control studies (Bilder et al., 2009; Larsson et al., 2005; Lee et al., 2012; Maimburg & Væth, 2006), clinically based case–control studies (Visser et al., 2013), prospective cohorts (Caramaschi et al., 2018; Nilsen et al., 2013) and meta-analyses (Jung et al., 2017; Rosen et al., 2015; Tang et al., 2015) from a range of geographic locations in North America and Europe. Except for one that used Mendelian randomization and an epigenetic measure (Caramaschi et al., 2018), however, these studies relied on self-reported measures of maternal smoking for exposure assessment. That our study arrived at similar results instead using prenatal maternal cotinine as a

TABLE 3 Association of maternal cotinine and childhood autism, stratified by the presence of intellectual disability (ID) or ADHD in the case, among Finnish births between 1987 and 2005

	OR[a]	95% CI	*p*-Value	OR[a]	95% CI	*p*-Value	*p*-Value for interaction
	With ID in the case ($N = 274$ cases, 274 controls)			**Without ID in the case ($N = 688$ cases, 688 controls)**			
Cotinine as continuous							
ln(cotinine)	0.95	(0.88, 1.03)	0.19	1.00	(0.95, 1.04)	0.85	0.24
Cotinine as categorical							
Ref (<20 ng/ml)	1.00	Ref	–	1.00	Ref	–	
Moderate (20–50 ng/ml)	0.85	(0.23, 3.14)	0.81	0.63	(0.28, 1.41)	0.26	0.37
Heavy (>50 ng/ml)	0.89	(0.56, 1.42)	0.63	1.03	(0.76, 1.39)	0.87	0.35
	With ADHD in the case ($N = 53$ cases, 53 controls)			Without ADHD in the case ($N = 909$ cases, 909 controls)			
Cotinine as continuous							
ln(cotinine)	1.19	(0.97, 1.46)	0.11	0.97	(0.93, 1.01)	0.19	0.07
Cotinine as categorical							
Ref (<20 ng/ml)	1.00	Ref	–	1.00	Ref	–	
Moderate (20–50 ng/ml)	1.68	(0.20, 13.85)	0.63	0.70	(0.34, 1.44)	0.33	0.30
Heavy (>50 ng/ml)	4.54	(0.87, 23.88)	0.07	0.93	(0.72, 1.20)	0.57	0.07
	Male ($N = 760$ cases, 760 controls)			Female ($N = 202$ cases, 202 controls)			
Cotinine as continuous							
ln(cotinine)	0.97	(0.93, 1.02)	0.25	1.03	(0.95, 1.13)	0.48	0.21
Cotinine as categorical							
Ref (<20 ng/ml)	1.00	Ref	–	1.00	Ref	–	
Moderate (20–50 ng/ml)	0.72	(0.34, 1.54)	0.40	1.03	(0.22, 4.88)	0.97	0.37
Heavy (>50 ng/ml)	0.96	(0.72, 1.27)	0.76	1.15	(0.65, 2.04)	0.63	0.34
	With history of parental psychiatric disorder[b] ($N = 57$ cases, 51 controls)			No history of parental psychiatric disorder ($N = 716$ cases, 775 controls)			
Cotinine as continuous							
ln(cotinine)	0.94	(0.88, 1.02)		1.00	(0.95, 1.05)	0.90	0.22
Cotinine as categorical							
Ref (<20 ng/ml)	1.00	Ref	–	1.00	Ref	–	
Moderate (20–50 ng/ml)	1.13	(0.34, 3.74)	0.85	0.58	(0.25, 1.37)	0.21	0.38
Heavy (>50 ng/ml)	0.76	(0.48, 1.20)	0.24	1.10	(0.81, 1.50)	0.55	0.20

[a]Adjusted for maternal age, paternal age, previous births, and any history of parental psychiatric disorder.
[b]ICD-10 codes F10-F19, F20-F25, F28-F29, F30-F34, F38-F39, F40-F45; F48; F50-53; F55; F59-66; F68-69, F84, F99.

biomarker of nicotine exposure supports the findings of these prior studies based on a measure with reduced exposure misclassification. A recent California study that examined prenatal cotinine levels in a population with low smoking prevalence (<4%) did not find evidence that these levels were associated with offspring autism (Berger et al., 2021). This comports with our findings of no association between maternal cotinine levels and offspring autism within the range of levels consistent with ETS exposure.

Conversely, some studies have demonstrated associations of prenatal smoking with offspring ASD, although none used a biomarker of smoking. A recent birth cohort study from California reported a statistically significant association suggesting nearly a 60% increased odds of autistic disorder with heavy maternal smoking (defined as 20 or more cigarettes per day in any trimester of pregnancy, as recorded in the birth record; von Ehrenstein et al., 2020). Based on 924 pairs, 28% of which were discordant, for comparison of the heavy cotinine exposure versus reference groups, assuming alpha = 0.05 and a two-sided test, our study had 96% power to detect an OR of 1.6, thus this difference between findings cannot readily be attributed to a relative lack of power by our study.

Explanations for the difference in findings may include our ability to adjust for parental psychiatric disorders. Given the elevated prevalence of smoking among individuals with psychiatric disorders (Prochaska et al., 2017) and the strong familial co-aggregation between autism and other psychiatric illnesses (Jokiranta et al., 2013), this represents an important potential confounder. A comparison of the OR estimates for high

maternal cotinine from our primary adjusted model omitting (aOR = 1.03) versus including (aOR = 0.98) adjustment for parental psychiatric disorders suggests that indeed this was a positive confounder; however, we note that both ORs were close to 1.0 and the 95% CIs included 1.0. The California study also reported an elevated odds ratio for autism associated with heavy maternal smoking in a sibling comparison analysis, which addresses confounding by shared familial factors, such as maternal psychiatric disorders. Although the OR was 1.97, the 95% confidence interval was wide (0.53, 7.38) and included 1.0 (von Ehrenstein et al., 2020), meaning that the association did not reach statistical significance.

It also may be that there is true population heterogeneity in the characteristics of ascertained cases between the two populations. For instance, in our study, there was limited evidence for a stronger association among those with ADHD diagnoses in the case, but this characteristic was not examined in the California study, so the possible impact on their results is not known. Finally, variation in patterns of confounding between populations may result in heterogeneity of the estimates of association for relationships that are explained primarily by confounding (Sellers et al., 2020). The prevalence of self-reported maternal smoking during pregnancy among controls was 16.6% in our study versus only 2.4% in the California study, which suggests such population differences in the exposure.

Other prior studies that reported positive associations of maternal smoking with ASD were limited to much smaller numbers of cases (Hultman et al., 2002; Larsson et al., 2009; Ronald et al., 2010). A previous study also based in the FiPS, and including an overlapping sample reported an association of maternal self-reported smoking during pregnancy with PDD-NOS but not childhood autism (Tran et al., 2013). In the present study, we examined only childhood autism, and our findings using a biomarker of exposure suggest that the lack of an association in this case group by Tran et al. was not due to exposure misclassification.

Our lack of association between prenatal maternal cotinine levels and autism suggests that prenatal smoking, at least during the first to early second trimester of pregnancy is not a risk factor for autism. The absence of a dose–response association, or increase in risk associated with moderate or lower-decile levels of maternal cotinine, suggests that ETS exposure is not a risk factor for autism either, since a biomarker of maternal smoking in contrast to self-report provides the capacity to assess ETS. While smoking and the use of tobacco products during pregnancy is not recommended due to negative maternal and fetal impacts including psychiatric outcomes (Niemelä et al., 2016; Sourander et al., 2019), this may provide some reassurance to families of children exposed to prenatal maternal smoking or ETS at least with regard to risk of autism.

The relationship that we observed between the birth register variables for maternal smoking during pregnancy and the classification based on maternal cotinine levels underscores the importance of using biomarkers. These measures were highly associated, with 74.7% of controls in the heavy cotinine exposure group reporting maternal smoking during pregnancy, versus only 3.7% of the reference cotinine group. This also indicates, however, that over one quarter of those classified as having heavy exposure based on maternal cotinine levels would have been classified as unexposed based on self-reported maternal smoking, a proportion which is consistent with previously reported misclassification rates (Ashford et al., 2017). Our use of a biomarker, in contrast to the previous studies using maternal self-report, therefore should have reduced bias due to misclassifications as well as allowing for evaluation of the dose–response relationship across the full range of exposure.

While we did not observe an association between maternal cotinine levels and autism overall, there was a greater than fourfold increased odds of autism with ADHD associated with heavy cotinine exposure, which approached statistical significance. While this finding should be interpreted with caution given the limited size of the stratum, we have previously documented an association between higher prenatal maternal cotinine and risk of ADHD in a separate population-based case–control study in the FiPS (Sourander et al., 2019). An estimated 25–30% of people with autism have co-morbid ADHD (Hossain et al., 2020) and shared genetic risk loci for the disorders have been identified in genome-wide analyses (Smoller et al., 2013). This suggests that the marginal association we observed with maternal cotinine may be due to ADHD as opposed to autism in the cases. On the other hand, given that distinct neuropsychological characteristics have been described in ASD with co-morbid ADHD versus ASD alone (Colombi & Ghaziuddin, 2017), the possibility that maternal smoking increases the risk for a distinct subtype of autism co-morbid with ADHD cannot be ruled out.

In addition to the use of a biomarker of prenatal exposure, the strengths of this study include a large, well-powered sample, based on a national birth cohort, and information from national comprehensive registries. Critically, the information available includes additional diagnoses (ID, ADHD) in cases with autism to address phenotypic heterogeneity, and on psychiatric disorders in the parents, allowing for adjustment of potential confounding. Additionally, the diagnosis examined was previously validated with high specificity and was given in the context of a national health system with universal coverage, reducing the probability of selection bias.

Limitations of the data include exposure assessment based on one time point during the first to early second trimester of pregnancy. As the half-life of cotinine in

serum as well as other bio-samples is generally estimated at <24 h (Centers for Disease Control and Prevention & U.S. Department of Health and Human Services, 2006), this may have misclassified as unexposed some women with only intermittent exposure. However, misclassification is likely to be reduced relative to maternal self-report. We examined only childhood autism as an outcome and did not include other diagnoses considered part of ASD. While this narrower phenotype may have an advantage given reduced heterogeneity, it may affect the comparability with other studies that used broader measures of ASD. As with any observational study, we cannot rule out the presence of confounding by an unmeasured factor, and the power of sub-group analyses, such as autism with ADHD, was limited by small sample sizes.

In conclusion, this national birth cohort study does not provide evidence for an association between maternal cotinine, a biomarker of maternal smoking and ETS, and risk of autism. Our negative findings using a biomarker suggest that the lack of association observed in various previous studies was not attributable to bias due to misclassification. Given the number and range of other studies, including meta-analyses, that also did not find associations of maternal smoking with risk of autism, the preponderance of evidence does not support a relationship between maternal smoking, at least during the early to middle stages of pregnancy, and autism in the offspring. We observed some suggestion of an association between heavy maternal cotinine exposure and with ADHD, although our analysis of this sub-group had limited statistical power and the association was not significant. Future studies focused on this specific sub-group may be warranted. Of particular interest would be study designs with detailed case phenotyping and those that address potential confounding by maternal characteristics including genetic risk for autism and ADHD.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Keely Cheslack-Postava https://orcid.org/0000-0001-7945-407X

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