HHS Public Access

Author manuscript

Autism Res. Author manuscript; available in PMC 2023 December 27.

Published in final edited form as:

Autism Res. 2021 September ; 14(9): 2017–2026. doi:10.1002/aur.2561.

The association of in utero tobacco smoke exposure, quantified by serum cotinine, and Autism Spectrum Disorder

Kimberly Berger[1], **Michelle Pearl**[2], **Marty Kharrazi**[2], **Ying Li**[3], **Josephine DeGuzman**[3], **Jianwen She**[3], **Paramjit Behniwal**[3], **Kristen Lyall**[4], **Gayle Windham**[2]

[1]Sequoia Foundation, La Jolla, California, USA

[2]Environmental Health Investigations Branch, California Department of Public Health, Richmond, California, USA

[3]Environmental Health Laboratory Branch, California Department of Public Health, Richmond, California, USA

[4]A.J. Drexel Autism Institute, Drexel University, Philadelphia, Pennsylvania, USA

Abstract

Previous studies on in utero exposure to maternal environmental tobacco smoke (ETS) or maternal active smoking and Autism Spectrum Disorder (ASD) have not been entirely consistent, and no studies have examined in utero cotinine concentrations as an exposure classification method. We measured cotinine in stored second trimester maternal serum for 498 ASD cases and 499 controls born in California in 2011–2012. We also obtained self-reported maternal cigarette smoking during and immediately prior to pregnancy, as well as covariate data, from birth records. Using unconditional logistic regression, we found no association between log10 cotinine concentrations and odds for developing ASD among children of non-smokers (aOR: 0.93 [95% CI: 0.69, 1.25] per ng/ml), which represents exposure to ETS, though there may be a possible interaction with race. We found no association between cotinine-defined smoking (≥3.08 ng/ml vs. <3.08 ng/ml) (adjusted odds ratio [aOR]: 0.73 (95% confidence interval [95% CI]: 0.35, 1.54)) or self-reported smoking (aOR: 1.64 [95% CI: 0.65, 4.16]) and ASD. In one of the few studies of ETS and the first with measured cotinine, our results indicate no overall relationship between in utero exposure to tobacco smoke from maternal ETS exposure or active smoking, and development of ASD.

Lay Summary:

This study found that women who smoke or are exposed to tobacco smoke during pregnancy are not more likely to have children with Autism Spectrum Disorder (ASD). This is the first

Correspondence: Kimberly Berger, Sequoia Foundation, La Jolla, CA, USA. kimberly.berger@cdph.ca.gov.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

This study was approved by the California Health and Human Services Agency Committee for the Protection of Human Subjects, and the Vital Statistics Advisory Committee (Protocol #16-04-2529).

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

ASD study to measure a chemical in the mother's blood during pregnancy to identify exposure to tobacco smoke.

Keywords

Autism Spectrum Disorder; cotinine; pregnancy; smoking; tobacco smoking

INTRODUCTION

The causes of increasing Autism Spectrum Disorder (ASD) prevalence are still largely undetermined and are important to elucidate. Factors contributing to the development of ASD appear to be many and varied, and while several genetic components have been identified (Vorstman et al., 2017), environmental exposures may have a substantial impact (Bölte et al., 2019; Lyall et al., 2017). In utero exposures are considered to be particularly relevant as prenatal neurodevelopment impacts later neurological disease, and the fetus is susceptible to the mother's environment (Miguel et al., 2019). Several prenatal environmental exposures have been associated with later development of ASD (Ornoy et al., 2015).

Environmental tobacco smoke (ETS) exposure, also called secondhand smoke or passive smoking, is the inhalation of smoke from either the tip of a lit cigarette or of another person's exhaled smoke. Its constituents overlap those of active smoking, though not entirely (Scherer et al., 1992), and exposure during pregnancy has been associated with a variety of adverse cardiovascular, respiratory, and obstetric health outcomes for mother and child (Gould et al., 2020). ETS exposure may be related to ASD: two case–control studies found increased odds of ASD associated with prenatal self-reported ETS (Liu et al., 2015; Zhang et al., 2010). ETS is an understudied component of total tobacco smoke exposure (Jung et al., 2017), and there are no reliable self-reporting methods. The gold standard method of assessing both ETS and active tobacco smoke exposure is via quantification of cotinine, a biomarker for nicotine exposure (Benowitz, 1996). To our knowledge, only one study has reported on cotinine in relation to ASD: cotinine measured in the urine of 320 Korean elementary school children was positively associated with ASD diagnosis, Autism Spectrum Screening Questionnaire (ASSQ) score, and relevant subscales of the Behavioral Assessment System for Children, second edition (BASC-2) (Kim et al., 2018). This study, however, was unable to measure prenatal cotinine.

Maternal cigarette smoking during pregnancy has been associated with children's neurodevelopmental impairments, including ADHD (Huizink & Mulder, 2006), though literature on ASD risk is somewhat conflicting. Recent meta-analyses on smoking and ASD (Jung et al., 2017; Wang et al., 2017), examining 22 and 9 studies respectively, found null results overall associated with self-reported smoking, as did several individual studies (Bilder et al., 2009; Burstyn et al., 2010; Caramaschi et al., 2018; Dodds et al., 2011; Lee et al., 2012; Lei et al., 2015; Maimburg & Væth, 2006; Mandic-Maravic et al., 2019; Nilsen et al., 2013). Positive associations were found in a few national registries (Hultman et al.,

2002; Nilsen et al., 2013; Tran et al., 2013) and smaller case–control studies (Duan et al., 2014; Jiang et al., 2016; Mrozek-Budzyn et al., 2013; Saunders et al., 2019).

Prenatal nicotine exposure has been shown to have teratogenic effects on developing animal brains through irregular cell differentiation and proliferation, mitotic arrest, and decreased numbers of neural cells (Shea & Steiner, 2008; Slotkin, 2008). Nicotine is largely thought to act on the fetal brain through nicotinic acetylcholine receptors (Dwyer et al., 2009). Additionally, higher plasma levels of heavy metals, also found in cigarette smoke, have been associated with higher risk for having ASD (Ashraf, 2012; Qin et al., 2018). Prenatal exposure to tobacco smoke may also lead to epigenetic modifications (Banik et al., 2017; Ladd-Acosta et al., 2016), including changes associated with neurological functioning in adolescents (Toledo-Rodriguez et al., 2010). Maternal smoking may also indirectly affect prenatal brain development through placental vasoconstriction, reduced umbilical supply, and hypoxia (Pauly & Slotkin, 2008; Shea & Steiner, 2008). Intrapartum hypoxia has been associated with higher risk of developing ASD (Kolevzon et al., 2007).

This is the first study to use a biomarker to further elucidate the potential relationship between prenatal exposure to ETS or active smoking and risk for developing ASD. Taking advantage of an existing case–control study of ASD, we added measurement of cotinine using a sensitive assay in banked prenatal maternal serum samples for mother–child pairs.

METHODS

Study population

The study population was a subset of a parent study on the association between polyunsaturated fatty acids and ASD (Lyall et al., 2021). Participants for this study were drawn from children born in 2011–2012 whose mothers had serum banked through participation in California's Prenatal Screening (PNS) Program. The PNS Program offers blood screening tests to all pregnant women between 15 and 20 weeks gestation in order to detect certain congenital abnormalities (Cunningham & Tompkinson, 1999). The California Biobank Program banks PNS specimens from women residing in Fresno, Madera, Kings, Tulare, Kern, Orange, and San Diego counties. ASD cases were identified through the California Department of Developmental Services (DDS), which provides support services to Californian children with developmental disabilities. DDS serves an estimated 75–80% of Californian children with ASD, encompassing the more severe cases (Croen et al., 2002). Children were defined as cases if they had a DDS record of provided services for ASD at the time of data linkage (February 2017, mean child age at linkage: 5.24 years). Diagnosis of ASD within DDS has been previously validated (Windham et al., 2011). Controls were selected from birth certificate data of children who did not link to DDS client data. California birth certificate data was linked to data from DDS and PNS Program records after excluding infant deaths. Covariate data was obtained primarily from birth records, as well as PNS records. From these linked data sets, 500 cases were randomly selected and frequency matched to 501 controls on birth year, birth month, and sex. Only singleton births were sampled. As four serum samples were unable to be analyzed, the final study sample included 498 cases and 499 controls.

Exposure

Maternal serum samples from the California PNS Program (Cunningham & Tompkinson, 1999) were retrieved from the California Department of Public Health's Biobank Program. Samples were collected in serum separator tubes, refrigerated upon receipt at the Newborn and Prenatal Screening Testing Laboratories, and frozen at −20°C within 14–30 days for Biobank storage. All samples analyzed in the current study went through a previous freeze/thaw cycle for analysis in our parent study and were immediately frozen to −80°C upon receipt at our laboratory until they could be thawed and analyzed for the current study. At the time of sample collection, participants signed consent forms that specified their sample may be used for legitimate research purposes with appropriate Institutional Review Board approval unless they chose to opt out.

100 μl of sample was used for cotinine measurement. Samples were spiked with an isotope labeled internal standard, then precipitated and deconjugated with formic acid. After solid phase extraction, samples were dried and reconstituted with methanol. Liquid chromatography–mass spectrometry with positive electron spray ionization and multiple reaction monitoring was used to quantify cotinine concentrations. The limit of detection (LOD) was 0.015 ng/ml.

Samples were analyzed in eight batches, each containing cases and controls. During sample testing laboratorians were blind to case status. Due to variation in calibration curves and distributions across batches, final cotinine concentrations were determined based on analytic methods to harmonize the batches. As part of method validation, we analyzed 10 samples that were previously analyzed by the Centers for Disease Control and Prevention and found the concentrations determined by the two labs were highly correlated ($R^2 = 0.998$). For quality control we analyzed spiked samples and found the coefficient of variance for our determined concentrations to be ≤20% for low level samples (0.1 ng/ml) and ≤15% for medium (1 ng/ml) and high (10 ng/ml) level samples. As part of method validation, we analyzed 10 samples that were previously analyzed by the Centers for Disease Control and Prevention and found the concentrations determined by the two labs were highly correlated ($R^2 = 0.998$). For quality control we analyzed spiked samples and found the coefficient of variance for our determined concentrations to be ≤20% for low level samples (0.1 ng/ml) and ≤15% for medium (1 ng/ml) and high (10 ng/ml) level samples.

Statistical methods

Concentrations detected below the LOD were assigned the value of LOD/√2, which equals 0.011 ng/ml. We classified mothers as cotinine-defined smokers and cotinine-defined non-smokers dichotomizing cotinine concentrations into below 3.08 ng/ml (including values below the LOD) versus 3.08 ng/ml or above, respectively, based on cut-points from a prior study (Benowitz et al., 2009). The nadir of the bimodal cotinine distribution in this study population fell between 2.28 and 6.03 ng/ml. To examine ETS exposure among non-smokers, we analyzed cotinine concentrations as a continuous variable as well as defined quartiles of concentrations below 3.08, but above the LOD, to be compared with values below the LOD. We further categorized the cotinine-defined smokers into "heavy smokers" (above 50th percentile cotinine value for smokers in the study population [35 ng/ml]) and

"light smokers" groups (at or below 50th percentile). We obtained self-reported maternal smoking status from birth certificates; mothers were asked how many cigarettes they smoked in each trimester, as well as during the 3 months before becoming pregnant. Mothers were defined as self-reported smokers if they indicated smoking cigarettes during any of the assessed time periods.

We used cotinine-defined smoking as the gold-standard to evaluate the sensitivity, specificity, positive predictive value, and negative predictive value of self-reported smoking on birth records among our sample. To evaluate associations with ETS exposure, we examined the defined non-smokers only and conducted unconditional logistic regression with continuous log10 cotinine as well as with quartiles of cotinine concentrations between the LOD and 3.08 ng/ml relative to those below LOD. We also conducted unconditional logistic regression to assess the relationship between ASD and cotinine-defined smoking relative to non-smoking and relative to those below the LOD; cotinine-defined heavy and light smoking relative to non-smokers; and self-reported smoking status relative to self-reported non-smoking status. The continuous analysis was not conducted across all participants because the chemical exposures from ETS are somewhat different than those from smoking (Scherer et al., 1992). Cotinine below 3.08 ng/ml was neither normally nor log-normally distributed but was log10 transformed in continuous models for ease of interpretability due to its skewed distribution. We found no evidence for non-linearity in the relationship between cotinine and ASD. Crude regressions controlled only for matching factors (birth year, birth month, and sex) and adjusted models additionally controlled for the following as determined using a Directed Acyclic Graph: maternal age, maternal education, maternal race/ethnicity, and health insurance type (public vs. private) as a proxy for socioeconomic status (SES). We explored possible interactive effects by child sex and maternal race/ethnicity by including interaction terms for these variables in adjusted models. Due to low numbers of participants in some race/ethnicity groups, we recategorized this variable into: Non-Hispanic white; Hispanic; and Non-Hispanic Asian, Black, and Other.

We also conducted sensitivity analyses excluding the 66 case children with co-occurring intellectual disabilities (ID), defined as DDS records of a composite score under 70 on standardized cognitive and functional tests. In addition, we conducted a post hoc computation of the minimum and maximum detectable odds ratios associated with logged cotinine exposure in the ETS range with our sample size, using an alpha value of 0.05 and setting power at 0.80.

RESULTS

Demographic characteristics of participants can be found in Table 1. Compared to control parents, case parents tended to be older, nulliparous or primiparous, covered by public health insurance, and more likely to have a high school degree or some college. Twelve case mothers (2%) and 10 control mothers (2%) reported smoking any cigarettes during pregnancy or the 3 months prior. The race/ethnicity composition of our sample is representative of the racial/ethnic makeup of all births in the sampled counties during 2011–2012. Of the 515 children whose mothers identified as Hispanic, 410 (80%) of their mothers identified with Mexican heritage, 28 (5%) identified with Central or South

American heritage, and the remaining 77 identified with another Hispanic heritage. Of the 156 children whose mothers identified as Asian, 47 (30%) of their mothers identified with Vietnamese heritage, 34 (22%) identified with Filipino heritage, 19 (12%) identified with Indian heritage, 15 (10%) identified with Chinese heritage, and the remaining 41 identified with another Asian heritage. The distribution of cotinine concentrations can be found in Table 2. Among both cases and controls, 19% of participants had concentrations of cotinine below the LOD and 77% had concentrations in the ETS range (LOD-3.08 ng/ml). Among children of cotinine-defined smokers (4%), cases had higher cotinine values than controls (case median: 53.9 ng/ml, control median: 35.0 ng/ml), though medians were similar among children of cotinine-defined nonsmokers (case median: 0.050 ng/ml, control median: 0.053 ng/ml). See Table S1 for cotinine distributions by race/ethnicity. Black controls had the highest median of cotinine concentrations (0.399 ng/ml), followed by controls (0.129 ng/ml) and cases (0.119 ng/ml) from the Other race/ethnic group, while cases in the Asian group had the lowest concentrations (0.047 ng/ml) followed by Asian controls (0.052 ng/ml) and Hispanic cases (0.052 ng/ml). Self-reported smoking had a sensitivity of 27% and a specificity of 99% relative to cotinine-defined smoking. The positive and negative predictive values were 45% and 97% respectively.

Cotinine was not associated with odds of having ASD in crude or adjusted regression models (Table 3). In Model 1, no association was seen between continuous log10 cotinine and ASD (aOR: 0.93 [95% CI: 0.69, 1.25] per ng/ml) for children of cotinine-defined non-smokers, representing ETS. Quartiles of cotinine between the LOD and 3.08 ng/ml, also representing ETS, were not associated with ASD in Model 2, and effect estimates decreased with increasing concentration. Children of cotinine-defined smokers did not have elevated odds of ASD compared to only those with cotinine below the LOD in Model 2 (aOR: 0.76 [95% CI: 0.34, 1.68]) or compared to those of cotinine-defined non-smokers in Model 3 (adjusted odds ratio [aOR]: 0.73 (95% Confidence Interval [CI]: 0.35, 1.54)). No association was seen with light or heavy smoker groups compared to nonsmokers in Model 4, though the effect estimates suggest a difference between the two smoker groups (light smokers: aOR: 0.38 (95% CI: 0.12, 1.17); heavy smokers: aOR: 1.25 (95% CI: 0.46, 3.45)). Self-identification as a smoker anytime during pregnancy or the 3 months prior to becoming pregnant was also not associated with odds of children having ASD in Model 5 (aOR: 1.64 [95% CI: 0.65, 4.16]).

Models including interaction terms showed no evidence for interaction of any smoke exposure variable with child sex (Table S2). Table 4 shows odds ratios calculated from maternal race interaction terms. Cotinine-defined smoking in the Non-Hispanic Asian, Black, and Other group was associated with lower odds of children having ASD compared to non-smokers (aOR compared to non-smokers: 0.25 [95% CI: 0.06, 0.97], *p*-value for interaction: 0.06), while no associations were seen in other groups (Hispanic OR: 0.96 [95% CI: 0.19, 4.98]; Non-Hispanic white OR: 1.27 [95% CI: 0.43, 3.80]). Similar results were seen when compared to mothers with undetectable cotinine as the referent group.

Results were similar in sensitivity analyses excluding children with co-occurring ID (see Table S3). Our post hoc power calculation indicated our study was powered to find odds

ratios higher than 1.30 for continuous logged cotinine in the ETS range as statistically significant, or lower than 0.77.

DISCUSSION

We found no association between in utero serum cotinine concentrations, examined in a number of ways as a measure of exposure to ETS or active maternal smoking, and odds of developing ASD. We further found no association between maternal self-reported smoking during pregnancy and odds of children developing ASD, although the number of smokers in our study was small. We found an interaction with race/ethnicity that suggests children whose mothers identify as Non-Hispanic Asian, Black, or Other may have lower odds of developing ASD associated with in utero cotinine concentrations than those of other race groups.

We did not see associations with cotinine concentrations reflecting ETS exposure, nor did we see a trend with higher levels, in contrast to two prior case–control studies which found increased odds of ASD associated with prenatal, self-reported ETS exposure (Liu et al., 2015; Zhang et al., 2010). The only prior study to have examined cotinine in relation to ASD found a significant positive cross-sectional association between childhood cotinine concentrations and diagnosis and performance on the ASSQ and BASC-2 (Kim et al., 2018). These studies were conducted in China and Korea, which have a higher rate of smoking and heavier smoking than the U.S. (Ng et al., 2014) and the Korean study reflects a different time window of exposure.

Our null findings for maternal smoking are similar to results from many other studies and meta-analyses (Bilder et al., 2009; Burstyn et al., 2010; Caramaschi et al., 2018; Dodds et al., 2011; Jung et al., 2017; Lee et al., 2012; Lei et al., 2015; Maimburg & Væth, 2006; Mandic-Maravic et al., 2019; Nilsen et al., 2013; Wang et al., 2017), but this is the first study to confirm this by use of a tobacco biomarker. Of the prior studies reporting positive findings (Duan et al., 2014; Hultman et al., 2002; Jiang et al., 2016; Mrozek-Budzyn et al., 2013; Nilsen et al., 2013; Saunders et al., 2019; Tran et al., 2013), only one other study controlled for SES (Tran et al., 2013). Smoking is differential across SES (Hiscock et al., 2012) and thus a relationship between smoking and ASD in these studies may be an indicator of a relationship between SES and ASD. We were able to indirectly control for SES via insurance payment type and maternal education. Prior positive findings in literature may also be confounded by familial patterns: a recent analysis of a Danish cohort showed a positive association in adjusted models only between self-reported maternal smoking during pregnancy and ASD diagnosis that was completely attenuated once models controlled for familial patterns of smoking and developmental disabilities (Kalkbrenner et al., 2020).

We did find an interaction with race/ethnicity, suggesting an inverse association of maternal cotinine-defined smoking and ASD among children whose mothers identify as Non-Hispanic Asian, Black, or Other, though with a very limited sample size. Although this group is mostly comprised of Asian mothers in our study, the association may be driven by the high cotinine concentration among Black controls relative to Black cases. Kalkbrenner et al. (2012) found lower risk of ASD associated with maternal prenatal smoking among U.S.

children of Non-Hispanic Black mothers. Because of our low sample size, we were unable to create smaller categorizations for race/ethnicity, though each category is comprised of a diverse set of people. In this study, of the 273 Hispanic children whose mothers were born outside of the U.S., the highest proportion [247 (90%)] of those mothers were born in Mexico. Of the 122 Asian children whose mothers were born outside of the U.S., the highest proportions of those mothers were born in Vietnam (41 [34%]), the Philippines (24 [20%]), India (15 [12%]), and South Korea (11 [9%]). Heterogeneity in exposure effects could be due to differences in cotinine-containing products used across race/ethnic groups, unmeasured co-exposures, or differences in confounding variables across or within race/ethnic groups. People of different races may also metabolize cotinine at different rates (Signorello et al., 2009). Given limited power discussed below, this finding should be interpreted with caution and further explored in future studies.

An advantage of the current study is our ability to measure serum cotinine, allowing us to evaluate exposure to ETS and cotinine-defined smoking. We found self-reported smoking to have low sensitivity, though high specificity, for cotinine-defined smoking which was also reported in a Canadian study (Burstyn et al., 2009). Further, underreporting of smoking on birth certificates may be differential by demographic factors: mothers with higher educational attainment may be less likely to report their prenatal smoking (Vinikoor et al., 2010) and mothers with low birth weight infants may be less likely to complete the field (Dietz et al., 1998). Assessing associations with a biomarker reduces reliance both on participants' memories of their exposure and willingness to report smoking truthfully. Additionally, our self-reported smoking was collected at birth and is thus not subject to recall bias with respect to ASD. A disadvantage, however, is that cotinine is a biomarker only of nicotine and may reflect exposures other than tobacco smoke, such as nicotine patches. In addition, the cut-off of 3.08 ng/ml that we used to define active smoking may not apply to each individual or race/ethnic group (Benowitz et al., 2009), and therefore introduces potential exposure misclassification. Nicotine and cotinine may also be metabolized differently across participants and may not consistently reflect exposure. Additionally, tobacco smoke contains hundreds of compounds, including metals and particulate matter, that may be more impactful than nicotine with regard to ASD; cotinine can only function as an indirect measure of exposure to these compounds. Finally, we only have one in utero measurement of cotinine which may not represent exposure across the whole of pregnancy or all relevant prenatal critical windows.

There are several additional limitations to the current study. Our sample size did not provide enough power to fully explore differences among race/ethnic groups, and we had very few cotinine-defined or self-reported smokers. Though the main purpose of the study was to evaluate associations with ETS exposure, we did capture fewer active smokers than anticipated. Power calculations indicated our study was powered to find odds ratios higher than 1.30 for continuous logged cotinine in the ETS range as statistically significant, which captures the odds ratio of 1.89 found in Kim et al., 2018, the only other study to relate cotinine to ASD, although cross-sectionally in childhood (Jung et al., 2017; Kim et al., 2018). Future studies assessing active smoking should plan on larger sample sizes. While we did not see evidence of differential association depending on ID status of cases, our conclusions are limited by the small number of ASD cases with co-occurring ID. Future

studies should explore relationships by ASD severity or symptom heterogeneity. DDS does not usually provide services to children with milder forms of ASD and therefore our results may only be generalizable to more severe cases. Additionally, lengthier follow-up may have revealed that some of the children in the control group were later diagnosed with ASD and became DDS clients, though this would be a very small number, unlikely to affect results because developmental disabilities are relatively rare (Zablotsky et al., 2017). It is also possible that control children may have moved out of California before being diagnosed with ASD and would thus not be captured in DDS records, though again we expect this number to be small. We were unable to assess parental mental health; both smoking during pregnancy (Agrawal et al., 2010) and having children with ASD (Totsika et al., 2011) are more likely among women who have mental health issues. Finally, while the methods for linking birth record data to PNS and DDS data are standard methods used by the California Department of Public Health's Biobank and state-wide ASD surveillance, respectively, there may be errors in the linkage process. Linkage methods used were conservative, with nonlinks more likely than false links. As more than 90% of PNS records matched to a birth record, linkage errors are unlikely to explain our observed results.

We found no overall association between in utero serum cotinine concentrations or self-reported smoking during pregnancy and odds for children developing ASD, generally confirming prior studies. This is the first study to examine prenatal cotinine as a marker for maternal smoking or maternal ETS exposure in relation to ASD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This study was funded by the Cigarette & Tobacco Products Surtax Fund. The parent study was funded by the U.S. Army Medical Research Acquisition Activity's Autism Research Program-Idea Development Award AR150143 and National Institutes of Health grants K22 ES026235 and R03 CA211820. The biospecimens and data used in this study were obtained from the California Biobank Program (SIS Biobank Request ID number 656), Section 6555(b), 17CCR. The California Department of Public Health is not responsible for the results or conclusions drawn by the authors of this publication, nor do they represent the official position of the California Department of Public Health.

REFERENCES

Agrawal A, Scherrer JF, Grant JD, Sartor CE, Pergadia ML, Duncan AE, Madden PAF, Haber JR, Jacob T, Bucholz KK, & Xian H (2010). The effects of maternal smoking during pregnancy on offspring outcomes. Preventive Medicine, 50(1–2), 13–18. [PubMed: 20026103]

Ashraf MW (2012). Levels of heavy metals in popular cigarette brands and exposure to these metals via smoking. The Scientific World Journal, 2012. https://www.hindawi.com/journals/tswj/2012/729430/.

Banik A, Kandilya D, Ramya S, Stünkel W, Chong YS, & Dheen ST (2017). Maternal factors that induce epigenetic changes contribute to neurological disorders in offspring. Genes, 8 (6), 150. [PubMed: 28538662]

Benowitz NL (1996). Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiologic Reviews, 18(2), 188–204. [PubMed: 9021312]

Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, & Wang J (2009). Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in

the United States between 1999 and 2004. American Journal of Epidemiology, 169(2), 236–248. [PubMed: 19019851]

Bilder D, Pinborough-Zimmerman J, Miller J, & McMahon W (2009). Prenatal, perinatal, and neonatal factors associated with autism spectrum disorders. Pediatrics, 123(5), 1293–1300. [PubMed: 19403494]

Bölte S, Girdler S, & Marschik PB (2019). The contribution of environmental exposure to the etiology of autism spectrum disorder. Cellular and Molecular Life Sciences, 76(7), 1275–1297. [PubMed: 30570672]

Burstyn I, Kapur N, Shalapay C, Bamforth F, Wild TC, Liu J, & LeGatt D (2009). Evaluation of the accuracy of self-reported smoking in pregnancy when the biomarker level in an active smoker is uncertain. Nicotine & Tobacco Research, 11(6), 670–678. [PubMed: 19395685]

Burstyn I, Sithole F, & Zwaigenbaum L (2010). Autism spectrum disorders, maternal characteristics and obstetric complications among singletons born in Alberta, Canada. Chronic Diseases in Canada, 30(4), 125–134. [PubMed: 20946713]

Caramaschi D, Taylor AE, Richmond RC, Havdahl KA, Golding J, Relton CL, Munafò MR, Smith GD, & Rai D (2018). Maternal smoking during pregnancy and autism: Using causal inference methods in a birth cohort study. Translational Psychiatry, 8(1), 1–10. [PubMed: 29317594]

Croen LA, Grether JK, Hoogstrate J, & Selvin S (2002). The changing prevalence of autism in California. Journal of Autism and Developmental Disorders, 32(3), 207–215. [PubMed: 12108622]

Cunningham GC, & Tompkinson DG (1999). Cost and effectiveness of the California triple marker prenatal screening program. Genetics in Medicine, 1(5), 199–206. [PubMed: 11256673]

Dietz PM, Adams MM, Kendrick JS, & Mathis MP (1998). Completeness of ascertainment of prenatal smoking using birth certificates and confidential questionnaires: Variations by maternal attributes and infant birth weight. American Journal of Epidemiology, 148(11), 1048–1054. [PubMed: 9850126]

Dodds L, Fell DB, Shea S, Armson BA, Allen AC, & Bryson S (2011). The role of prenatal, obstetric and neonatal factors in the development of autism. Journal of Autism and Developmental Disorders, 41(7), 891–902. [PubMed: 20922473]

Duan G, Yao M, Ma Y, & Zhang W (2014). Perinatal and background risk factors for childhood autism in central China. Psychiatry Research, 220(1–2), 410–417. [PubMed: 25085792]

Dwyer JB, McQuown SC, & Leslie FM (2009). The dynamic effects of nicotine on the developing brain. Pharmacology & Therapeutics, 122(2), 125–139. [PubMed: 19268688]

Gould GS, Havard A, Lim LL, & Kumar R (2020). Exposure to tobacco, environmental tobacco smoke and nicotine in pregnancy: A pragmatic overview of reviews of maternal and child outcomes, effectiveness of interventions and barriers and facilitators to quitting. International Journal of Environmental Research and Public Health, 17(6), 2034. [PubMed: 32204415]

Hiscock R, Bauld L, Amos A, Fidler JA, & Munafò M (2012). Socioeconomic status and smoking: A review. Annals of the New York Academy of Sciences, 1248(1), 107–123. [PubMed: 22092035]

Huizink AC, & Mulder EJ (2006). Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring. Neuroscience & Biobehavioral Reviews, 30(1), 24–41. [PubMed: 16095697]

Hultman CM, Sparén P, & Cnattingius S (2002). Perinatal risk factors for infantile autism. Epidemiology, 13(4), 417–423. [PubMed: 12094096]

Jiang H, Liu L, Sun D, Yin X, Chen Z, Wu C, & Chen W (2016). Interaction between passive smoking and folic acid supplement during pregnancy on autism spectrum disorder behaviors in children aged 3 years. Zhonghua liu xing bing xue za zhi= Zhonghua liuxingbingxue zazhi, 37(7), 940–944. [PubMed: 27453101]

Jung Y, Lee AM, McKee SA, & Picciotto MR (2017). Maternal smoking and autism spectrum disorder: Meta-analysis with population smoking metrics as moderators. Scientific Reports, 7 (1), 1–10. [PubMed: 28127051]

Kalkbrenner AE, Braun JM, Durkin MS, Maenner MJ, Cunniff C, Lee L-C, Pettygrove S, Nicholas JS, & Daniels JL (2012). Maternal smoking during pregnancy and the prevalence of autism

spectrum disorders, using data from the autism and developmental disabilities monitoring network. Environmental Health Perspectives, 120(7), 1042–1048. [PubMed: 22534110]

Kalkbrenner AE, Meier SM, Madley-Dowd P, Ladd-Acosta C, Fallin MD, Parner E, & Schendel D (2020). Familial confounding of the association between maternal smoking in pregnancy and autism spectrum disorder in offspring. Autism Research, 13(1), 134–144. [PubMed: 31464107]

Kim KM, Lim MH, Kwon H-J, Yoo S-J, Kim E-J, Kim JW, Ha M, & Paik KC (2018). Associations between urinary cotinine and symptoms of attention deficit/hyperactivity disorder and autism spectrum disorder. Environmental Research, 166, 481–486. [PubMed: 29957501]

Kolevzon A, Gross R, & Reichenberg A (2007). Prenatal and perinatal risk factors for autism: A review and integration of findings. Archives of Pediatrics & Adolescent Medicine, 161(4), 326–333. [PubMed: 17404128]

Ladd-Acosta C, Shu C, Lee BK, Gidaya N, Singer A, Schieve LA, Schendel DE, Jones N, Daniels JL, Windham GC, Newschaffer CJ, Croen LA, Feinberg AP, & Fallin MD (2016). Presence of an epigenetic signature of prenatal cigarette smoke exposure in childhood. Environmental Research, 144, 139–148. [PubMed: 26610292]

Lee BK, Gardner RM, Dal H, Svensson A, Galanti MR, Rai D, Dalman C, & Magnusson C (2012). Brief report: Maternal smoking during pregnancy and autism spectrum disorders. Journal of Autism and Developmental Disorders, 42(9), 2000–2005. [PubMed: 22173844]

Lei G, Xi QQ, Wu J, Han Y, Dai W, Su YY, & Zhang X (2015). Association between prenatal environmental factors and child autism: A case control study in Tianjin, China. Biomedical and Environmental Sciences, 28(9), 642–650. [PubMed: 26464251]

Liu D, Zhan J-Y, & Shao J (2015). Environmental risk factors for autism spectrum disorders in children. Zhongguo dang dai er ke za zhi= Chinese journal of contemporary pediatrics, 17(11), 1147–1153. [PubMed: 26575869]

Lyall K, Croen L, Daniels J, Fallin MD, Ladd-Acosta C, Lee BK, Park BY, Snyder NW, Schendel D, Volk H, Windham GC, & Newschaffer C (2017). The changing epidemiology of autism spectrum disorders. Annual Review of Public Health, 38, 81–102.

Lyall K, Windham GC, Snyder NW, Kuskovsky R, Xu P, Bostwick A, Robinson L, & Newschaffer CJ (2021). Association between midpregnancy polyunsaturated fatty acid levels and offspring autism spectrum disorder in a California population-based case-control study. American Journal of Epidemiology, 190 (2), 265–276. [PubMed: 33524118]

Maimburg RD, & Væth M (2006). Perinatal risk factors and infantile autism. Acta Psychiatrica Scandinavica, 114(4), 257–264. [PubMed: 16968363]

Mandic-Maravic V, Coric V, Mitkovic-Voncina M, Djordjevic M, Savic-Radojevic A, Ercegovac M, Matic M, Simic T, Lecic-Tosevski D, Toskovic O, Pekmezovic T, Pljesa-Ercegovac M, & Pejovic-Milovancevic M (2019). Interaction of glutathione S-transferase polymorphisms and tobacco smoking during pregnancy in susceptibility to autism spectrum disorders. Scientific Reports, 9(1), 1–9. [PubMed: 30626917]

Miguel PM, Pereira LO, Silveira PP, & Meaney MJ (2019). Early environmental influences on the development of children's brain structure and function. Developmental Medicine & Child Neurology, 61(10), 1127–1133. [PubMed: 30740660]

Mrozek-Budzyn D, Majewska R, & Kieltyka A (2013). Prenatal, perinatal and neonatal risk factors for autism-study in Poland. Central European Journal of Medicine, 8(4), 424–430.

Ng M, Freeman MK, Fleming TD, Robinson M, Dwyer-Lindgren L, Thomson B, Wollum A, Sanman E, Wulf S, Lopez AD, Murray CJL, & Gakidou E (2014). Smoking prevalence and cigarette consumption in 187 countries, 1980–2012. JAMA, 311(2), 183–192. [PubMed: 24399557]

Nilsen RM, Surén P, Gunnes N, Alsaker ER, Bresnahan M, Hirtz D, Hornig M, Lie KK, Lipkin WI, Reichborn-Kjennerud T, Roth C, Schjølberg S, Smith GD, Susser E, Vollset SE, Øyen A-S, Magnus P, & Stoltenberg C (2013). Analysis of self-selection bias in a population-based cohort study of autism spectrum disorders. Paediatric and Perinatal Epidemiology, 27(6), 553–563. [PubMed: 23919580]

Ornoy A, Weinstein-Fudim L, & Ergaz Z (2015). Prenatal factors associated with autism spectrum disorder (ASD). Reproductive Toxicology, 56, 155–169. [PubMed: 26021712]

Pauly JR, & Slotkin TA (2008). Maternal tobacco smoking, nicotine replacement and neurobehavioural development. Acta Paediatrica, 97(10), 1331–1337. [PubMed: 18554275]

Qin Y-Y, Jian B, Wu C, Jiang C-Z, Kang Y, Zhou J-X, Yang F, & Liang Y (2018). A comparison of blood metal levels in autism spectrum disorder and unaffected children in Shenzhen of China and factors involved in bioaccumulation of metals. Environmental Science and Pollution Research, 25(18), 17950–17956. [PubMed: 29680891]

Saunders A, Woodland J, & Gander S (2019). A comparison of prenatal exposures in children with and without a diagnosis of autism spectrum disorder. Cureus, 11(7), e5223. [PubMed: 31565625]

Scherer G, Conze C, Tricker A, & Adlkofer F (1992). Uptake of tobacco smoke constituents on exposure to environmental tobacco smoke (ETS). The Clinical Investigator, 70(3–4), 352–367. [PubMed: 1521050]

Shea AK, & Steiner M (2008). Cigarette smoking during pregnancy. Nicotine & Tobacco Research, 10(2), 267–278. [PubMed: 18236291]

Signorello LB, Cai Q, Tarone RE, McLaughlin JK, & Blot WJ (2009). Racial differences in serum cotinine levels of smokers. Disease Markers, 27(5), 187–192. [PubMed: 20037205]

Slotkin TA (2008). If nicotine is a developmental neurotoxicant in animal studies, dare we recommend nicotine replacement therapy in pregnant women and adolescents? Neurotoxicology and Teratology, 30(1), 1–19. [PubMed: 18380035]

Toledo-Rodriguez M, Lotfipour S, Leonard G, Perron M, Richer L, Veillette S, Pausova Z, & Paus T (2010). Maternal smoking during pregnancy is associated with epigenetic modifications of the brain-derived neurotrophic factor-6 exon in adolescent offspring. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 153(7), 1350–1354.

Totsika V, Hastings RP, Emerson E, Lancaster GA, & Berridge DM (2011). A population-based investigation of behavioural and emotional problems and maternal mental health: Associations with autism spectrum disorder and intellectual disability. Journal of Child Psychology and Psychiatry, 52 (1), 91–99. [PubMed: 20649912]

Tran PL, Lehti V, Lampi KM, Helenius H, Suominen A, Gissler M, Brown AS, & Sourander A (2013). Smoking during pregnancy and risk of autism spectrum disorder in a Finnish National Birth Cohort. Paediatric and Perinatal Epidemiology, 27 (3), 266–274. [PubMed: 23574415]

Vinikoor LC, Messer LC, Laraia BA, & Kaufman JS (2010). Reliability of variables on the North Carolina birth certificate: A comparison with directly queried values from a cohort study. Paediatric and Perinatal Epidemiology, 24(1), 102–112. [PubMed: 20078836]

Vorstman JA, Parr JR, Moreno-De-Luca D, Anney RJ, Nurnberger JI Jr., & Hallmayer JF (2017). Autism genetics: Opportunities and challenges for clinical translation. Nature Reviews Genetics, 18(6), 362.

Wang C, Geng H, Liu W, & Zhang G (2017). Prenatal, perinatal, and postnatal factors associated with autism: A meta-analysis. Medicine, 96(18), e6696. [PubMed: 28471964]

Windham GC, Anderson MC, Croen LA, Smith KS, Collins J, & Grether JK (2011). Birth prevalence of autism spectrum disorders in the San Francisco Bay area by demographic and ascertainment source characteristics. Journal of Autism and Developmental Disorders, 41(10), 1362–1372. [PubMed: 21264681]

Zablotsky B, Black LI, & Blumberg SJ (2017). Estimated prevalence of children with diagnosed developmental disabilities in the United States, 2014–2016. NCHS Data Brief, no 291. Hyattsville, MD: National Center for Health Statistics. https://www.cdc.gov/nchs/products/databriefs/db291.htm?fbclid=IwAR0MwhWDgawPU

Zhang X, Lv C-C, Tian J, Miao R-J, Xi W, Hertz-Picciotto I, & Qi L (2010). Prenatal and perinatal risk factors for autism in China. Journal of Autism and Developmental Disorders, 40(11), 1311–1321. [PubMed: 20358271]

TABLE 1

Parental demographic characteristics of study participants, born in certain Californian counties in 2011–2012 (N = 977)

	ASD		Controls		
	N	**%**	***N***	**%**	**Chi2 *p*-value**
Maternal age (years)					0.01
<20	25	(5.0)	32	(6.4)	
20–24	85	(17.1)	113	(22.7)	
25–29	132	(26.5)	133	(26.7)	
30–34	155	(31.1)	156	(31.3)	
35+	101	(20.3)	65	(13.0)	
Paternal age (years)					0.01
<20	9	(1.8)	11	(2.2)	
20–24	49	(9.8)	68	(13.6)	
25–29	100	(20.1)	121	(24.3)	
30–34	136	(27.3)	145	(29.1)	
35+	204	(41.0)	154	(30.9)	
Maternal education					0.01
Missing	17	(3.4)	24	(4.8)	
Less than high school	86	(17.3)	114	(22.9)	
High school/Some college/Associate’s degree	272	(54.6)	223	(44.7)	
Bachelor’s degree or higher	123	(24.7)	138	(27.7)	
Maternal race					0.43
Non-Hispanic white	126	(25.3)	126	(25.3)	
Non-Hispanic Black	17	(3.4)	20	(4.0)	
Non-Hispanic Asian	88	(17.7)	68	(13.6)	
Hispanic	251	(50.4)	264	(52.9)	
Other	16	(3.2)	21	(4.2)	
Parity category					0.06
0	212	(42.6)	205	(41.1)	
1	179	(35.9)	156	(31.3)	
2+	107	(21.5)	138	(27.7)	

	ASD		Controls		Chi2 *p*-value
	N	%	*N*	%	
Insurance type					0.87
Public	276	(55.4)	274	(54.9)	
Private	222	(44.6)	225	(45.1)	
Self-reported smoking during pregnancy or 3 months prior					0.55
Missing	1	(0.2)	3	(0.6)	
Non-smoker	485	(97.4)	486	(97.4)	
Smoker	12	(2.4)	10	(2.0)	

Abbreviation: ASD, Autism Spectrum Disorder.

TABLE 2

Distribution of serum cotinine concentration among study participants, born in certain Californian counties in 2011–2012 (N = 997)

	N	%	% <LOD (0.015 ng/ml)	Geometric mean	95% CI for geometric mean	Percentiles					Kruskal-Wallis *p*-value
						5th	25th	50th	75th	95th	
All											0.38
ASD	498	50.0	18.9	0.060	(0.052, 0.069)	0.011	0.026	0.053	0.097	0.423	
Controls	499	50.1	19.4	0.065	(0.056, 0.076)	0.011	0.024	0.055	0.113	1.230	
Cotinine-defined smokers (≥3.08 ng/ml)											0.06
ASD	16	43.2	0.0	52.870	(36.088, 77.456)	12.100	32.000	53.900	87.400	198.000	
Controls	21	56.8	0.0	29.163	(19.066, 44.606)	7.030	16.800	35.000	55.800	95.200	
Cotinine-defined non-smokers (<3.08 ng/ml)											0.51
ASD	482	50.2	19.5	0.048	(0.044, 0.052)	0.011	0.026	0.050	0.092	0.233	
Controls	478	49.8	20.3	0.050	(0.045, 0.055)	0.011	0.022	0.053	0.098	0.265	

Abbreviations: ASD, Autism Spectrum Disorder; LOD, limit of detection.

TABLE 3

Associations between serum cotinine concentrations, smoking status, and Autism Spectrum Disorder in selected models for study participants, born in certain Californian counties in 2011–2012

	Unadjusted[a]				Adjusted[b]			
	N		Odds ratio	(95% Confidence interval)	*N*		Odds ratio	(95% Confidence interval)
	ASD	Controls			ASD	Controls		
Model 1								
Continuous cotinine among cotinine-defined non-smokers (<3.08 ng/ml)	482	478	0.91	(0.69, 1.21)	481		0.93	(0.69, 1.25)
Model 2								
Cotinine below LOD	94	97	Ref		89	92	Ref	
Cotinine-defined non-smoker Q1[c]	101	92	1.13	(0.76, 1.70)	95	89	1.06	(0.69, 1.62)
Cotinine-defined non-smoker Q2[c]	103	89	1.20	(0.80, 1.80)	102	83	1.24	(0.81, 1.89)
Cotinine-defined non-smoker Q3[c]	97	96	1.04	(0.70, 1.56)	96	90	1.14	(0.75, 1.73)
Cotinine-defined non-smoker Q4[c]	87	104	0.86	(0.58, 1.30)	84	103	0.83	(0.54, 1.26)
Cotinine-defined smoker (≥3.08 ng/ml)	16	21	0.78	(0.38, 1.60)	15	18	0.76	(0.34, 1.68)
Model 3								
Cotinine-defined non-smoker (<3.08 ng/ml)	482	478	Ref		466	457	Ref	
Cotinine-defined smoker (≥3.08 ng/ml)	16	21	0.75	(0.39, 1.46)	15	18	0.73	(0.35, 1.54)
Model 4								
Cotinine-defined non-smokers	482	478	Ref		466	457	Ref	
Low-cotinine smokers (≤35 ng/ml)	5	11	0.44	(0.15, 1.29)	5	11	0.38	(0.12, 1.17)
High-cotinine smokers (>35 ng/ml)	11	10	1.10	(0.46, 2.62)	10	7	1.25	(0.46, 3.45)
Model 5								
Self-identified non-smoker	485	486	Ref		468	465	Ref	
Self-identified smoker	12	10	1.20	(0.51, 2.82)	12	8	1.64	(0.65, 4.16)

Abbreviations: ASD, Autism Spectrum Disorder; LOD, limit of detection.

[a] Adjusted for matching factors only (birth year, birth month, child sex).

[b] Adjusted for birth year, birth month, child sex, insurance type, maternal age, maternal education, maternal race.

[c]Quartiles of cotinine concentration between the LOD and 3.08 were defined as Q1: ≤0.0411 ng/ml; Q2: >Q1 and ≤0.0634 ng/ml; Q3: >Q2 and ≤0.112 ng/ml; Q4: >Q3 and <3.08 ng/ml.

TABLE 4

Effect estimates calculated from interaction terms by race/ethnicity for associations between cotinine-defined smoking and Autism Spectrum Disorder in two models for study participants, born in certain Californian counties in 2011–2012

		N					
		Cotinine-defined smokers		**Cotinine-defined non-smokers**			
		ASD	**Controls**	**ASD**	**Controls**	**Adjusted odds ratio[a]**	**(95% Confidence interval)**
Cotinine-defined smoker (≥3.08 ng/ml) versus <3.08 ng/ml	Non-Hispanic white	9	6	110	116	1.27	(0.43, 3.80)
	Hispanic	3	3	246	254	0.96	(0.19, 4.98)
	Non-Hispanic Asian, Black, and Other	3	9	110	87	0.25	(0.06, 0.97)
		N					
		Cotinine-defined smokers		**<LOD**			
		ASD	**Controls**	**ASD**	**Controls**	**Adjusted odds ratio[a]**	**(95% Confidence interval)**
Cotinine-defined smoker (≥3.08 ng/ml)versus < LOD	Non-Hispanic white	9	6	20	25	1.56	(0.46, 5.30)
	Hispanic	3	3	48	48	0.90	(0.17, 4.84)
	Non-Hispanic Asian, Black, and Other	3	9	21	19	0.27	(0.06, 1.19)

Abbreviation: LOD, Limit of detection.

[a]Adjusted for birth year, birth month, child sex, insurance type, maternal age, maternal education.