HHS Public Access

Author manuscript

Autism Res. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

Autism Res. 2018 January ; 11(1): 69–80. doi:10.1002/aur.1885.

Joint Effects of Prenatal Air Pollutant Exposure and Maternal Folic Acid Supplementation on Risk of Autism Spectrum Disorder

Amanda J. Goodrich[1], **Heather E. Volk**[1,*], **Daniel J. Tancredi**[2], **Rob McConnell**[1], **Fred W. Lurmann**[4], **Robin L. Hansen**[2,3], and **Rebecca J. Schmidt**[3,5]

[1]Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California, USA

[2]Department of Pediatrics, University of California Davis, Sacramento, CA, USA

[3]MIND (Medical Investigation of Neurodevelopmental Disorders) Institute, University of California Davis, Davis, CA, USA

[4]Sonoma Technology, Inc, Petaluma, California, USA

[5]Department of Public Health Sciences, University of California Davis, Davis, CA, USA

Scientific Abstract

Background—Independent studies report that periconceptional folic acid (FA) may decrease the risk of autism spectrum disorder (ASD) while exposure to air pollution may increase ASD risk. We examined the joint effects of gestational FA and air pollution exposures in association with ASD.

Methods—We studied 346 ASD cases and 260 typically developing controls from the CHARGE case-control study. Self-reported FA intake for each month of pregnancy was quantified. Estimates of exposure to near roadway air pollution (NRP) and criteria air pollutant measures were assigned based on maternal residential history.

Results—Among mothers with high FA intake (>800 μg) in the first pregnancy month, exposure to increasing levels of all air pollutants, except ozone, during the first trimester was associated

Corresponding author information: Dr. Heather Volk, Department of Mental Health, Wendy Klag Center for Autism and Developmental Disabilities, Bloomberg School of Public Health, Johns Hopkins University, 624 N. Broadway, HH833, Baltimore, MD 21210, Phone: 410-614-9404, Fax: 410-614-7569, hvolk1@jhu.edu.
Amanda J. Goodrich, University of Southern California, Health Sciences Campus, 2001 N. Soto St. Space 312-19, Los Angeles, CA 90032
Heather E. Volk, Department of Mental Health, Wendy Klag Center for Autism and Developmental Disabilities, Bloomberg School of Public Health, Johns Hopkins University, 624 N. Broadway, HH833, Baltimore, MD 21210
Daniel J. Tancredi, Associate Professor in Residence, UC Davis Department of Pediatrics and Center for Healthcare Policy and Research (CHPR), 2103 Stockton Blvd, Suite 2224, Sacramento, California 95817
Rob McConnell, University of Southern California, Health Sciences Campus, SSB 230D 2001 N. Soto Street, Mail Code:9237, Los Angeles, California 90033
Fred W. Lurmann, Sonoma Technology, Inc., 1435 N. McDowell Blvd., Suite 300, Petaluma, CA 94954
Robin L. Hansen, UC Davis MIND Institute, 2825 50th Street, Sacramento, CA 95817
Rebecca J. Schmidt, Department of Public Health Sciences, School of Medicine, University of California Davis, 123 Med Sci 1C, One Shields Ave, Davis, California 95616-8638
*Dr. Volk is currently with the Department of Mental Health, Wendy Klag Center for Autism and Developmental Disabilities, Bloomberg School of Public Health, Johns Hopkins University

with decreased ASD risk, while increased ASD risk was observed for the same pollutant among mothers with low FA intake (≤800 μg). This difference was statistically significant for NO_2 (e.g. NO_2 and low FA intake: OR=1.53 (0.91, 2.56) vs NO_2 and high FA intake: OR=0.74 (0.46, 1.19), p-interaction=0.04). Mothers exposed to higher levels (≥ median) of any air pollutant during the first trimester of pregnancy and who reported low FA intake were at a higher ASD risk compared to mothers exposed to lower levels of that air pollutant and who reported high first month FA intake. Joint effects showed significant (alpha<0.10) departures from expected interaction for NRP and NO_2.

Conclusions—Our results suggest that periconceptional FA intake may reduce ASD risk in those with high prenatal air pollution exposure. Further study is needed to replicate these findings in larger sample sizes and to understand mechanisms of this potential relationship.

Keywords

Autism; ASD; folic acid; air pollution; prenatal exposure; environmental exposure

Introduction

Autism spectrum disorders (ASD) are neurodevelopmental disorders characterized by difficulties in social interaction, verbal and nonverbal communication, and repetitive behaviors, affecting an estimated 1 in 68 children (1 in 42 boys) in the United States (Centers for Disease Control and Prevention (CDC), 2014; Christensen et al., 2016). Evidence supports large heritable contributions to the etiology of ASD, and most studies suggest that both environmental and genetic factors play a role (Bailey et al., 1995; Lyall, Schmidt, & Hertz-Picciotto, 2014; E. R. Ritvo, Freeman, Mason-Brothers, Mo, & Ritvo, 1985).

In recent years, pre- and perinatal air pollution exposure has been found to be associated with an increased risk of ASD among studies from the United States (Becerra, Wilhelm, Olsen, Cockburn, & Ritz, 2013; Ehrenstein, Aralis, Cockburn, & Ritz, 2014; Kalkbrenner et al., 2010; 2015; Raz et al., 2014; Roberts et al., 2013; Talbott, Arena, et al., 2015a; Talbott, Marshall, et al., 2015b; Volk, Hertz-Picciotto, Delwiche, Lurmann, & Mcconnell, 2010; Volk, Lurmann, Penfold, Hertz-Picciotto, & Mcconnell, 2013; Windham, Zhang, Gunier, Croen, & Grether, 2006). Research evaluating the relationship between ASD risk and measures related to vehicular traffic, via dispersion models, distance to freeway, or assignment of diesel exhaust particle concentration via the Hazardous Air Pollutant monitoring network, have shown an increased risk with increasing exposure (Roberts et al., 2013; Volk et al., 2010; 2013; Windham et al., 2006). Other studies have examined the relationship between ASD and criteria air pollutant exposures capitalizing on the EPA's Air Quality Monitoring network to evaluate the effects of particles (both less than 10 and less than 2.5 microns in diameter (PM_{10}), ($PM_{2.5}$)), and nitrogen dioxide (NO_2), and ozone. Associations have been reported between increased exposure to both PM10 and PM2.5 (Becerra et al., 2013; Kalkbrenner et al., 2015; Raz et al., 2014; Talbott, Arena, et al., 2015a; Volk et al., 2013), and NO_2 (Becerra et al., 2013; Volk et al., 2013), further supporting a relationship between ASD and air pollutant exposure. Results from Asia demonstrate

associations (Jung, Lin, & Hwang, 2013), while findings from Europe, in contrast, have not shown associations with ASD (Gong et al., 2014).

Other factors may help prevent ASD. Periconceptional folic acid supplements are known to prevent up to 70% of neural tube defects (MRC Vitamin Study Research Group, 1991), and have been associated with decreased childhood behavioral problems (Roza et al., 2010), hyperactivity and peer problems (Schlotz et al., 2009), and improved scores on neurodevelopmental measures, including social competence, attention, verbal, and verbal-executive function (Julvez et al., 2009). Additionally, we, and others, have shown that mothers reporting the use of a prenatal supplement or folic acid supplement near conception had decreased risk of having a child with ASD (Braun et al., 2014; Raghavan, Fallin, & Wang, 2016; Schmidt et al., 2012; Surén et al., 2013). However, not all studies have observed this association (Virk et al., 2015). Other studies did not find a statistically significant association between serum folate and ASD after adjusting for confounders (Braun et al., 2014; Steenweg-de Graaff, Ghassabian, Jaddoe, Tiemeier, & Roza, 2015). The discrepancy of these results suggests that specific timing and dose of folate during pregnancy may alter risk of ASD. In the United States, it is recommended that women consume at least 600 μg of folic acid during pregnancy (National Institutes of Health, 2016). Most prenatal vitamins contain 800 μg of folic acid or more.

In CHARGE we have observed both that increasing folate intake during the two months prior or the first month of pregnancy decreases risk of ASD and that increasing exposure to air pollution during pregnancy increases risk of ASD (Schmidt et al., 2012; Volk et al., 2010; 2013). The mechanism by which folic acid supplementation protects neurodevelopment remains unclear, however, given that folate serves as a primary methyl-donor, DNA methylation pathways have been proposed and supported by several lines of evidence (Cooney, Dave, & Wolff, 2002; Gardiki-Kouidou & Seller, 2008; Heijmans et al., 2008; Reik & Walter, 2001; Shaw, Carmichael, Yang, Selvin, & Schaffer, 2004; Steegers-Theunissen et al., 2009; Wolff, Kodell, Moore, & Cooney, 1998). It has also been shown that exposure to higher ambient levels of traffic particles can decrease DNA methylation (Baccarelli et al., 2009). Therefore, we hypothesized that supplemental folic acid may attenuate neurodevelopmental effects of air pollution by increasing the levels of methylation, thereby reducing the risk of ASD. In this study, we investigated the joint effects of maternal-reported folic acid intake and prenatal air pollutant exposures on ASD risk.

Methods

Study Population

The Childhood Autism Risk from Genetics and the Environment (CHARGE) study is a large population-based case-control study focusing on the association between environmental exposures, as well as their interactions with genes, and risk for ASD. As described previously (Hertz-Picciotto et al., 2006), children were eligible if they were between the ages of 2 and 5 years, born in California, and currently living with at least one biologic parent in the catchment areas of a specified list of regional centers (RCs) in California. RCs are private nonprofit corporations that contract with the California Department of

Developmental Services (DDS) to coordinate or provide services for individuals with developmental disabilities.

Children with autism and developmental delay (DD) were identified through RCs that contract with DDS to determine eligibility. General population controls were identified from state birth files and were frequency-matched to the autism cases by age, sex, and broad residential RC catchment area distribution. All births were between 1997 and 2008.

As described previously (Hertz-Picciotto et al., 2006), children with a DDS diagnosis of autism were evaluated using the Autism Diagnostic Observation Schedule-Generic (ADOS) (Lord et al., 2000) and parents were administered the Autism Diagnostic Interview-Revised (ADI-R) (Lord, Rutter, & Le Couteur, 1994) to confirm the child's diagnostic group. In the children recruited from the general population or those with developmental delays, the Social Communication Questionnaire (SCQ) was used to screen for autism, with a cut-off score of 15; any children scoring above this point were administered the ADOS and ADI-R. Children sampled from the general population were defined as typically developing (TD) controls if they received a score ≤ 15 on the SCQ and scored in the normal range on the Mullen Scales of Early Learning and Vineland Adaptive Behavior Scales, thereby showing no evidence of other types of delay (cognitive or adaptive).

Assessments were conducted in English or Spanish at the Medical Investigation of Neurodevelopmental Disorders (M.I.N.D.) Institute by trained research-reliable staff. Final autism case status was defined as scoring at or above the total cut off for autistic disorder on the ADOS Module 1 or 2, and meeting criteria on the Communication, Social, and Repetitive Behavior domains of the ADI–R. Autism spectrum disorder was defined using the ASD2 criteria of Risi *et al.*

A total of 856 CHARGE subjects (510 cases meeting either autism or ASD ((348) autism, (162) ASD) and 346 TD controls) were eligible for study. Scoring methods for the ADOS for the 90 Southern California participants were not consistent with methods used for the rest of the population; therefore, they were excluded from analysis. This results in a final sample of 297 autism and 143 ASD cases and 326 TD controls eligible here.

Quantification of nutrient intake

As reported previously (Schmidt et al., 2012), mothers were asked, via telephone interviews, if they had consumed multivitamins, prenatal vitamins, nutrient-specific vitamins, cereals (i.e. breakfast cereals, granolas, and hot cereals), and other supplements (i.e. breakfast shakes and protein bars), for the index period, defined as 3 months before conception through the end of breastfeeding. A total value of folic acid intake was calculated for each woman for each month of the index period, based on whether she reported consuming each supplement for each month, the reported brands, dose, and frequency of consumption of each product (reported for the entire period) as described previously (Schmidt et al., 2012). Using the manufacturer's label or data from the USDA National Nutrient Database for Standard Reference (U S Dept Agriculture, 2010), amounts of folic acid (and other nutrients) were assigned to each brand or product. We focused on maternal folic acid intake reported for the first month of pregnancy, given this was the critical period of interest

previously (Schmidt et al., 2012). Folic acid intake during the first pregnancy month was available on 442 children with ASD and 288 TD controls.

Air pollution exposure assessment

In telephone interviews, parents were asked for their residential history (including addresses and dates lived at each location) beginning 3 months before conception and extending to the most recent place of residence. Using this information and the conception date for each child, based on gestational age from ultrasonographic measurements or the date of last menstrual period as determined from prenatal records, air pollution exposure values were assigned for each trimester of pregnancy, and an all pregnancy average (described previously in (Volk et al., 2013). When more than 1 address fell into a time interval, we created a weighted average to reflect the exposure level of the participant across the time of interest, taking into account changes in residence.

Addresses were geo-coded using the Tele Atlas database and software (Tele Atlas, Inc., Boston, CA, www.na.teleatlas.com). The CALINE4 line-source air quality dispersion model was used to obtain model-based estimates of average exposure to near roadway air pollution (NRP) (Benson, 1992). NRP was assigned based on the required inputs reflecting change in each address over the study period. The CALINE4 model uses roadway geometry, link-based traffic volumes, vehicle emission rates, wind speed and direction, atmospheric stability, and mixing heights to estimate average concentrations for the specific locations and time periods examined. Roadway geometry data and annual average daily traffic counts were obtained from Tele Atlas/Geographic Data Technology in 2005, and represent traffic counts collected between 1995 and 2000. Counts were scaled for our specific years of interest based on estimated growth in county average vehicle-miles-traveled. Meteorological data from 56 local monitoring stations were matched to the dates and locations of interest. It should be noted that we used this NRP model to estimate nitrogen oxides (NOx), but that these model-based concentrations should be viewed as an indicator of the estimated near-roadway pollutant mixture rather than of effects of NOx or any other specific pollutant.

Criteria air pollutant measures (i.e. $PM_{2.5}$, PM_{10}, ozone, and NO_2) were assigned using the United States Environmental Protection Agency's (EPA) Air Quality System data (http://www.epa.gov/ttn/airs/airsaqs) supplemented by University of Southern California Children's Health Study data for 1997 through 2009 (Alcorn & Lurmann, 2003). Monthly measurements from up to four monitoring stations located within 50 km of each residence were used to assess average exposure based on spatial interpolation of ambient concentrations via inverse distance-squared weighting. If one or more stations were located within 5 km of a residence, only data from those stations were used. For ozone, we used the average range of measurements from 1000 to 1800 hours (reflecting the high 8-hour daytime) while the measurements for the other three pollutants correspond to daily (24hr average) collection.

Statistical analyses

Unconditional logistic regression was used to evaluate the primary associations between all potential confounders, air pollutant variables, folate intake, and the risk of ASD. We

evaluated potential confounders by adding them to the primary model and we retained covariates causing a 10% or greater change in the estimated log of the exposure odds ratio. All variables in Table 1 were assessed for confounding. Additionally, we examined if calculated values of other available nutrients were confounders of this relationship due to their correlated nature with folic acid. Final models were adjusted for self-reported financial hardship between the 3 months before pregnancy to the time of interview (defined as a time when it was hard to pay for basic needs like food, housing, medical care, and heating and reported (yes/no)), child's year of birth, vitamin A and zinc intake during the first month of pregnancy. We obtained 95% confidence intervals (CIs) as measures of precision and determined statistical significance for associations using an alpha level of 0.05. Analyses were conducted using Stata Version 13 (StataCorp, College Station, TX). The institutional review boards of the University of Southern California and the University of California, Davis, approved the research.

For the purpose of this analysis, average total daily folic acid intake for the first month of pregnancy was dichotomized at 800 μg that was similar to the median intake for the controls (801.74 μg). We classified subjects with folic acid above 800 μg as having "high" folic acid intake and those at or below 800 μg as "low" folic acid intake.

In models where air pollution was treated as a continuous variable, values above the upper 2.5% and below the lower 2.5% of each air pollutant were recoded to equal the value associated with the upper and lower 2.5 percentile, respectively, to reduce the influence of extreme values for continuous air pollution models. For reparameterized models, all air pollutants were dichotomized at the median, based on the total pregnancy period (NRP = 15.58 ppb; NO_2 = 14.18 ppb; PM_{10} = 22.88 μg/cm^3; $PM_{2.5}$ = 12.40 μg/cm^3; Ozone = 33.41 μg/cm^3). Values above the median were considered "high" exposure levels and values at or below the median were considered "low" exposure levels.

We first tested for a statistical multiplicative interaction between air pollution (as a continuous variable) and folic acid intake (less than or equal to 800 μg vs greater than 800 μg) comparing logistic regression model with and without a multiplicative term of both exposures. A likelihood ratio *p*-value of less than 0.10, for the addition of the multiplicative term, was considered significant evidence for an interaction. Effect estimates were scaled to twice the standard deviation of the distribution for all pregnancy period estimates.

Subjects were also reparameterized into one of four groups based on both air pollution exposure and folic acid intake: low air pollution and high folic acid intake (referent group); low air pollution and low folic acid intake; high air pollution and high folic acid intake; high air pollution and low folic acid intake. These reparameterized variables were then used in an unconditional multivariate logistic regression model to assess the combined risk of air pollution and nutrient intake on the risk of autism. All models were fitted separately for each of several time periods (i.e. first trimester, second trimester, third trimester, and whole pregnancy period). First trimester air pollution exposure was of primary interest given the early timing of the folic acid intake periconceptional critical window of interest. Additional analyses were performed evaluating the reparameterized joint effects of both air pollution

exposure and folic acid intake in which each air pollutant was dichotomized at the 75th percentile.

Expected joint effects under an additive model were calculated by adding the ORs of the groups with only one exposure and subtracting 1. Expected joint effects under a multiplicative model were calculated by multiplying the ORs of the groups with only one exposure. The relative excess risk due to interaction (RERI), the attributable proportion (AP) due to interaction, and their p-values were calculated using the "ic" package in Stata Version 13 (Andersson, Alfredsson, Källberg, Zdravkovic, & Ahlbom, 2005; Hosmer & Lemeshow, 1992).

Results

Participant Characteristics

Of 766 enrolled children who were eligible for the study and whose mothers were interviewed by November 2011 (when the interview was revised) (326 TD controls and 440 ASD cases), 51 lacked complete data on air pollution exposure, 112 were missing data on periconceptional folic acid intake. In addition, one person was missing data on periconceptional vitamin A and one person was missing data on periconceptional zinc intake. This resulted in complete data from 606 subjects (346 case and 260 controls), and all analyses included these subjects. All demographic characteristics of the 160 subjects that were missing exposure data were similar to the 606 subjects with complete data, with the exception of child's year of birth and mother's age (≥ 35 years or < 35 years) (Supplemental Table 1).

The majority of the children in our study were male (84%), and non-Hispanic white (52%) (Table 1). Given delayed enrollment of controls, cases and controls differed statistically on year of birth, where TD children were more likely to be born in later birth years than ASD children. Families of TD children were more likely to own their residence at time of study participation and their mothers were more likely to be born in the US than ASD children, while families of ASD children were more likely to report financial hardship during the index period.

Distribution of exposures

Mothers of TD children were more likely to have taken prenatal vitamins during the three months before conception or the first month of pregnancy, and took a higher amount folic acid in the first month of pregnancy than mothers of ASD children (Table 1). Folic acid intake had a moderate to high correlation with all other nutrients except Vitamin B12 (Supplemental Table 2.) Folic acid, vitamin C, vitamin E, and vitamin B6 had different median values across ASD cases and controls.

The distribution of near roadway air pollution was relatively constant across trimesters of pregnancy (Supplemental Figure). Near roadway air pollution, during both the first trimester and the whole pregnancy period, was highly correlated with nitrogen dioxide, moderately correlated with $PM_{2.5}$ and PM_{10}, and inversely correlated with ozone. Among the regional pollutant measures, nitrogen dioxide, $PM_{2.5}$, and PM_{10} were all highly correlated for both

time periods. Correlations with ozone were low to moderate and often negative, demonstrating an inverse relationship (Supplemental Table 3). Increasing exposure to NO_2, PM_{10}, $PM_{2.5}$, and ozone during pregnancy was associated with increased ASD risk (Supplemental Table 4). Increased ASD risk was also seen when comparing high (≥ median value) exposure to low (<median) exposure for NRP and NO_2 during pregnancy (Supplemental Table 4).

Joint Effects of Air Pollutants and Periconceptional Folic Acid Intake

We found elevated, though non-statistically significant, risk for ASD among mothers with increasing NRP, NO_2, PM_{10}, and $PM_{2.5}$ exposure in the first trimester when there was also low periconceptional folic acid intake (Table 2). In contrast, ASD risk for increasing air pollutant exposure among mothers with high periconceptional folic acid intake was near null or protective. For example, increasing NO_2 among mothers with high periconceptional folic acid was associated with ASD in a protective direction (OR=0.74, 95% Confidence Interval (CI) (0.46–1.19), while increasing NO_2 among the low periconceptional folate group was associated with an increased risk of ASD (OR=1.53, 95% CI (0.91–2.56) though these associations did not reach statistical significance after adjustment. Notably, a statistically significant multiplicative interaction between NO_2 during the first trimester and periconceptional folic acid intake was identified (p=0.039) (Table 2). Similar findings were observed when air pollution exposures were averaged across all of pregnancy, or examined for specific trimesters (Supplemental Table 5).

When using reparameterized models including both dichotomous air pollutant and folic acid, we found that the mothers exposed to higher levels of NRP, NO_2, PM_{10}, and $PM_{2.5}$ during the first trimester of pregnancy and who reported low periconceptional folic acid intake were at a higher risk of ASD compared to those mothers exposed to lower levels of the same air pollutant and who reported high periconceptional folic acid intake (Table 3). For example, high NO_2 exposure and low folic acid intake was associated with an increased, though non-statistically significant ASD risk (1.38 (0.69, 2.75)) while high NO_2 and high folic acid intake showed protective effects (0.71 (0.44, 1.12)), both as compared to subjects with low NO_2 exposure and high folic acid. Paradoxically, an increased ASD risk was also seen among subjects with low $PM_{2.5}$ (1.56 (1.03, 2.38)) and ozone (1.54 (0.99, 2.40)) exposure as well as low folic acid. This same pattern of effects was present, but did not maintain statistical significance after adjustment. Additionally, for adjusted models, the odds ratios were higher than would be expected assuming additive or multiplicative interaction for NRP, NO_2, PM_{10}, and ozone (Table 3). Only for NRP and NO_2 did these effects indicate a significant departure from expected interaction (p<0.10). Results were consistent for the air pollution exposure during pregnancy (Supplemental Table 6), second (Supplemental Table 7) and the third trimester (Supplemental Table 8).

To determine if our findings were sensitive to categorization of air pollution exposure we examined the joint effects of folic acid intake and each air pollutant dichotomized at the 75th percentile. These models produced similar results for NRP, NO_2, and PM_{10} for the first trimester. Increased ASD risk was seen for the high air pollutant/low folic acid group for all pollutants in unadjusted models, and for subjects with low ozone exposure as well as low

folic acid. However, these associations did not remain statistically significant after adjustment (Supplemental Table 9). These adjusted joint effects were greater than expected additive and multiplicative interaction effects for all pollutants but ozone, and significant departures from additivity and multiplicativity were observed for NRP, as well as suggestions of departure from additivity for NO_2. During the whole pregnancy period, the high air pollution exposure / low folic acid group was at increased ASD risk in unadjusted models. An increased ASD risk was also seen among subjects with low $PM_{2.5}$ exposure as well as low folic acid. These results did not maintain statistical significance after adjustment. However, adjusted joint effects were greater than expected for both additive and multiplicative interactions for all pollutants except $PM_{2.5}$, though none demonstrated statistically significant departures (Supplemental Table 10).

Discussion

Our study examined the joint effects of perinatal folic acid intake and prenatal air pollution exposure, drawing on the rich exposure data at developmentally relevant time-points in the CHARGE study. Our results suggest that the associated risk of having a child with ASD may be attenuated in mothers exposed to high levels of air pollution (2 SD increase or above median levels of NRP, NO_2, PM_{10}, or $PM_{2.5}$) during the first trimester, if their reported folic acid during the first month of pregnancy was above 800μg. Specifically, it appears that the increased ASD risk from NO_2 may be attenuated by increased periconceptional folic acid. It is important to note that while the risk for ASD associated with high air pollution levels is attenuated among mothers with high folic acid in the first month of pregnancy, only two models showed statistical evidence for interaction and none of our re-parameterized effects reached statistical significance. As such these findings, while provocative, may be due to chance.

While the qualitative directions of effect we see here broadly suggest the potential for interaction, our sample size to test this hypothesis was limited likely resulting in imprecise estimates of effect. For reparameterized models it is difficult to determine if the associations with ASD presented here are truly different across exposure groups. We suggest these be interpreted as a set of estimates, rather than a single stratum, and in light of tests of departures from expected joint multiplicative and additive effects.

In this context, we interpret individual group comparisons with caution. For some pollutants, high exposure to air pollution (for example NRP, ozone, and NO_2), together with higher folic acid, show associations with ASD in the protective direction. While surprising that increasing air pollution exposure may show a protective association with ASD risk, these estimates may reflect imprecise statistical estimation or lack of association. They might also suggest that air pollution, particularly those pollutants mostly likely to induce oxidative stress, and folic acid together operate in susceptible subgroups of individuals where the protective effect of folic acid might swamp any deleterious effect of air pollution given that the joint effects for high air pollutant exposure and low folic acid show an elevated ASD risk. We also find that low air pollution exposure (for example $PM_{2.5}$) and low folic acid are associated with positive ORs. In this stratum ASD risk may perhaps be best explained by additional factors unexamined here.

Child's year of birth was added to all models because it substantially changed the magnitude of the primary association between NRP and ASD. Confounding by year of birth is likely an artifact of differential enrollment, because cases and controls were frequency matched on age, not year of birth, and enrollment of controls was delayed compared to enrollment of cases. In fact, children with later birth years were more likely to be a control, in contrast to the fact that ASD has been reportedly increasing over time. While cases and controls were similar for many characteristics, they did differ based on home ownership, self-report financial hardship, and maternal birthplace. These variables were included as potential confounders in our analysis as they may relate to air pollution exposure levels or prenatal folic acid intake as they reflect socio-economic status, which might reflect residential location and hence air pollution exposure level, or access to prenatal care. We believe that failure to account for such factors could have exacerbated estimates of association in this sample away from the null.

Effect modification of periconceptional folic acid intake on prenatal air pollution exposure was notably observed in air pollution exposures in the first trimester of pregnancy. Given that the half-life of folate in the body is ~100 days (Nijhout, Reed, Budu, & Ulrich, 2004) and the moderate to high correlations of our air pollution exposure assignments over time, it is reasonable to hypothesize that these measures may overlap in time beyond the first trimester of pregnancy. This is also congruent with the most critical time period for expectant mothers to be taking high amounts of folic acid (>600 μg/day), near and before conception for neural tube defects (NTDs) and potentially ASD protection (Centers for Disease Control (CDC), 1991; Schmidt et al., 2012). This also overlaps with previous findings suggesting that the association between folic acid and reduced ASD is primarily near conception (Schmidt et al., 2012; 2011; Surén et al., 2013) and other studies providing evidence for early pregnancy origins of ASD (Lyall et al., 2014; Rodier, Ingram, Tisdale, Nelson, & Romano, 1996; Schmidt, Lyall, & Hertz-Picciotto, 2014). However, we caution that it is difficult to evaluate potential protection of this recommended folic acid level in the context of air pollution exposure effects on health as no data on this topic exist to our knowledge and only one folic acid categorization was examined here.

Air pollutants have been shown to induce oxidative stress in *in vitro* studies, animal studies, and epidemiologic studies (Ghio, Carraway, & Madden, 2012; Kelly, 2003; Risom, Møller, & Loft, 2005). Additionally, studies have shown that children with ASD, and their mothers, have increased oxidative stress compared to controls (James et al., 2004; 2008). Because folic acid exhibits antioxidant properties and corrects conditions of oxidative stress (Joshi, Adhikari, Patro, Chattopadhyay, & Mukherjee, 2001), this could also explain the attenuated risk of ASD in mothers whose periconceptional folic acid intake was above 800 μg.

Folic acid has been shown to protect against the harmful developmental and reproductive effects exhibited by other environmental toxins, including bisphenol A (BPA) (Dolinoy, Huang, & Jirtle, 2007) and methomyl insecticide (Shalaby, Zorba, & Ziada, 2010) in animal studies, and pesticides (Ouyang et al., 2014) and arsenic (Gamble et al., 2006; Peters et al., 2016) in human studies. DNA methylation was demonstrated as the mechanism behind the protection for BPA (Dolinoy et al., 2007), and was proposed for methomyl insecticide (Shalaby et al., 2010) and arsenic (Howe et al., 2014; Lambrou et al., 2012; Tsang et al.,

2012). Exposure to higher ambient levels of traffic particles can decrease DNA methylation (Baccarelli et al., 2009). Furthermore, children with autism and their parents have been shown to have genome-wide DNA hypomethylation (James et al., 2004; 2008; 2010). It is possible that the attenuation of risk observed in this study was a result of increased methylation due to the higher levels of folate, thereby reducing the neurodevelopmental effects of air pollution. A recent crossover study of 10 adults showed changes in DNA methylation in 10 loci following two hours of controlled PM2.5 exposure. These changes were not observed in the same 10 loci when PM2.5 exposure followed four weeks of B vitamin supplementation, including high doses of folic acid (2.5mg/d) and vitamin B6 (50 mg/d) (Zhong et al., 2017).

Although we controlled for multiple socioeconomic factors, our results could also be affected by unmeasured confounding factors associated with autism, exposure to air pollutants, and folic acid supplementation. These factors might include lifestyle or other residential exposures. We have also not explored indoor sources of pollution, such as indoor nitrogen oxide or second-hand tobacco smoke. In addition, there could be residual confounding if proximity to diagnosing physicians or treatment centers was also associated with the exposures. We cannot also rule out the possibility that our limited sample size has affected the precision associated with our estimates of effect.

Previous air pollution analyses in CHARGE categorized all air pollutants using quartiles (Volk et al., 2013). Due to limited power when looking at combined effects of air pollution and folic acid, we categorized air pollutants instead using a median split to increase analytic power. In supplemental analysis, we examined the joint effects of FA and each air pollutant dichotomized that the 75th percentile. Though many of the results were similar, it should be noted that different thresholds with more extreme exposures might be needed in further analyses for some pollutants due to varying manifestation of biological effects. Additionally, the number of subjects included in this sample differed slightly from previous CHARGE publications and includes both autism and ASD in the case definition, where previous papers focused on autism alone. Additionally, this analysis includes only subjects from the middle range of CHARGE birth years that may limit the range of potential exposures in when air pollution is examined continuously.

Due to the retrospective reporting of vitamin and supplement information in this study, in which mothers were asked about a period several years before the interview and after the child's developmental status was known, there is a potential for recall bias. However, our previous study suggested that findings were not likely to be entirely due to recall bias for folic acid and ASD (Schmidt et al., 2012). Though differential recall bias cannot be ruled out, we are examining the joint effects of folic acid intake with air pollution. Therefore, in order for recall bias to be present in this study, recall differences between cases and controls would have to have occurred primarily in those with high air pollution exposure.

Our findings suggest that taking 800μg of supplemental folic acid (about the amount in a typical prenatal vitamin) in the first month of pregnancy may reduce the child's susceptibility for ASD if the mother is living in a highly polluted area. However, this was not the case for every air pollutant in every pregnancy period, nor did it reduce associated risk

completely. Replication of these analyses in samples with improved statistical power is needed to determine if our findings reflect an important potential to modify ASD risk or are due to chance. Future research should also seek to enhance understanding of the mechanisms behind any effect modification of folic acid and air pollution exposure on ASD, especially in studies with prospectively collected diet and supplement data and/or biomarkers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of financial support: Financial Support from The M.I.N.D. Institute matching funds and pilot grant program; NIEHS: ES015359, ES11269, ES021330, and ES019002; NICHD: 1U54HD079125; NIH: T32-MH073124; EPA Star-R: 829388, 833292;

We thank CHARGE families for participating in this research and CHARGE study investigators and staff for their time and effort. We also thank Irva Hertz-Picciotto for her advice, collaboration, and data access to the CHARGE study.

References

Alcorn SH, Lurmann FW. Southern California Children's Health Study exposure database. Petaluma, CA: Sonoma Technology, Inc, Technology Report STI- 95230-2453-FR3. 2003

Andersson T, Alfredsson L, Källberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. European Journal of Epidemiology. 2005; 20(7):575–579. http://doi.org/10.1007/s10654-005-7835-x. [PubMed: 16119429]

Baccarelli A, Wright RO, Bollati V, Tarantini L, Litonjua AA, Suh HH, et al. Rapid DNA Methylation Changes after Exposure to Traffic Particles. American Journal of Respiratory and Critical Care Medicine. 2009; 179(7):572–578. http://doi.org/10.1164/rccm.200807-1097OC. [PubMed: 19136372]

Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M. Autism as a strongly genetic disorder: evidence from a British twin study. Psychological Medicine. 1995; 25(01):63–77. http://doi.org/10.1017/S0033291700028099. [PubMed: 7792363]

Becerra TA, Wilhelm M, Olsen J, Cockburn M, Ritz B. Ambient air pollution and autism in Los Angeles county, California. Environmental Health Perspectives. 2013; 121(3):380–386. http://doi.org/10.1289/ehp.1205827. [PubMed: 23249813]

Benson PE. A review of the development and application of the CALINE3 and 4 models. Atmospheric Environment. Part B. Urban Atmosphere. 1992; 26(3):379–390.

Braun, JM., Froehlich, T., Kalkbrenner, A., Pfeiffer, CM., Fazili, Z., Yolton, K., Lanphear, BP. Brief Report: Are Autistic-Behaviors in Children Related to Prenatal Vitamin Use and Maternal Whole Blood Folate Concentrations?. Journal of Autism and Developmental Disorders. 2014. http://doi.org/10.1007/s10803-014-2114-x

Centers for Disease Control (CDC). Use of folic acid for prevention of spina bifida and other neural tube defects--1983–1991. MMWR. Morbidity and Mortality Weekly Report. 1991; 40(30):513–516. [PubMed: 2072886]

Centers for Disease Control and Prevention (CDC). Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. Morbidity and Mortality Weekly Report. Surveillance Summaries (Washington, D.C.: 2002). 2014; 63(2):1–21.

Christensen DL, Baio J, Braun KVN, Bilder D, Charles J, Constantino JN, et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network 11 Sites, United States, 2012. Morbidity and

Mortality Weekly Report. Surveillance Summaries (Washington, D.C.: 2002). 2016; 65(3):1–23. http://doi.org/10.15585/mmwr.ss6503a1.

Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. The Journal of Nutrition. 2002; 132(8 Suppl):2393S–2400S. [PubMed: 12163699]

Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proceedings of the National Academy of Sciences. 2007; 104(32):13056–13061. http://doi.org/10.1073/pnas.0703739104.

Ehrenstein von OS, Aralis H, Cockburn M, Ritz B. In utero exposure to toxic air pollutants and risk of childhood autism. Epidemiology. 2014; 25(6):851–858. http://doi.org/10.1097/EDE.0000000000000150. [PubMed: 25051312]

Gamble MV, Liu X, Ahsan H, Pilsner JR, Ilievski V, Slavkovich V, et al. Folate and arsenic metabolism: a double-blind, placebo-controlled folic acid-supplementation trial in Bangladesh. The American Journal of Clinical Nutrition. 2006; 84(5):1093–1101. [PubMed: 17093162]

Gardiki-Kouidou P, Seller MJ. Amniotic fluid folate, vitamin B12 and transcobalamins in neural tube defects. Clinical Genetics. 2008; 33(6):441–448. http://doi.org/10.1111/j.1399-0004.1988.tb03478.x.

Ghio AJ, Carraway MS, Madden MC. Composition of air pollution particles and oxidative stress in cells, tissues, and living systems. Journal of Toxicology and Environmental Health. Part B, Critical Reviews. 2012; 15(1):1–21. http://doi.org/10.1080/10937404.2012.632359.

Gong T, Almqvist C, Bölte S, Lichtenstein P, Anckarsäter H, Lind T, et al. Exposure to Air Pollution From Traffic and Neurodevelopmental Disorders in Swedish Twins. Twin Research and Human Genetics. 2014; 17(06):553–562. http://doi.org/10.1017/thg.2014.58. [PubMed: 25229653]

Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proceedings of the National Academy of Sciences. 2008; 105(44):17046–17049. http://doi.org/10.1073/pnas.0806560105.

Hertz-Picciotto I, Croen LA, Hansen R, Jones CR, van de Water J, Pessah IN. The CHARGE Study: An Epidemiologic Investigation of Genetic and Environmental Factors Contributing to Autism. Environmental Health Perspectives. 2006; 114(7):1119–1125. http://doi.org/10.1289/ehp.8483. [PubMed: 16835068]

Hosmer DW, Lemeshow S. Confidence Interval Estimation of Interaction. Epidemiology. 1992; 3(5):452. [PubMed: 1391139]

Howe CG, Niedzwiecki MM, Hall MN, Liu X, Ilievski V, Slavkovich V, et al. Folate and Cobalamin Modify Associations between S-adenosylmethionine and Methylated Arsenic Metabolites in Arsenic-Exposed Bangladeshi Adults. The Journal of Nutrition. 2014; 144(5):690–697. http://doi.org/10.3945/jn.113.188789. [PubMed: 24598884]

James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, Gaylor DW, Neubrander JA. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. The American Journal of Clinical Nutrition. 2004; 80(6):1611–1617. [PubMed: 15585776]

James SJ, Melnyk S, Jernigan S, Hubanks A, Rose S, Gaylor DW. Abnormal Transmethylation/transsulfuration Metabolism and DNA Hypomethylation Among Parents of Children with Autism. Journal of Autism and Developmental Disorders. 2008; 38(10):1976. http://doi.org/10.1007/s10803-008-0614-2. [PubMed: 19011973]

James SJ, Melnyk S, Jernigan S, Pavliv O, Trusty T, Lehman S, et al. A functional polymorphism in the reduced folate carrier gene and DNA hypomethylation in mothers of children with autism. American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: the Official Publication of the International Society of Psychiatric Genetics. 2010; 153B(6):1209–1220. http://doi.org/10.1002/ajmg.b.31094.

Joshi R, Adhikari S, Patro BS, Chattopadhyay S, Mukherjee T. Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. Free Radical Biology and Medicine. 2001; 30(12):1390–1399. http://doi.org/10.1016/S0891-5849(01)00543-3. [PubMed: 11390184]

Julvez J, Fortuny J, Mendez M, Torrent M, Ribas-Fitó N, Sunyer J. Maternal use of folic acid supplements during pregnancy and four-year-old neurodevelopment in a population-based birth

cohort. Paediatric and Perinatal Epidemiology. 2009; 23(3):199–206. http://doi.org/10.1111/j.1365-3016.2009.01032.x. [PubMed: 19775381]

Jung C-R, Lin Y-T, Hwang B-F. Air Pollution and Newly Diagnostic Autism Spectrum Disorders: A Population-Based Cohort Study in Taiwan. PLoS ONE. 2013; 8(9):e75510. http://doi.org/10.1371/journal.pone.0075510.t005. [PubMed: 24086549]

Kalkbrenner AE, Daniels JL, Chen J-C, Poole C, Emch M, Morrissey J. Perinatal Exposure to Hazardous Air Pollutants and Autism Spectrum Disorders at Age 8. Epidemiology. 2010; 21(5): 631–641. http://doi.org/10.1097/EDE.0b013e3181e65d76. [PubMed: 20562626]

Kalkbrenner AE, Windham GC, Serre ML, Akita Y, Wang X, Hoffman K, et al. Particulate matter exposure, prenatal and postnatal windows of susceptibility, and autism spectrum disorders. Epidemiology. 2015; 26(1):30–42. http://doi.org/10.1097/EDE.0000000000000173. [PubMed: 25286049]

Kelly FJ. Oxidative stress: its role in air pollution and adverse health effects. Occupational and Environmental Medicine. 2003; 60(8):612–616. [PubMed: 12883027]

Lambrou A, Baccarelli A, Wright RO, Weisskopf M, Bollati V, Amarasiriwardena C, et al. Arsenic Exposure and DNA Methylation Among Elderly Men. Epidemiology. 2012; 23(5):668–676. http://doi.org/10.1097/EDE.0b013e31825afb0b. [PubMed: 22833016]

Lord C, Risi S, Lambrecht L, Cook EH, Leventhal BL, DiLavore PC, et al. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. Journal of Autism and Developmental Disorders. 2000; 30(3):205–223. [PubMed: 11055457]

Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. Journal of Autism and Developmental Disorders. 1994; 24(5):659–685. [PubMed: 7814313]

Lyall K, Schmidt RJ, Hertz-Picciotto I. Maternal lifestyle and environmental risk factors for autism spectrum disorders. International Journal of Epidemiology. 2014; 43(2):443–464. http://doi.org/10.1093/ije/dyt282. [PubMed: 24518932]

MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. Lancet. 1991; 338(8760): 131–137. [PubMed: 1677062]

National Institutes of Health. [Retrieved June 16, 2016] Dietary Supplement Fact Sheet: Folate — Health Professional Fact Sheet. 2016. from https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/

Nijhout HF, Reed MC, Budu P, Ulrich CM. A mathematical model of the folate cycle: new insights into folate homeostasis. The Journal of Biological Chemistry. 2004; 279(53):55008–55016. http://doi.org/10.1074/jbc.M410818200. [PubMed: 15496403]

Ouyang F, Longnecker MP, Venners SA, Johnson S, Korrick S, Zhang J, et al. Preconception serum 1,1,1-trichloro-2,2,bis(p-chlorophenyl)ethane and B-vitamin status: independent and joint effects on women's reproductive outcomes. American Journal of Clinical Nutrition. 2014; 100(6):1470–1478. http://doi.org/10.3945/ajcn.114.088377. [PubMed: 25411282]

Peters BA, Hall MN, Liu X, Parvez F, Sanchez TR, van Geen A, et al. Folic Acid and Creatine as Therapeutic Approaches to Lower Blood Arsenic: A Randomized Controlled Trial. Environmental Health Perspectives. 2016; 123(12):1–1. http://doi.org/10.1289/ehp.1409396.

Raghavan, R., Fallin, MD., Wang, X. Maternal plasma folate, vitamin B12 levels and multivitamin supplementation during pregnancy and risk of Autism Spectrum Disorder in the Boston Birth Cohort. Presented at the In: Proceedings of Experimental Biology Meeting; 2–6 April 2016; San Diego, CA, USA. Federation of American Societies for Experimental Biology; 2016. p. 151.6-151.6.http://doi.org/10.1096/fj.1530-6860

Raz, R., Roberts, AL., Lyall, K., Hart, JE., Just, AC., Laden, F., Weisskopf, MG. 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. Environmental Health Perspectives. 2014. http://doi.org/10.1289/ehp.1408133

Reik W, Walter J. Genomic imprinting: parental influence on the genome. Nature Reviews Genetics. 2001; 2(1):21–32. http://doi.org/10.1038/35047554.

Risom L, Møller P, Loft S. Oxidative stress-induced DNA damage by particulate air pollution. Mutation Research. 2005; 592(1–2):119–137. http://doi.org/10.1016/j.mrfmmm.2005.06.012. [PubMed: 16085126]

Ritvo ER, Freeman BJ, Mason-Brothers A, Mo A, Ritvo AM. Concordance for the syndrome of autism in 40 pairs of afflicted twins. The American Journal of Psychiatry. 1985; 142(1):74–77. [PubMed: 4038442]

Roberts, AL., Lyall, K., Hart, JE., Laden, F., Just, AC., Bobb, JF., et al. Perinatal Air Pollutant Exposures and Autism Spectrum Disorder in the Children of Nurses' Health Study II Participants. Environmental Health Perspectives. 2013. http://doi.org/10.1289/ehp.1206187

Rodier PM, Ingram JL, Tisdale B, Nelson S, Romano J. Embryological origin for autism: developmental anomalies of the cranial nerve motor nuclei. Journal of Comparative Neurology. 1996; 370(2):247–261. [PubMed: 8808733]

Roza SJ, van Batenburg-Eddes T, Steegers EAP, Jaddoe VWV, Mackenbach JP, Hofman A, et al. Maternal folic acid supplement use in early pregnancy and child behavioural problems: The Generation R Study. The British Journal of Nutrition. 2010; 103(3):445–452. http://doi.org/10.1017/S0007114509991954. [PubMed: 19772683]

Schlotz W, Jones A, Phillips DIW, Gale CR, Robinson SM, Godfrey KM. Lower maternal folate status in early pregnancy is associated with childhood hyperactivity and peer problems in offspring. Journal of Child Psychology and Psychiatry. 2009; 51(5):594–602. http://doi.org/10.1111/j.1469-7610.2009.02182.x. [PubMed: 19874428]

Schmidt RJ, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tancredi DJ, et al. Prenatal Vitamins, One-carbon Metabolism Gene Variants, and Risk for Autism. Epidemiology. 2011; 22(4):476–485. http://doi.org/10.1097/EDE.0b013e31821d0e30. [PubMed: 21610500]

Schmidt RJ, Lyall K, Hertz-Picciotto I. Environment and autism: current state of the science. Cutting Edge Psychiatry in …. 2014; 19(11):1220–1226. http://doi.org/10.1038/mp.2014.18.

Schmidt RJ, Tancredi DJ, Ozonoff S, Hansen RL, Hartiala J, Allayee H, et al. Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. American Journal of Clinical Nutrition. 2012; 96(1):80–89. http://doi.org/10.3945/ajcn.110.004416. [PubMed: 22648721]

Shalaby MA, Zorba, El HY, Ziada RM. Reproductive toxicity of methomyl insecticide in male rats and protective effect of folic acid. Food and Chemical Toxicology: an International Journal Published for the British Industrial Biological Research Association. 2010; 48(11):3221–3226. http://doi.org/10.1016/j.fct.2010.08.027. [PubMed: 20813150]

Shaw GM, Carmichael SL, Yang W, Selvin S, Schaffer DM. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. American Journal of Epidemiology. 2004; 160(2):102–109. http://doi.org/10.1093/aje/kwh187. [PubMed: 15234930]

Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, Lindemans J, Siebel C, Steegers EA, et al. Periconceptional Maternal Folic Acid Use of 400 μg per Day Is Related to Increased Methylation of the IGF2 Gene in the Very Young Child. PLoS ONE. 2009; 4(11):e7845. http://doi.org/10.1371/journal.pone.0007845. [PubMed: 19924280]

Steenweg-de Graaff J, Ghassabian A, Jaddoe VWV, Tiemeier H, Roza SJ. Folate concentrations during pregnancy and autistic traits in the offspring. The Generation R Study. European Journal of Public Health. 2015; 25(3):431–433. http://doi.org/10.1093/eurpub/cku126. [PubMed: 25085472]

Surén P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, et al. Association Between Maternal Use of Folic Acid Supplements and Risk of Autism Spectrum Disorders in Children. JAMA: the Journal of the American Medical Association. 2013; 309(6):570–577. http://doi.org/10.1001/jama.2012.155925. [PubMed: 23403681]

Talbott EO, Arena VC, Rager JR, Clougherty JE, Michanowicz DR, Sharma RK, Stacy SL. Fine particulate matter and the risk of autism spectrum disorder. Environmental Research. 2015a; 140:414–420. http://doi.org/10.1016/j.envres.2015.04.021. [PubMed: 25957837]

Talbott EO, Marshall LP, Rager JR, Arena VC, Sharma RK, Stacy SL. Air toxics and the risk of autism spectrum disorder: the results of a population based case-control study in southwestern Pennsylvania. Environmental Health: a Global Access Science Source. 2015b; 14(1):80. http://doi.org/10.1186/s12940-015-0064-1. [PubMed: 26444407]

Tsang V, Fry RC, Niculescu MD, Rager JE, Saunders J, Paul DS, et al. The epigenetic effects of a high prenatal folate intake in male mouse fetuses exposed in utero to arsenic. Toxicology and Applied Pharmacology. 2012; 264(3):439–450. http://doi.org/10.1016/j.taap.2012.08.022. [PubMed: 22959928]

U S Dept Agriculture, A. R. S. [Retrieved May 11, 2012] USDA National Nutrient Database for Standard Reference, Release 23. 2010 Jan 7. from http://www.ars.usda.gov/ba/bhnrc/ndl

Virk J, Liew Z, Olsen J, Nohr EA, Catov JM, Ritz B. Preconceptional and prenatal supplementary folic acid and multivitamin intake and autism spectrum disorders. Autism: the International Journal of Research and Practice. 2015; 20(6):710–718. http://doi.org/10.1177/1362361315604076. [PubMed: 26408631]

Volk HE, Hertz-Picciotto I, Delwiche L, Lurmann F, Mcconnell R. Residential Proximity to Freeways and Autism in the CHARGE Study. Environmental Health Perspectives. 2010; 119(6):873–877. http://doi.org/10.1289/ehp.1002835. [PubMed: 21156395]

Volk HE, Lurmann F, Penfold B, Hertz-Picciotto I, Mcconnell R. Traffic-Related Air Pollution, Particulate Matter, and Autism. JAMA Psychiatry. 2013; 70(1):71. http://doi.org/10.1001/jamapsychiatry.2013.266. [PubMed: 23404082]

Windham GC, Zhang L, Gunier R, Croen LA, Grether JK. Autism Spectrum Disorders in Relation to Distribution of Hazardous Air Pollutants in the San Francisco Bay Area. Environmental Health Perspectives. 2006; 114(9):1438–1444. http://doi.org/10.1289/ehp.9120. [PubMed: 16966102]

Wolff GL, Kodell RL, Moore SR, Cooney CA. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 1998; 12(11):949–957. [PubMed: 9707167]

Zhong J, Karlsson O, Wang G, Li J, Guo Y, Lin X, et al. B vitamins attenuate the epigenetic effects of ambient fine particles in a pilot human intervention trial. Proceedings of the National Academy of Sciences of the United States of America. 2017; 114(13):3503–3508. [PubMed: 28289216]

Lay Summary

We examined interactions between periconceptional folic acid (FA) and air pollution exposure on risk of ASD. Mothers exposed to higher levels of air pollution during the first trimester of pregnancy and who reported low supplemental FA intake during the first pregnancy month were at a higher ASD risk compared to mothers exposed to lower levels of air pollution and who reported high first month FA intake. Our results suggest that periconceptional FA intake may reduce ASD risk in those with high prenatal air pollution exposure.

Table 1

Demographic characteristics of CHARGE cases with Autism spectrum disorder(ASD) and controls with typical development (n = 606).

Demographic Variable	N (%) Controls (n=260)	Cases (n=346)	Chi-square *p*-value
Male sex	213 (81.9)	297 (85.8)	0.191
Child race/ethnicity			
White	136 (52.3)	179 (51.7)	
Hispanic	51 (19.6)	65 (18.8)	0.921
Other	73 (28.1)	102 (29.5)	
Child's Year of Birth			
1998 – 2001	34 (13.1)	122 (35.3)	
2002 – 2003	108 (41.5)	100 (28.9)	<0.001
2004 – 2007	118 (45.4)	124 (35.8)	
Financial Hardship[a] (% yes)	29 (11.2)	70 (20.2)	0.003
Home Ownership (% yes)[b]	205 (79.2)	234 (67.8)	0.002
Maximum education in home			
Some college or less	104 (40.0)	137 (39.6)	0.920
Bachelor degree	156 (60.0)	209 (60.4)	
Mother's Birthplace			
Born in USA	216 (83.1)	263 (76.0)	0.034
Born outside USA	44 (16.9)	83 (24.0)	
Maternal age ≥ 35 years	51 (19.6)	82 (23.7)	0.229
Gestational Diabetes (% yes)[c]	11 (4.3)	28 (8.1)	0.058
Preterm delivery (< 37 weeks)[d]	22 (8.6)	29 (8.6)	0.998
Parity[e]			
1	114 (43.9)	156 (45.4)	
2	92 (35.4)	131 (38.1)	0.605
3	39 (15.0)	40 (11.6)	
4+	15 (5.8)	17 (4.9)	
Prenatal vitamin use during 1st month of pregnancy	160 (61.5)	179 (51.7)	0.016
Folic Acid intake during 1st month of pregnancy			
0–800 μg	109 (41.9)	180 (52.0)	0.014
801+ μg	151 (58.1)	166 (48.0)	

Abbreviations: CHARGE, Childhood Autism Risk from Genetics and the Environment;

[a]Self report of financial hardship during index period;

[b]Missing 1 control, 1 case

[c]Missing 2 controls,

[d]Missing 3 controls, 7 cases;

[e] Missing 2 cases.

Table 2

Odds of ASD for 606 Children based on Continuous Pollutant Exposure by Periconceptional Folic Acid Intake[a]

Air pollutant	Month 1 FA (< or ≥ 800 μg)	Odds Ratio (95% CI)[b] 1st Trimester	
		Unadjusted	Adjusted[c]
NRP	Low	1.87 (1.09, 3.19)	1.57 (0.92, 2.70)
	High	0.98 (0.63, 1.51)	0.92 (0.59, 1.45)
	Interaction p	0.066	0.137
NO_2	Low	1.87 (1.14, 3.09)	1.53 (0.91, 2.56)
	High	1.05 (0.67, 1.64)	0.74 (0.46, 1.19)
	Interaction p	0.088	0.039
PM_{10}	Low	1.49 (0.92, 2.42)	1.33 (0.81, 2.19)
	High	1.11 (0.71, 1.74)	0.94 (0.59, 1.49)
	Interaction p	0.384	0.311
$PM_{2.5}$	Low	1.27 (0.80, 2.01)	1.13 (0.70, 1.83)
	High	1.16 (0.73, 1.85)	0.97 (0.60, 1.59)
	Interaction p	0.782	0.664
Ozone	Low	1.16 (0.73, 1.84)	1.07 (0.66, 1.73)
	High	1.29 (0.82, 2.04)	1.14 (0.71, 1.82)
	Interaction p	0.737	0.868

Abbreviations: ASD, autism spectrum disorder; FA, folic acid; NRP, near roadway air pollution; NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter < 2.5 μm; PM_{10}, particulate matter < 10 μm.

[a] Folic acid intake is dichotomized at 800 μg (high vs. low) and includes all folic acid intake during the first month of pregnancy.

[b] Air pollution effects reflect odds of autism based on 2 SDs from the mean value, for each pollutant at each time period, as reflected in Supplemental Figure.

[c] Model was adjusted for self-reported financial hardship between 3 months before pregnancy to time of interview (yes/no), child's year of birth, vitamin A and zinc intake during the first month of pregnancy.

Table 3

Joint associations between ASD, folic acid (FA) intake[a] and air pollution (near roadway air pollution (NRP) and regional pollutants)[b] during the first trimester of pregnancy. N=606

				Unadjusted Model	Adjusted Model[c]					
						Additive interaction			Multiplicative Interaction	
Poll. < or ≥ median		FA < or ≥ 800 μg	N ASD/ Control	OR (95% CI)	OR (95% CI)	Expected Joint OR	RERI p-value	AP p-value	Expected Joint OR	Interaction p-value
NRP	Low	High	85/71	1.0 (ref)	1.0 (ref)					
		Low	78/61	1.07 (0.67, 1.69)	0.88 (0.45, 1.73)					
	High	High	81/80	0.85 (0.54, 1.32)	0.75 (0.47, 1.19)					
		Low	102/48	1.77 (1.11, 2.83)	1.18 (0.58, 2.37)	0.63	0.076	0.059	0.66	0.091
NO_2	Low	High	87/74	1.0 (ref)	1.0 (ref)					
		Low	82/67	1.04 (0.67, 1.63)	0.78 (0.40, 1.51)					
	High	High	79/77	0.87 (0.56, 1.36)	0.71 (0.44, 1.12)					
		Low	98/42	1.98 (1.23, 3.20)	1.38 (0.69, 2.75)	0.49	0.011	<0.001	0.55	0.008
PM_{10}	Low	High	93/86	1.0 (ref)	1.0 (ref)					
		Low	86/62	1.28 (0.83, 1.99)	1.02 (0.53, 1.96)					
	High	High	73/65	1.04 (0.67, 1.62)	0.89 (0.56, 1.42)					
		Low	94/47	1.85 (1.17, 2.92)	1.28 (0.65, 2.51)	0.91	0.294	0.262	0.91	0.319
$PM_{2.5}$	Low	High	95/96	1.0 (ref)	1.0 (ref)					
		Low	102/66	1.56 (1.03, 2.38)	1.26 (0.66, 2.39)					
	High	High	71/55	1.30 (0.83, 2.05)	1.19 (0.74, 1.90)					
		Low	78/43	1.83 (1.15, 2.93)	1.33 (0.67, 2.68)	1.45	0.793	0.798	1.50	0.742
Ozone	Low	High	90/83	1.0 (ref)	1.0 (ref)					
		Low	95/57	1.54 (0.99, 2.40)	1.19 (0.61, 2.30)					
	High	High	76/68	1.03 (0.66, 1.61)	0.88 (0.56, 1.40)					
		Low	85/52	1.51 (0.96, 2.38)	1.08 (0.56, 2.08)	1.07	0.971	0.971	1.05	0.923

Abbreviations: ASD, autism spectrum disorder; FA, folic acid; NRP, near roadway air pollution; NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter < 2.5 μm; PM_{10}, particulate matter < 10 μm.

[a] Folic acid intake is dichotomized at 800 μg (high vs. low) and includes all folic acid intake during the first month of pregnancy.

[b] All pollutants were dichotomized at their whole pregnancy period median (high vs low) (NRP = 15.58 ppb; NO_2 = 14.18 ppb; PM_{10} = 22.88 μg/cm^3; $PM_{2.5}$ = 12.40 μg/cm^3; Ozone = 33.41 μg/cm^3).

[c] Model was adjusted for self-reported financial hardship between 3 months before pregnancy to time of interview (yes/no), child's year of birth, vitamin A and zinc intake during the first month of pregnancy.