American Journal of Epidemiology

Vol. 190, No. 5
DOI: 10.1093/aje/kwaa182
Advance Access publication:
August 24, 2020

Original Contribution

Maternal Prenatal Smoking and Autism Spectrum Disorder in Offspring: A California Statewide Cohort and Sibling Study

Ondine S. von Ehrenstein*, Xin Cui, Qi Yan, Hilary Aralis, and Beate Ritz

* Correspondence to Dr. Ondine S. von Ehrenstein, Fielding School of Public Health, University of California, Los Angeles, P.O. Box 951772, Los Angeles, CA 90095-1772 (e-mail: ovehren@ucla.edu).

Initially submitted January 2, 2019; accepted for publication June 3, 2020.

We examined associations between maternal smoking and autism spectrum disorder (ASD) in children in a statewide population-based cohort and sibling-comparison design using California birth records ($n = 2{,}015{,}104$) with information on maternal smoking, demographic factors, and pregnancy (2007–2010). ASD cases ($n = 11{,}722$) were identified through California Department of Developmental Services records with diagnoses based on the *Diagnostic and Statistical Manual of Mental Disorders*–IV-TR. We estimated odds ratios for ASD with and without intellectual disability in the full cohort using logistic regression and in a sibling comparison using conditional logistic regression. In the full cohort, the adjusted odds ratio for ASD and maternal smoking 3 months before/during pregnancy compared with nonsmoking was 1.15 (95% confidence interval (CI): 1.04, 1.26), and it was similar in cases with (odds ratio = 1.12, 95% CI: 0.84, 1.49) and without intellectual disability (odds ratio = 1.15, 95% CI: 1.04, 1.27). Heavy prenatal smoking (≥20 cigarettes/day in any trimester) was related to an odds ratio of 1.55 (95% CI: 1.21, 1.98). In the sibling comparison, the odds ratio for heavy smoking was similarly elevated but the confidence interval was wide. Our findings are consistent with an increased risk for ASD in offspring of mothers who smoked ≥20 cigarettes/day during pregnancy; associations with lighter smoking were weaker.

autism spectrum disorder; cohort study; maternal smoking; sibling comparison

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; DDS, Department of Developmental Services; SES, socioeconomic status.

Editor's note: *An invited commentary on this article appears on page 738, and the authors' response appears on page 742.*

Autism spectrum disorder (ASD) is a severe and heterogeneous developmental disorder characterized by atypical socialization, restricted interests, and repetitive behavior. Prenatal maternal smoking has been suspected to be a risk factor for ASD but previous studies have resulted in equivocal findings. Several studies found no associations (1), while others suggested small to moderate increases in risk for ASD (2–4) related to maternal smoking. A recent meta-analysis reported a pooled odds ratio of 1.02 (95% confidence interval (CI): 0.93, 1.12); however, there was large heterogeneity in study designs, covariate adjustments, classification of exposure, case definitions, and effect estimate sizes (5). A number of studies reported associations between ASD and prenatal air pollution exposures that contain many combustion-derived toxics also found in cigarette smoke (6–8). Biological mechanisms relating maternal smoking to ASD include mitochondrial dysfunction, epigenetic changes, (9, 10) gene expression (11), and fetal testosterone concentrations (12). Additionally, maternal smoking has been related to impaired fetal brain development (13, 14), adverse behavioral outcomes (15), attention deficit hyperactivity disorder (16), and neuropsychological function related to inhibitory control (17).

Research has established adverse impacts of maternal smoking on fetal brain development, including impairments in cognitive function (18). Several biological mechanisms that are thought to affect brain function and morphology (13, 19), including inflammatory and epigenetic mechanisms (20, 21), might also be involved in the pathology of ASD (22). However, previous studies have not considered

whether ASD phenotypes with intellectual disability (which is considered a marker of severity of impairment in ASD) are differentially affected or particularly susceptible to maternal prenatal smoking. There is ongoing debate as to whether associations between maternal smoking and child neurobehavioral outcomes might be confounded by shared familial and genetic factors. Women who smoke in pregnancy might be affected by neuropsychological conditions affecting smoking behavior and might carry genetic traits potentially associated with neuropsychiatric outcomes in offspring, including autism (23–27). Therefore, to examine associations between maternal smoking in pregnancy and child's diagnosis of ASD, we conducted a California-statewide population-based cohort study of all births, and we embedded a sibling comparison aiming to assess the role of shared familial confounding factors. We consider comorbid intellectual disability diagnosis, which is a marker of ASD severity and developmental impairment.

METHODS

We used a cohort registry linkage design including all births during 2007–2010 in California; data were retrieved from Office of Vital Statistics birth rolls. ASD cases were identified using records maintained at the California Department of Developmental Services (DDS) and collected by 21 regional centers through December 31, 2013. We included only cases with a primary diagnosis of "autistic disorder" based on the *Diagnostic and Statistical Manual of Mental Disorders* (code 299.00 in DSM IV-TR—the standard diagnostic instrument until December 31, 2013) (28), as reported on the DDS Client Development Evaluation Report. Validation studies have established the reliability and validity of the Client Development Evaluation Report in California (29). Eligibility for DDS services does not depend on citizenship or financial status (i.e., services are available to all children). We linked DDS case records to California birth records using a probabilistic linkage based on child and parental identifiers (e.g., first/last name, birth date, and sex) to estimate the probability/likelihood that 2 records are for the same person, assigning total scores for a linkage as the sum of scores generated from matching individual fields using the National Program of Cancer Registries Link Plus Software (30) (linkage rate: 86.3%). We manually checked pairs with borderline scores; nonlinkage was mainly due to missing information on records. We excluded 455 case and 82,100 noncase records with missing or implausible/nonviable gestational ages (included: 147–322 days) or birth weights (included: 500–7,000 g); after restricting to singleton births, this yielded a final sample of 11,722 cases and 2,003,382 noncases. Among these, siblings (born 2007–2010) were identified using a similar approach based on child and maternal identifiers (mandatory) and paternal when available, to estimate the probability/likelihood that 2 birth records relate to the same mother/parents.

We classified ASD cases based on DDS evaluation records by "intellectual disability" status (recorded as "mental retardation" at the time of study, diagnosed according to *Diagnostic and Statistical Manual of Mental Disorders–IV* criteria corresponding to *International Classification of Diseases, Ninth Revision*) applying the last evaluation record for each child. We determined age at diagnosis in the CDER; the later recorded diagnosis date was used for 44 cases with 2 recorded dates.

This research was approved by the University of California, Los Angeles, Office of the Human Research Protection Program and the California Committee for the Protection of Human Subjects, and was exempted from informed consent requirements because there was no contact with human subjects.

Smoking exposure and pregnancy variables

Information on pregnancy characteristics such as gestational age, birth weight, and pregnancy complications, as well as sociodemographic data, were retrieved from birth records. Smoking information was recorded (since 2007) on California birth certificates as number of cigarettes smoked per day 3 months prior to pregnancy and in each trimester.

Statistical analysis

Odds ratios and 95% confidence intervals were estimated for maternal smoking and ASD using logistic regression. Smoking was assessed according to developmental period. All models adjusted for year of birth and sex, and additionally for potential confounders selected based on prior knowledge (31–33), including maternal age, education, race/ethnicity, parity, and pregnancy complications (definitions displayed in Table 1). We conducted sensitivity analyses adjusting for additional covariates, including preterm birth, birthweight, type of insurance (as proxy for socioeconomic status (SES) (34)), maternal birth place (United States/non–United States), and DDS Regional Center catchment area (in a subsample); because none of these variables changed the estimates of interest by >5%, they were not retained in final models. Further we stratified by ASD with and without intellectual disability comorbidity to examine phenotypes by severity. We also conducted analyses restricted to term births and to diagnosis received at or before age 3 years, and not adjusting for pregnancy complications.

To examine the role of confounding by unmeasured shared familial factors, we implemented a sibling comparison restricting to the group of cases with siblings ("case families"). That is, from the original full cohort, we selected all ASD cases with a sibling ($n = 2{,}705$) and the nonaffected siblings of ASD cases (born 2007–2010; $n = 2{,}639$); these subjects were considered to belong to the same families (case families, $n = 2{,}611$) (Figure 1). Conditional logistic regression clustering on maternal identifier as the family indicator for sets of siblings was applied. We conducted sensitivity analyses to compare the findings from the case-family only analyses with those using all siblings (Figure 1).

If associations seen in the full cohort persist in the sibling comparison subcohort, this supports the hypothesis that maternal smoking in pregnancy increases offspring's ASD risk even after controlling for family-specific factors. To the extent that associations are attenuated, at least 2 explanations

Table 1. Basic Characteristics in Autism Spectrum Disorder Cases and Noncases in the Full Cohort and Case-Sibling Cohort[a] Births, California, 2007–2010

Characteristic	Full Cohort				Sibling Cohort[a]			
	Case ($n = 11,722$)		Noncase ($n = 2,003,382$)		Case ($n = 2,705$)		Noncase ($n = 2,639$)	
	No.	%	No.	%	No.	%	No.	%
Sex								
Male	8,720	74.4	1,024,163	51.1	1,987	73.5	1,282	48.6
Female	3,002	25.6	979,206	48.9	718	26.5	1,357	51.4
Missing	0	0.0	13	0.0	0	0.0	0	0.0
Birth year								
2007	3,066	26.2	523,897	26.2	717	26.5	734	27.8
2008	3,394	29.0	511,530	25.5	753	27.8	533	20.2
2009	3,061	26.1	490,423	24.5	683	25.2	569	21.6
2010	2,201	18.8	477,532	23.8	552	20.4	803	30.4
Maternal age, years								
≤18	416	3.5	113,262	5.7	106	3.9	107	4.1
19–25	3,360	28.7	615,843	30.7	943	34.9	873	33.1
26–30	3,161	27.0	543,822	27.1	759	28.1	772	29.3
31–35	2,889	24.6	456,801	22.8	589	21.8	585	22.2
>35	1,896	16.2	273,620	13.7	308	11.4	302	11.4
Missing	0	0.0	34	0.0	0	0.0	0	0.0
Maternal race/ethnicity								
White[b]	3,072	26.2	532,259	26.6	750	27.7	739	28.0
Latina	5,745	49.0	1,043,033	52.1	1,327	49.1	1,277	48.4
African American	784	6.7	104,285	5.2	157	5.8	172	6.5
Asian	1,392	11.9	205,861	10.3	327	12.1	316	12.0
Others	547	4.7	87,221	4.4	110	4.1	106	4.0
Unknown	182	1.6	30,723	1.5	34	1.3	29	1.1
Maternal education[c], years								
≤8	714	6.1	169,649	8.5	134	5.0	133	5.0
9–11	1,702	14.5	336,292	16.8	452	16.7	429	16.3
12	3,054	26.1	518,328	25.9	716	26.5	731	27.7
13–15	3,088	26.3	446,226	22.3	690	25.5	656	24.9
≥16	2,788	23.8	467,872	23.4	635	23.5	617	23.4
Missing	376	3.2	65,015	3.2	78	2.9	73	2.8
Pregnancy complications[d]								
Yes	6,878	58.7	1,178,691	58.8	1,586	58.6	1,535	58.2
No	4,843	41.3	824,440	41.2	1,119	41.4	1,104	41.8
Missing	1	0.0	251	0.0	0	0.0	0	0.0
Smoking[e] before[f] or during pregnancy								
Yes	464	4.0	71,366	3.6	89	3.3	84	3.2
No	11,035	94.1	1,898,355	94.8	2,563	94.8	2,513	95.2
Missing	223	1.9	33,661	1.7	53	2.0	42	1.6

Table continues

Table 1. Continued

Characteristic	Full Cohort				Sibling Cohort[a]			
	Case ($n = 11,722$)		Noncase ($n = 2,003,382$)		Case ($n = 2,705$)		Noncase ($n = 2,639$)	
	No.	%	No.	%	No.	%	No.	%
Smoking[e] during pregnancy								
Yes	301	2.6	48,110	2.4	64	2.4	58	2.2
No	11,217	95.7	1,923,831	96.0	2,591	95.8	2,543	96.4
Missing	204	1.7	31,441	1.6	50	1.8	38	1.4
No. of cigarettes/day[g]								
Prepregnancy	10 (5–20)		10 (4–20)		10 (4–20)		10 (4–20)	
First trimester	10 (4–12)		6 (3–10)		8 (3–15)		6 (3–10)	
Second trimester	6 (3–10)		5 (3–10)		5 (3–10)		5 (3–10)	
Third trimester	5 (3–10)		5 (3–10)		5 (3–10)		5 (3–10)	

[a] Includes all siblings with at least 1 case among the siblings.
[b] Non-Hispanic White.
[c] Years of education numbering 12, 13–15, and ≥16 corresponded to high-school graduate, some degrees less than college, and college or more than college.
[d] Pregnancy complications included any conditions recorded on birth certificates.
[e] Smoking defined as 1 or more cigarettes/day.
[f] Prepregnancy defined as 3 months prior to pregnancy.
[g] Among smokers, reported as median, and interquartile range.

should be considered; first, the full cohort associations did at least in part result from familial confounding; second, nondifferential measurement error of the exposure (i.e., random misclassification of maternal smoking on the birth record). While the sibling comparison should adjust for shared familial factors by design (35), the approach has several limitations; it relies on within-family discordance, is prone to exposure misclassification and to unmeasured nonfamilial confounding, and usually has less power and generalizability (35, 36). Analyses were conducted using SAS, version 9.4 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Characteristics of the full cohort, and of the case-sibling subcohort, are displayed by case status in Table 1 and by smoking status in Web Table 1 (available at https://doi.org/10.1093/aje/kwaa182). Case mothers were on average older than noncase mothers, more educated, and less frequently identified as Latina/Hispanic compared with mothers of noncases in the full cohort (Table 1). These demographic factors were similar for cases with and without a sibling born during the study period (Web Table 2). Among cases with a sibling, demographic characteristics were similar comparing autism cases with and without intellectual disability comorbidity (Web Table 3). Demographic characteristics of the "all siblings" noncases and the full cohort or the case-sibling controls were also similar (Web Table 4).

In the full cohort, we estimated an adjusted odds ratio for ASD in relation to smoking 3 months before or during pregnancy ("ever smokers") of 1.15 (95% CI: 1.04, 1.26) compared with nonsmoking mothers. Odds ratios for smoking only prior to pregnancy, and prior and during pregnancy ("continuous smokers"), were similarly elevated (Table 2). The point estimates for maternal smoking and ASD in the sibling comparison were generally similar but less stable, and the 95% confidence intervals were wide due to the relatively small number of discordant sibling pairs (Table 2).

For heavier smokers (≥20 cigarettes/day), we estimated elevated odds ratios in the full cohort and in the sibling comparison. Smoking 20 or more cigarettes/day during any trimester period during pregnancy was associated with greater odds (odds ratio = 1.55, 95% CI: 1.21, 1.98) for developing the disorder in the full cohort. The estimated association related to 1–19 cigarettes/day was weaker (Table 2).

Stratifying by intellectual disability comorbidity for ever-smoking, estimated odds ratios were similar for cases with intellectual disability (odds ratio = 1.12, 95% CI: 0.84, 1.49) and without (odds ratio = 1.15, 95% CI: 1.04, 1.27) in the full cohort (Table 3). In the sibling comparisons, numbers in some cells were too small for models to converge (among "with intellectual disability") and generally 95% confidence intervals were wide (Table 3).

In sensitivity analyses, restricting to term births or to cases diagnosed by age 3 years, or not adjusting for pregnancy complications, did not change our estimated associations appreciably (data not shown). We also evaluated the entire

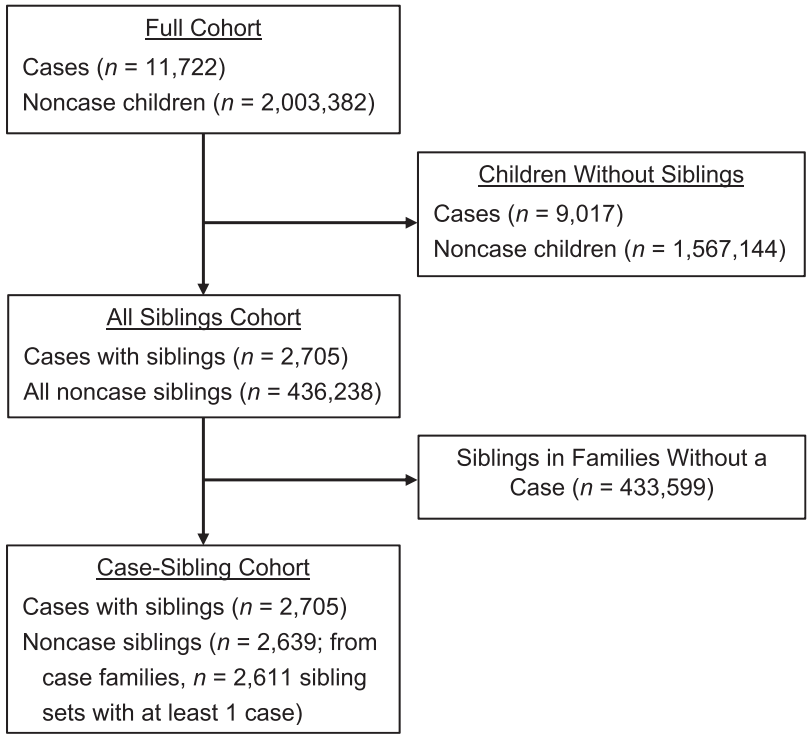

Figure 1. Flow chart of sample derivation for the maternal smoking and autism spectrum disorder study, among births in California, 2007–2010.

sample of siblings within the full cohort. Compared with case-family siblings, estimated odds ratios based on all siblings (by design not a "sibling comparison") were generally similar; all confidence intervals were wide and overlapped (Web Table 5).

DISCUSSION

To our knowledge, this study is the first in the United States to examine maternal smoking during pregnancy in relation to ASD in offspring, considering intellectual disability comorbidity, in a statewide cohort involving all births. Additionally, we used a sibling-comparison design aimed at assessing the potential role of familial confounding. In the full cohort, with over 11,000 cases, we estimated a small to moderate increase in risk for ASD related to maternal prenatal smoking (defined as at least 1 cigarette/day), with more strongly elevated odds ratios related to smoking $\geq$20 cigarettes/day than for smoking <20 cigarettes/day. Associations were similar for cases with and without intellectual disability. In the sibling comparison, point estimates were quite similar, but confidence intervals were wide due to the small number of discordant siblings. The findings based on our full cohort involving over 2,000,000 births are consistent with the conclusion that heavy maternal smoking increases risk for ASD in offspring. There was little indication of differences in associations with smoking by phenotype (with or without intellectual disability). While the estimates using the sibling-comparison design are generally consistent with findings from the full cohort, the small number of discordant siblings resulted in imprecise estimations, thus limiting our ability to evaluate family-based confounding; if there is a maternal characteristic that determines both pregnancy smoking behavior and offspring autism risk, we cannot rule out this potential confounding.

Earlier studies examining ASD and maternal smoking were inconclusive and have limitations, including heterogeneous disease definitions (including milder cases) and diagnostic changes during long study periods that might have affected results (1, 3, 37, 38). Phenotype severity was rarely assessed, and sibling comparisons have also not been considered. Smoking was usually recorded as "yes/no" and not as number of cigarettes/day and by trimester. Of the earlier studies, most were Scandinavian registry-based studies using cohort designs that examined ASD related to maternal smoking recorded on birth records (1, 37, 38), similar to our study design. Two studies—from Finland and Denmark—reported no associations (37, 38). In Sweden, birth registry data (1984–2003), linked with multisource identification of ASD, suggested associations with smoking similar to our cohort findings, but after adjusting for SES-related variables associations became null in the Swedish study (1). We observed little change in our estimated odds ratios and confidence intervals when we included different indicators of SES measures, after adjustment for birth year and sex, maternal age, and parity. In another Swedish study with a nested case-control design (1987–1994) and infantile autism

Table 2. Maternal Smoking and Autism Spectrum Disorder in the Full Cohort and Case-Sibling-Cohort[a] Births, California, 2007–2010

Smoking Status	Full Cohort				Sibling Cohort[a]			
	No. of Cases	No. of Noncases	OR[b]	95% CI	No. of Cases	No. of Noncases	OR[b]	95% CI
Never smokers	11,035	1,898,355	1.00	Referent	2,563	2,513	1.00	Referent
Ever smokers[c]	464	71,366	1.15	1.04, 1.26	89	84	1.03	0.64, 1.68
Prepregnancy smokers[d]	172	23,952	1.21	1.04, 1.41	26	27	0.75	0.36, 1.58
Continued smokers[e]	282	45,718	1.11	0.98, 1.26	61	56	1.40	0.71, 2.73
During pregnancy[f], no. of cigarettes/day								
1–19	233	40,007	1.05	0.92, 1.20	52	50	1.30	0.66, 2.54
≥20	68	8,103	1.55	1.21, 1.98	12	8	1.97	0.53, 7.38
Pregnancy Periods								
Prepregnancy[g], no. of cigarettes/day								
1–19	321	51,863	1.08	0.96, 1.21	60	63	0.97	0.58, 1.64
≥20	133	17,907	1.36	1.14, 1.62	27	20	1.52	0.64, 3.59
First trimester, no. of cigarettes/day								
1–19	229	38,849	1.06	0.93, 1.21	52	49	1.29	0.66, 2.54
≥20	65	7,614	1.58	1.23, 2.03	10	8	1.60	0.46, 5.56
Second trimester, no. of cigarettes/day								
1–19	173	30,538	1.04	0.89, 1.22	38	41	0.92	0.43, 2.00
≥20	30	3,731	1.46	1.00, 2.13	6	6	1.06	0.24, 4.76
Third trimester, no. of cigarettes/day								
1–19	161	28,697	1.04	0.89, 1.22	36	34	1.02	0.44, 2.39
≥20	25	3,066	1.52	1.02, 2.28	7	5	1.21	0.21, 7.00

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Includes all siblings with at least 1 case among the siblings.
[b] Adjusted for birth year, sex, maternal age, maternal race/ethnicity, maternal education, parity, and pregnancy complications.
[c] Smoked 3 months before pregnancy or any time during pregnancy.
[d] Smoked 3 months before pregnancy but quit smoking during pregnancy.
[e] Smoked 3 months before pregnancy and kept smoking during pregnancy.
[f] Smoking was defined as maximum number of cigarettes/day during any trimester.
[g] Prepregnancy was defined as 3 months prior to pregnancy.

as the outcome (*International Classification of Diseases, Ninth Revision*, code 299A), the authors adjusted for pregnancy, maternal, and SES-related variables, and reported an odds ratio of 1.4 (95% CI: 1.1, 1.8) for "daily smoking" (3), similar to our findings. Two recent meta-analyses, including 15 studies, reported a pooled odds ratio for autism related to prenatal maternal smoking of 1.02 (95% CI: 0.93, 1.12) (5, 39). However, heterogeneity between studies was large, and calculating a pooled estimate might not have been appropriate. A systematic review including a subset of 7 studies that controlled for SES also suggested no overall increase in risk (40). In 2 of the studies included, a slightly increased odds ratio for ASD without intellectual disability but no increase for ASD with intellectual disability was reported. However, the diagnosis included milder cases on the autism spectrum (2), thus the case definitions are not directly comparable to ours. Previous research is equivocal with regard to maternal smoking and intellectual disability in offspring, with some studies suggesting impairment following heavy maternal smoking in pregnancy (41, 42) but other studies failing to corroborate this (43). We lack data to examine associations with intellectual disability separately.

Table 3. Maternal Smoking and Autism Spectrum Disorder According to Intellectual Disability Status in the Full Cohort and the Case-Sibling-Cohort[a] Births 2007–2010, California.

Smoking Status	With ID				Without ID			
	No. of Cases	No. of Noncases	OR[b]	95% CI	No. of Cases	No. of Noncases	OR[b]	95% CI
Full cohort								
Never smokers	1,144	1,898,355	1.00	Referent	9,891	1,898,355	1.00	Referent
Ever smokers[c]	51	71,366	1.12	0.84, 1.49	413	71,366	1.15	1.04, 1.27
Prepregnancy smokers[d]	13	23,952	0.81	0.46, 1.43	159	23,952	1.26	1.07, 1.48
Continued smokers[e]	37	45,718	1.29	0.92, 1.81	245	45,718	1.09	0.95, 1.24
During pregnancy[f], no. of cigarettes/day								
1–19	32	40,007	1.27	0.89, 1.82	201	40,007	1.02	0.88, 1.18
≥20	7	8,103	1.42	0.67, 3.01	61	8,103	1.57	1.21, 2.03
Sibling cohort[a]								
Never smokers	259	259	1.00	Referent	2,304	2,256	1.00	Referent
Ever smokers[c]	13	10	4.62	1.00, 21.44	76	74	0.82	0.50, 1.34
Prepregnancy smokers[d]	2	2	3.04	0.38, 24.52	24	25	0.87	0.43, 1.77
Continued smokers[e]	11	7	–[g]	–[g]	50	49	0.79	0.43, 1.46
During pregnancy[f], no. of cigarettes/day								
1–19	7	7	3.87	0.33, 46.10	45	43	0.83	0.45, 1.55
≥20	4	1	–[g]	–[g]	8	7	0.89	0.65, 1.53

Abbreviations: CI, confidence interval; ID, intellectual disability; OR, odds ratio.
[a] Includes all siblings with at least 1 case among the siblings.
[b] Adjusted for birth year, sex, maternal age, maternal race/ethnicity, maternal education, parity, and pregnancy complication.
[c] Smoked 3 months before pregnancy or any time during pregnancy.
[d] Smoked 3 months before pregnancy but stopped smoking during pregnancy.
[e] Smoked 3 months before pregnancy and kept smoking during pregnancy.
[f] Smoking was defined as maximum number of cigarettes/day during any trimester.
[g] Models did not converge due to distribution of n resulting in small n in some cells.

As a comorbidity with ASD, our findings suggest that both ASD phenotypes, with and without intellectual disability comorbidity, might be affected by maternal smoking.

There is biological evidence to support in utero impacts of maternal smoking on autism phenotypes (44). Interestingly, maternal smoking–related epigenetic changes in offspring were found at the same sites for smoking and more general impairment of child developmental (45), which points to possible epigenetic mechanisms that connect smoking and ASD. Effects of maternal smoking were shown on trophoblast-related processes suggesting dysregulation of nicotine receptors and metalloproteinase activity (46) and on umbilical-cord-tissue gene expression related to immune development (11), mechanisms suggested to be relevant to autism development (47–49). In addition, young adults exposed in utero to maternal smoking showed altered activity in certain brain regions (17), and prenatal smoking exposure was associated with changes of the precentral and superior frontal cortices, which are related to affective problems (50). Further support comes from animal data indicating behavioral changes in offspring (13), decreased myelin gene expression (51), attenuated γ-aminobutyric acid release (52), and decreased brain-derived neurotrophic factor/tyrosine kinase receptor B (53) after in utero exposure to maternal smoking. Overall, there is biological evidence supporting associations between maternal smoking and ASD, and future translational research should help to link epidemiologic and experimental data.

Several limitations need to be considered when interpreting our study findings. Even though we included all

births occurring over 4 years statewide in California (over 2,000,000), our data is limited for implementing sibling comparisons to assess the role of family-level confounding. Smoking in pregnancy is now a rather rare behavior among Californian women, and autism is a rare outcome. Also, the sibling design has less statistical power and requires a relatively large number of discordant pairs; thus, sample size limitations did not allow us to assess the role of familial confounding as intended. While it remains important to consider shared familial factors, which might influence both smoking behavior of the mother and autism in offspring (as suggested, e.g., for hyperactivity (23, 54)), the sibling-comparison design has several weaknesses, including the inability to assess nonfamilial confounding. As demonstrated by Frisell et al. (35), within–sibling pair estimates will not be confounded by factors perfectly shared by siblings, but the estimates might be more severely biased due to nonshared confounders than estimates from an unpaired design. In addition, within-sibling exposure-effect estimates might be more attenuated than nonsibling pair–based estimates due to nondifferential exposure misclassification (35). Among discordant pairs there is also possible bias ("carryover") if the outcome (autism) of the first birth influences exposure (smoking) or outcome of the subsequent birth (55). If smoking in an earlier pregnancy had long-term impacts that are related to the risk of autism in subsequent pregnancies, this would also result in bias (55). However, no such long-term biological mechanisms are known. In our full cohort, we addressed potential nonfamilial confounders by controlling for known and suspected confounders and by conducting a number of sensitivity analyses, which did not appreciably influence the estimated associations between maternal smoking and offspring autism. We cannot, however, rule out family-level confounding.

Further limitations include the self-reported nature of maternal smoking on birth certificates. However, the specificity of self-reported prenatal smoking was shown to be high in validation studies comparing pregnancy self-report with cotinine measurement (56), and reports on birth certificates have been confirmed by data from other sources (57). Moreover, the percentages of smokers on California birth certificates, in the California Maternal and Infant Health Survey (58), and in our own survey data, are similar (34, 59). However, we are still likely misclassifying some smokers who are underreporting smoking behavior in pregnancy, and differential misclassification cannot be ruled out. Women with high SES who are $\geq$30 years of age when giving birth are more likely to underreport prenatal smoking (60), which would lead to overall underestimation of risk, given that mothers of cases tend to be older than mothers of noncases. We do not have information on intellectual disability unrelated to ASD, but future research addressing this outcome would be informative. It is hard to examine a "most critical exposure period" for any smoking-related associations because those who smoke later in pregnancy also smoked already in the first trimester. Strengths of our study include the registry-based design including all births (over 2,000,000) in California in the study period, which prevents participation bias due to self-selection. Importantly, mothers' report of smoking was recorded at the time of birth—thus, before a diagnosis of autism—and all cases were diagnosed according to the same criteria (using *Diagnostic and Statistical Manual of Mental Disorders*–IV).

In conclusion, there is a long, ongoing debate on how to assess causal inference between maternal prenatal smoking and offspring neuropsychiatric health, and triangulation via sibling design approaches as we attempted herein has been suggested (27, 35, 36, 61). A critical limitation of this approach in population-based studies is that very large data sets are required with detailed information about maternal smoking behavior to ensure sufficient numbers of discordant siblings, which is a challenge for rare neurodevelopmental conditions such as autism. Overall our full population cohort findings support the hypothesis that rather heavy maternal smoking is associated with greater odds for ASD and suggests weaker impacts for lighter smoking.

ACKNOWLEDGMENTS

Author affiliations: Department of Community Health Sciences, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, California, United States (Ondine S. von Ehrenstein); Department of Epidemiology, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, California, United States (Ondine S. von Ehrenstein, Xin Cui, Qi Yan, Beate Ritz); Department of Biostatistics, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, California, United States (Hilary Aralis); Department of Neurology, Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, United States (Beate Ritz); and Department of Environmental Health, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, California, United States (Beate Ritz).

This work was partially funded by the National Institute of Environmental Health Sciences (grants R21ES022389 and R21ES025558).

Conflict of interest: none declared.

REFERENCES

1. 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.
2. 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.
3. Hultman CM, Sparén P, Cnattingius S. Perinatal risk factors for infantile autism. *Epidemiology*. 2002;13(4):417–423.
4. Larsson HJ, Eaton WW, Madsen KM, et al. Risk factors for autism: perinatal factors, parental psychiatric history, and socioeconomic status. *Am J Epidemiol*. 2005;161(10):916–925.

5. Rosen BN, Lee BK, Lee NL, et al. Maternal smoking and autism Spectrum disorder: a meta-analysis. *J Autism Dev Disord*. 2015;45(6):1689–1698.
6. Becerra TA, Wilhelm M, Olsen J, et al. Ambient air pollution and autism in Los Angeles county, California. *Environ Health Perspect*. 2013;121(3):380–386.
7. von Ehrenstein OS, Aralis H, Cockburn M, et al. In utero exposure to toxic air pollutants and risk of childhood autism. *Epidemiology*. 2014;25(6):851–858.
8. Raz R, Roberts AL, Lyall K, et al. Autism spectrum disorder and particulate matter air pollution before, during, and after pregnancy: a nested case-control analysis within the Nurses' Health Study II Cohort. *Environ Health Perspect*. 2015; 123(3):264–270.
9. Maccani JZ, Koestler DC, Houseman EA, et al. Placental DNA methylation alterations associated with maternal tobacco smoking at the *RUNX3* gene are also associated with gestational age. *Epigenomics*. 2013;5(6): 619–630.
10. Nielsen CH, Larsen A, Nielsen AL. DNA methylation alterations in response to prenatal exposure of maternal cigarette smoking: a persistent epigenetic impact on health from maternal lifestyle? *Arch Toxicol*. 2016;90(2): 231–245.
11. Hussain N, Krueger W, Covault J, et al. Effects of prenatal tobacco exposure on gene expression profiling in umbilical cord tissue. *Pediatr Res*. 2008;64(2):147–153.
12. Rizwan S, Manning JT, Brabin BJ. Maternal smoking during pregnancy and possible effects of in utero testosterone: evidence from the 2D:4D finger length ratio. *Early Hum Dev*. 2007;83(2):87–90.
13. Ekblad M, Korkeila J, Lehtonen L. Smoking during pregnancy affects foetal brain development. *Acta Paediatr*. 2015;104(1):12–18.
14. Habek D, Kovačević M. Adverse pregnancy outcomes and long-term morbidity after early fetal hypokinesia in maternal smoking pregnancies. *Arch Gynecol Obstet*. 2011;283(3): 491–495.
15. Indredavik MS, Brubakk AM, Romundstad P, et al. Prenatal smoking exposure and psychiatric symptoms in adolescence. *Acta Paediatr*. 2007;96(3):377–382.
16. Kovess V, Keyes KM, Hamilton A, et al. Maternal smoking and offspring inattention and hyperactivity: results from a cross-national European survey. *Eur Child Adolesc Psychiatry*. 2015;24(8):919–929.
17. Holz NE, Boecker R, Baumeister S, et al. Effect of prenatal exposure to tobacco smoke on inhibitory control: neuroimaging results from a 25-year prospective study. *JAMA Psychiat*. 2014;71(7):786–796.
18. Clifford A, Lang L, Chen R. Effects of maternal cigarette smoking during pregnancy on cognitive parameters of children and young adults: a literature review. *Neurotoxicol Teratol*. 2012;34(6):560–570.
19. El Marroun H, Schmidt MN, Franken IH, et al. Prenatal tobacco exposure and brain morphology: a prospective study in young children. *Neuropsychopharmacology*. 2014;39(4): 792–800.
20. de Vocht F, Simpkin AJ, Richmond RC, et al. Assessment of offspring DNA methylation across the lifecourse associated with prenatal maternal smoking using Bayesian mixture Modelling. *Int J Environ Res Public Health*. 2015;12(11): 14461–14476.
21. Breton CV, Byun HM, Wenten M, et al. Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation. *Am J Respir Crit Care Med*. 2009;180(5): 462–467.
22. Carter CJ, Blizard RA. Autism genes are selectively targeted by environmental pollutants including pesticides, heavy metals, bisphenol A, phthalates and many others in food, cosmetics or household products. *Neurochem Int*. 2016; S0197-0186(16):30197–30198.
23. Laucht M, Hohm E, Esser G, et al. Association between ADHD and smoking in adolescence: shared genetic, environmental and psychopathological factors. *J Neural Transm (Vienna)*. 2007;114(8):1097–1104.
24. Obel C, Zhu JL, Olsen J, et al. The risk of attention deficit hyperactivity disorder in children exposed to maternal smoking during pregnancy—a re-examination using a sibling design. *J Child Psychol Psychiatry*. 2016;57(4):532–537.
25. Thapar A, Harold G, Rice F, et al. Do intrauterine or genetic influences explain the foetal origins of chronic disease? A novel experimental method for disentangling effects. *BMC Med Res Methodol*. 2007;7:25.
26. Yerushalmy J, Palmer CE. On the methodology of investigations of etiologic factors in chronic diseases. *J Chronic Dis*. 1959;10(1):27–40.
27. Yerushalmy J. The relationship of parents' cigarette smoking to outcome of pregnancy—implications as to the problem of inferring causation from observed associations. *Am J Epidemiol*. 1971;93(6):443–456.
28. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision*. 4th ed. Washington, DC: American Psychiatric Association; 2000.
29. Hertz-Picciotto I, Croen LA, Hansen R, et al. The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism. *Environ Health Perspect*. 2006;114(7):1119–1125.
30. Centers for Disesae Control and Prevention, Atlanta, Georgia. *National Program of Cancer Registries. Registry Plus Software Programs for Cancer Registries, Registry Plus™ Link Plus*. https://www.cdc.gov/cancer/npcr/tools/registryplus/lp.htm. Accessed January 15, 2019.
31. Becerra TA, von Ehrenstein OS, Heck JE, et al. Autism spectrum disorders and race, ethnicity, and nativity: a population-based study. *Pediatrics*. 2014;134(1):e63–e71.
32. Burstyn I, Sithole F, Zwaigenbaum L. Autism spectrum disorders, maternal characteristics and obstetric complications among singletons born in Alberta. *Canada Dis Can*. 2010;30(4):125–134.
33. Windham GC, Anderson MC, Croen LA, et al. Birth prevalence of autism spectrum disorders in the San Francisco Bay area by demographic and ascertainment source characteristics. *J Autism Dev Disord*. 2011;41(10): 1362–1372.
34. Ritz B, Wilhelm M, Hoggatt KJ, et al. Ambient air pollution and preterm birth in the environment and pregnancy outcomes study at the University of California, Los Angeles. *Am J Epidemiol*. 2007;166(9):1045–1052.
35. Frisell T, Öberg S, Kuja-Halkola R, et al. Sibling comparison designs: bias from non-shared confounders and measurement error. *Epidemiology*. 2012;23(5):713–720.
36. Keyes KM, Smith GD, Susser E. Commentary: smoking in pregnancy and offspring health: early insights into family-based and 'negative control' studies? *Int J Epidemiol*. 2014;43(5):1381–1388.
37. Maimburg RD, Vaeth M. Perinatal risk factors and infantile autism. *Acta Psychiatr Scand*. 2006;114(4):257–264.
38. 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.

39. Tang S, Wang Y, Gong X, et al. A meta-analysis of maternal smoking during pregnancy and autism spectrum disorder risk in offspring. *Int J Environ Res Public Health*. 2015;12(9): 10418–10431.
40. Kalkbrenner AE, Schmidt RJ, Penlesky AC. Environmental chemical exposures and autism spectrum disorders: a review of the epidemiological evidence. *Curr Probl Pediatr Adolesc Health Care*. 2014;44(10):277–318.
41. Drews CD, Murphy CC, Yeargin-Allsopp M, et al. The relationship between idiopathic mental retardation and maternal smoking during pregnancy. *Pediatrics*. 1996;97(4): 547–553.
42. Braun JM, Daniels JL, Kalkbrenner A, et al. The effect of maternal smoking during pregnancy on intellectual disabilities among 8-year-old children. *Paediatr Perinat Epidemiol*. 2009;23(5):482–491.
43. Yang S, Decker A, Kramer MS. Exposure to parental smoking and child growth and development: a cohort study. *BMC Pediatr*. 2013;13:104.
44. Berko ER, Greally JM. How might epigenetic dysregulation in early embryonic life contribute to autism spectrum disorder? *Epigenomics*. 2015;7(1):1–4.
45. Xu CJ, Bonder MJ, Söderhäll C, et al. The emerging landscape of dynamic DNA methylation in early childhood. *BMC Genomics*. 2017;18(1):25.
46. Holloway AC, Salomon A, Soares MJ, et al. Characterization of the adverse effects of nicotine on placental development: in vivo and in vitro studies. *Am J Physiol Endocrinol Metab*. 2014;306(4):E443–E456.
47. Abdallah MW, Michel TM. Matrix metalloproteinases in autism spectrum disorders. *J Mol Psychiatry*. 2013;1(1):16.
48. Vafadari B, Salamian A, Kaczmarek L. MMP-9 in translation: from molecule to brain physiology, pathology, and therapy. *J Neurochem*. 2015;139(suppl 2):91–114.
49. Estes ML, McAllister AK. Immune mediators in the brain and peripheral tissues in autism spectrum disorder. *Nat Rev Neurosci*. 2015;16(8):469–486.
50. Basil P, Li Q, Dempster EL, et al. Prenatal maternal immune activation causes epigenetic differences in adolescent mouse brain. *Transl Psychiatry*. 2014;4(9):e434.
51. Cao J, Dwyer JB, Gautier NM, et al. Central myelin gene expression during postnatal development in rats exposed to nicotine gestationally. *Neurosci Lett*. 2013;553:115–120.
52. Damborsky JC, Griffith WH, Winzer-Serhan UH. Neonatal nicotine exposure increases excitatory synaptic transmission and attenuates nicotine-stimulated GABA release in the adult rat hippocampus. *Neuropharmacology*. 2015;88:187–198.
53. Xiao L, Kish VL, Benders KM, et al. Prenatal and early postnatal exposure to cigarette smoke decreases BDNF/TrkB signaling and increases abnormal behaviors later in life. *Int J Neuropsychopharmacol*. 2016;19(5):pyv117.
54. Agrawal A, Scherrer JF, Grant JD, et al. The effects of maternal smoking during pregnancy on offspring outcomes. *Prev Med*. 2010;50(1–2):13–18.
55. Sjölander A, Frisell T, Kuja-Halkola R, et al. Carryover effects in sibling comparison designs. *Epidemiology*. 2016; 27(6):852–858.
56. Kvalvik LG, Nilsen RM, Skjærven R, et al. Self-reported smoking status and plasma cotinine concentrations among pregnant women in the Norwegian Mother and Child Cohort study. *Pediatr Res*. 2012;72(1):101–107.
57. Ventura SJ, Hamilton BE, Mathews TJ, et al. Trends and variations in smoking during pregnancy and low birth weight: evidence from the birth certificate, 1990–2000. *Pediatrics*. 2003;111(5 Pt 2):1176–1180.
58. California Department of Public Health, Sacramento, California. *Maternal and Infant Health Assessment*. https://www.cdph.ca.gov/Programs/CFH/DMCAH/MIHA/Pages/Data-and-Reports.aspx?Name=AnnualReport. Accessed May 15, 2019.
59. von Ehrenstein OS, Wilhelm M, Wang A, et al. Preterm birth and prenatal maternal occupation: the role of Hispanic ethnicity and nativity in a population-based sample in Los Angeles, California. *Am J Public Health*. 2014;104(suppl 1): S65–S72.
60. Land TG, Landau AS, Manning SE, et al. Who underreports smoking on birth records: a Monte Carlo predictive model with validation. *PLoS One*. 2012;7(4):e34853.
61. Pearl J. *Causality: Models, Reasoning, and Inference*. Cambridge, UK: Cambridge University Press; 2000.