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Abstract

Background: Autism spectrum disorder (ASD) is a developmental disorder with increasing prevalence worldwide, yet with unclear etiology.

Objective: To explore the association between maternal exposure to particulate matter (PM) air pollution and odds of ASD in her child.

Methods: We conducted a nested case-control study of participants in the Nurses' Health Study II (NHS II), a prospective cohort of 116,430 US female nurses recruited in 1989, followed by biennial mailed questionnaires. Subjects were NHS II participants' children born 1990-2002 with ASD (n=245), and children without ASD (n=1522) randomly selected using frequency matching for birth years. ASD was based on maternal report, which was validated against the Autism Diagnostic Interview-Revised in a subset. Monthly averages of PM with diameters ≤2.5 μm ($PM_{2.5}$) and 2.5-10 μm ($PM_{10-2.5}$) were predicted from a spatiotemporal model for the continental US and linked to residential addresses.

Results: $PM_{2.5}$ exposure during pregnancy was associated with increased odds of ASD, with an adjusted odds ratio (OR) for ASD per interquartile range higher $PM_{2.5}$ (4.42 μg/m^3) of 1.57 (95% CI: 1.22, 2.03) among women with the same address before and after pregnancy (160 cases, 986 controls). Associations with $PM_{2.5}$ exposure 9 months before or after the pregnancy were weaker in independent models and null when all three time periods were included, while the association with the 9 months of pregnancy remained (OR=1.63; 95% CI: 1.08-2.47). The association between ASD and $PM_{2.5}$ was stronger for exposure during the third trimester (OR=1.42 per interquartile range increase in $PM_{2.5}$, 95% CI: 1.09, 1.86) than other trimesters (ORs 1.06 and 1.00) when mutually adjusted. There was little association between $PM_{10-2.5}$ and ASD.

Conclusions: Higher maternal exposure to $PM_{2.5}$ during pregnancy, in particular the third trimester, was associated with greater odds of her child having ASD.

Introduction

Autism spectrum disorder (ASD) is a developmental disorder with increasing reported prevalence worldwide (French et al. 2013). Though genetics plays a strong role in ASD, evidence suggests that environmental exposures, in particular *in utero* or during early life, also affect ASD risk (Grønborg et al. 2013; Hallmayer et al. 2011; Quaak et al. 2013). However, no specific environmental toxicant has been consistently associated with increased risk of ASD.

Air pollution contains various toxicants that have been found to be associated with neurotoxicity and adverse effects on the fetus in utero (Crump et al. 1998; Grandjean and Landrigan 2006; Rice and Barone 2000; Rodier 1995; Stillerman et al. 2008). Airborne particles are covered with various contaminants, and have been found to penetrate the subcellular environment and induce oxidative stress and mitochondrial damage *in vitro* (Li et al. 2002; MohanKumar et al. 2008). In rodents, they have also been found to stimulate inflammatory cytokine release systemically and in the brain, and alter the neonatal immune system (Hertz-Picciotto et al. 2005, 2008; MohanKumar et al. 2008) – processes that have been implicated in ASD (Depino 2013; Napoli et al. 2013).

Several studies have explored associations of air pollution with ASD, using the United States Environmental Protection Agency (USEPA) hazardous air pollutants models (Environmental Protection Agency), distance to freeway, or local models for specific pollutants. These studies suggest increased odds of having a child with ASD with higher exposures to diesel particulate matter (PM) (Roberts et al. 2013; Windham et al. 2006), several metals (Roberts et al. 2013; Windham et al. 2006), criteria pollutants (Becerra et al. 2013; Volk et al. 2013) and some organic materials as well as closer proximity to a freeway (Volk et al. 2011).

Our goal was to explore the association between ASD and exposure to PM during defined time periods before, during, and after pregnancy, within the Nurses' Health Study II (NHS II), a large, well-defined cohort with detailed residential history. This nested case-control study includes participants from across the continental United States (US) and exposure was linked to monthly data on two size fractions of PM.

Methods

Participants

The study population included offspring of participants in NHS II, a prospective cohort of 116,430 US female nurses aged 25-43 when recruited in 1989, followed biennially (Solomon et al. 1997). NHS II participants originally were recruited from 14 states in all regions of the continental US, but they now reside in all 50 states. The study was approved by the Partners Health Care Institutional Review Board and complied with all applicable U.S. regulations; return of completed questionnaires constituted consent to participate.

In 2005, NHS II participants were asked whether any of their children had been diagnosed with autism, Asperger's syndrome or 'other autism spectrum', and 839 women replied affirmatively. In 2007, we initiated a pilot follow-up study, shortly followed by a full-scale follow-up as described previously (Lyall et al. 2012). The follow-up questionnaire included questions about the pregnancy and birth, child's sex, and diagnosis. NHS II protocol only allows re-contacting nurses who responded to the most recent biennial questionnaire. Thus, this follow-up was attempted with the 756 mothers of ASD cases for whom this was the case. Mothers who reported having more than one child with ASD were directed to report about the youngest one. Controls were selected from among parous women not reporting a child with ASD in 2005. For each case

mother, controls were randomly selected from among those women who gave birth to a child in a matching birth year, to yield a total of 3,000 controls. Six hundred and thirty-six (84%) mothers of cases and 2,747 (92%) mothers of controls responded; 164 women (including 51 case mothers) declined to participate.

For the current study, only children whose estimated conception month was June 1989 or later were included since nurses' addresses before this month were unknown. Of the 265 children reported to have an ASD diagnosis who met this criterion we excluded 4 for whom ASD was not confirmed by the mother on the follow-up questionnaire, and another 2 with genetic syndromes associated with ASD (N=1, Down syndrome, N=1 Rett syndrome). The remaining 259 children were classified as ASD cases. There were 1640 control children who met the conception month criterion. We further excluded participants missing PM data because their addresses could not be geocoded (8 cases and 30 controls), controls who were reported to have ASD on the 2009 questionnaire (N=9), and children missing data on birth month (6 cases and 79 controls). The final study sample included 245 cases and 1522 controls born 1990 through 2002. The average (SD) year of diagnosis of the ASD cases was 1999 ± 3.3. None of these children were reported to have been adopted. Of 188 ASD cases with data on ASD in siblings, 7.4% were reported to have a sibling with ASD. Analyses excluding those 7.4% were similar to analyses including all children and are therefore not reported.

Case validation

ASD diagnosis was validated by telephone administration of the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al. 1994) in a subsample of 50 cases randomly selected from mothers who indicated on our follow-up questionnaire willingness to be contacted (81% of all case

mothers). In this sample, 43 children (86%) met full ADI-R criteria for autistic disorder (which is stricter than the broader "autism spectrum disorder" of current DSM-V criteria, or other autism spectrum disorders including PDD-NOS or Asperger syndrome of DSM-IV criteria), defined by meeting cutoff scores in all three domains (social interaction; communication and language; restricted and repetitive behaviors) and having onset by age 3. The remaining individuals met the onset criterion and communication domain cutoff and missed the autistic disorder cutoff by one point in one domain (n=5; 10%), or met cutoffs in one or two domains only (n=2; 4%), thus indicating presence of ASD traits (see Lord et al., 1994 for further details on scoring of ADI-R) (Lord et al. 1994). In addition, Social Responsiveness Scale scores (Constantino and Przybeck 2000), obtained for approximately 90% of eligible cases, also were indicative of accuracy of case ascertainment. While not a clinical diagnostic instrument, the SRS is a widely used measure of social functioning and autistic traits, and has been shown to have excellent validity as compared to ADI-R and ADOS (Constantino et al. 2003). Among our ASD cases, 93% met the SRS cutoff for ASD. In contrast, 93% of controls completing the same measure fell within the normative range. Therefore, both ADI-R and SRS scores support reliable ASD case ascertainment in our population. For all analyses only the maternal reports were used for determination of ASD status.

Exposure assessment

Residential locations of the nurses were determined from the mailing addresses used for the biennial NHS II questionnaire. Monthly ambient exposure predictions of airborne particulate matter with an aerodynamic diameter $\leq 10\mu m$ (PM_{10}) and $\leq 2.5\mu m$ ($PM_{2.5}$) were generated from nationwide expansions of previously validated spatiotemporal models (Yanosky et al. 2008, 2009, 2014). The models use monthly average PM_{10} and/or $PM_{2.5}$ data from USEPA's Air

Quality System, a nationwide network of continuous and filter-based monitors, as well as monitoring data from various other sources. The models also incorporated information on several geospatial predictors including distance to road, population density, point sources (e.g. power generating utilities, waste combustors), elevation and meteorology. All data were used in generalized additive statistical models (Yanosky et al. 2008) with smoothing terms of space and time to create separate PM prediction surfaces for each month. Since monitoring data on $PM_{2.5}$ is limited prior to 1999, $PM_{2.5}$ in the period before 1999 was modeled using data on PM_{10} and visibility data at airports (Yanosky et al. 2009, 2014). $PM_{10-2.5}$ predictions were calculated as the difference between monthly PM_{10} and $PM_{2.5}$ predictions. These models provide estimates for any geolocation in the conterminous US by monthly intervals. The models also have been shown to have low bias and high precision: the normalized mean bias factor for $PM_{2.5}$ is -1.6% and the absolute value of the prediction errors is 1.61. For $PM_{2.5-10}$ these values are -3.2% and 4.18, respectively (Yanosky et al. 2014).

For each child, exposures to $PM_{2.5}$ and $PM_{10-2.5}$ before, during, and after pregnancy were estimated by averaging monthly concentrations for the mother's residential address during the relevant months. The months of pregnancy were determined from the child's birth month and gestational age at birth, as reported by the mother. Exposures to PM during each pregnancy trimester were calculated similarly.

Covariates

The following covariates, all associated with autism in previous studies, were included in multivariable models: child's birth year, birth month and sex, maternal age at birth, paternal age at birth, and median census tract income in the birth year. Among these variables, only census

tract income (1.5%) and paternal age (10.6%) had missing data. We used the missing indicator method for missing data. We conducted sensitivity analyses to evaluate the influence of adjusting for gestational factors (premature birth, birth weight, gestational diabetes, preeclampsia), smoking during pregnancy, state, marital status, median census house value, paternal education and maternal grandparents' education. All covariate data except for census variables were from maternal self-report.

Statistical analyses

Logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI) of ASD by PM exposures modeled both using PM quartiles and as continuous variables, in separate models. Exposures to different PM size fractions were examined in separate models, and also together in a single model.

For nurses who moved between two questionnaires straddling pregnancy, we did not know the exact date of moving. Therefore, we conducted separate analyses for exposures assigned assuming the nurse was at the earlier address during the whole intervening period (pre-pregnancy address) or at the later address during the whole period (post-pregnancy address). In addition, in order to reduce misclassification of exposure, we conducted analyses that were limited to those mothers for whom the pre- and post-pregnancy addresses were identical (160 cases [65%] and 986 controls [65%], referred to here as "non-movers").

To examine temporal specificity of any associations between PM and ASD, we considered the association with $PM_{2.5}$ exposure during the 9 months before pregnancy, the pregnancy period, and the 9 months after birth. These examinations were restricted to non-movers with complete data for all exposure periods, and each time period was considered independently, and then also

in a single model that included all three time periods simultaneously. Because of differences in ASD rates by sex and prior suggestions that air pollution effects may be specific to boys, we a priori decided to also examine associations stratified by sex of the child. For simplicity, we did this only among the children whose mothers did not move during pregnancy. SAS version 9.3 (SAS Institute Inc.) was used for data extraction, and R version 3.0.1 for Linux-gnu was used for analyses. All analyses were conducted at the 0.05 alpha level.

Results

ASD cases were more likely to be male, to have been exposed to maternal preeclampsia or maternal smoking during gestation, and to be missing data on premature birth compared with controls (Table 1). The median (25th-75th percentile) year of birth for cases and controls was the same: 1993 (1991-1996). As expected given time trends in air pollution, control children born in earlier years were more likely to be in higher $PM_{2.5}$ quartiles. Census income and parental age also decreased slightly, but generally steadily by exposure, while there was little clear pattern of difference by exposure for other variables (Table 2).

The average (SD) levels of $PM_{2.5}$ and $PM_{10-2.5}$ during pregnancy were 14.6 ± 3.3 and 9.9 ± 4.9 $\mu g/m^3$, respectively. While $PM_{10-2.5}$ did not show a clear and consistent association with ASD, $PM_{2.5}$ was associated with ASD regardless of the address used for the PM estimation (Figure 1). Among non-movers, for whom misclassification of exposure because of an address change is reduced, the odds ratio (OR) was 2.06 (95% CI: 1.17, 3.63) in the 4th quartile, comparing to the 1st quartile. The results were also similar when limiting to non-movers and using continuous PM estimates, with an OR of 1.57 (95% CI: 1.22, 2.03) per inter-quartile range (IQR) increase in $PM_{2.5}$ (4.42 $\mu g/m^3$), and little association with $PM_{10-2.5}$ (OR=1.07 per $PM_{10-2.5}$ IQR [5.15 $\mu g/m^3$],

95% CI: 0.89, 1.28). When $PM_{2.5}$ and $PM_{10-2.5}$ were in the same model together the difference between the two was greater: OR=1.61 per $PM_{2.5}$ IQR (95% CI: 1.22-2.12); OR=0.96 per $PM_{10-2.5}$ IQR (95% CI: 0.79-1.17). The association with $PM_{2.5}$ among non-movers was slightly stronger for boys (OR=1.73, 95% CI: 1.29-2.31) than girls (OR=1.12, 95% CI: 0.59-2.12), but there were only 23 non-mover girls with ASD (137 non-mover boys) and the interaction p-value was 0.17.

When estimating the association with $PM_{2.5}$ exposure during the 9 months before pregnancy, the pregnancy period, and the 9 months after birth, all restricted to non-movers with exposure estimates for all 3 exposure periods, the associations with exposures before or after the pregnancy were lower compared to the association with exposure during pregnancy (Table 3). The partial correlation of $PM_{2.5}$ during pregnancy with $PM_{2.5}$ during the 9 months before or after pregnancy was 0.85 and 0.83, respectively. When we included all three $PM_{2.5}$ exposure periods together in a mutually adjusted model, ASD was significantly associated only with exposure during the pregnancy period (Table 3). This pattern did not change after further restriction to women who did not move during the whole period from 9 months before conception to 9 months after birth (data not shown).

When examining trimester-specific associations in non-movers, exposure to $PM_{2.5}$ was associated with ASD in all three trimesters, while $PM_{10-2.5}$ was not associated with ASD in any of the trimesters (Figure 2). The highest association with $PM_{2.5}$ was seen in the third trimester (OR=1.49 per $PM_{2.5}$ IQR, 95% CI: 1.20, 1.85) (Figure 2). In a model with all trimesters mutually adjusted, the only statistically significant association was seen with $PM_{2.5}$ in the third trimester (OR=1.42, 95% CI: 1.09, 1.86), while exposure during the first and second did not show

associations (OR= 1.06, 95% CI: 0.83, 1.35, and OR=1.00, 95% CI: 0.78, 1.30 respectively). When third trimester $PM_{2.5}$ and $PM_{10-2.5}$ were in the same model together the difference between the two was greater: OR=1.50 per $PM_{2.5}$ IQR (95% CI: 1.19-1.89); OR=0.89 per $PM_{10-2.5}$ IQR (95% CI: 0.81-1.19).

Odds ratios and CIs were comparable in separate analyses excluding premature births, or participants missing data on census tract income, or paternal age (data not shown). Adjusting for $PM_{10-2.5}$ also resulted in comparable estimates for $PM_{2.5}$ (data not shown). Results were also similar in models adjusted for (each in a separate model): gestational variables (premature birth, birth weight, gestational diabetes, preeclampsia), smoking during pregnancy, census tract house value, state, marital status of the nurse, or husband's/partner's education or maternal grandparents' education (data not shown). In addition, models limited to either mothers with white race/ethnicity (95% of the nurses) or children who had a full term pregnancy (i.e. excluding premature births and those with missing data on this variable) showed comparable estimates (data not shown).

Discussion

In our nested case-control study of nurses from across the continental US, ambient $PM_{2.5}$ concentrations during pregnancy were significantly associated with having a child diagnosed with ASD. Importantly, the association we found appeared specific to $PM_{2.5}$ during pregnancy; $PM_{2.5}$ exposure before or after pregnancy showed weaker associations with ASD, and $PM_{10-2.5}$ during pregnancy showed little association with ASD. In a model mutually adjusted for all three exposure periods, only the pregnancy period was associated with ASD. The change in the ORs with mutual adjustment did not appear to be an artifact of collinearity because the precision of

the mutually adjusted model was not substantially lower than the single exposure model (e.g., CI widths for an IQR change in PM2.5 during pregnancy of 2.3 versus 1.7, respectively). The 95% CI were not notably larger in this analysis, suggesting collinearity was not a significant problem. Moreover, during pregnancy we found the association to be specifically with the third trimester exposure in models that included exposure in all trimesters together. The specificity of the association to the prenatal period is in line with several other lines of evidence that suggest a prenatal origin of ASD including data on differences in brain cytoarchitecture in brains of children with ASD (McFadden and Minshew 2013; Stoner et al. 2014) and associations between maternal exposure to teratogens during pregnancy and ASD (Rodier 1995). Our results also suggest an association predominantly in boys, but this finding should be interpreted with caution, given the small number of girls with ASD in our sample.

These results generally agree with previous studies. A report from the CHildhood Autism Risks from Genetics and the Environment (CHARGE) study among 304 ASD cases and 259 controls, in several areas in California, used residential address history reported by parents to calculate distance to roads as a proxy for traffic-related air pollution exposure and found increased risk for ASD among women who lived in proximity to a freeway (Volk et al. 2011). Further analysis of the CHARGE study group in a subset of 279 cases and 245 controls using data from the USEPA Air Quality System, suggested positive associations of ASD with traffic-related air pollution during pregnancy, and specifically with $PM_{2.5}$ (Volk et al. 2013). ASD was also associated with pregnancy exposure to PM_{10}, and - in contrast to our results - the association with traffic-related air pollution exposure during the first year of life was higher than that found for the exposure during pregnancy. In the CHARGE study, associations were also seen with exposures in the year

after birth that were about as strong as exposures during pregnancy. Our findings suggested a weaker association with post-pregnancy exposure that was essentially null in models that included exposure during all time periods. In the CHARGE study, however, the pregnancy and post-pregnancy exposure periods were not included together in the same regression model.

Another study, from Los Angeles (LA) county, used birth certificate address and ASD cases identified from the Department of Developmental Services in California (Becerra et al. 2013). Using exposure data from the nearest monitoring stations and from a land use regression model (Su et al. 2009), they found a positive association between $PM_{2.5}$ exposure and autism (OR per 4.68 µg/m3 $PM_{2.5}$ = 1.15; 95% CI: 1.06, 1.24 in a model of exposure over the entire pregnancy and also adjusted for ozone levels). There was not a consistent association with PM_{10}. The LA study included many more ASD cases than any of the other studies and so the effect estimate could represent a more stable estimate of the true effects of PM. Alternatively, differences in the composition of PM in the LA area could result in smaller effects. Other differences in study design could also have led to smaller effect sizes in the LA study. The case definition was a primary diagnosis of autistic disorder, the most severe among ASD diagnoses, and the association with PM could be preferentially with milder forms of ASD. Slightly more measurement error from using a nearest monitor exposure assignment approach or addresses from the birth certificate could have biased results towards the null. Smaller associations in that study could also have occurred if there was under-ascertainment of cases among children of more highly exposed mothers. Lower socioeconomic status has been associated with under-ascertainment in ASD registries like that used in the LA study (Kalkbrenner et al. 2012). Although estimates were not much different when the sample was stratified by education level, if

residual socioeconomic differences were associated with $PM_{2.5}$ exposures (lower socioeconomic status with higher $PM_{2.5}$) this could lead to bias toward the null since the controls included all birth certificates in the region. The importance of the environment in the development of ASD was recently implicated in a comparison of concordance rates between monozygotic and dizygotic twins that found that the shared environment accounted for 58% (95% CI: 30-80%) of the broader autism phenotype (Hallmayer et al. 2011). In line with these findings, a comparison of sibling ASD recurrence risk in a different population revealed much higher rate of ASD recurrence in half-siblings with the same mother (2.4, 95% CI: 1.4-4.1) compared with half-siblings with the same father (1.5, 95% CI: 0.7-3.4) (Grønborg et al. 2013). This finding may be attributed either to maternal factors affecting the in utero environment or to common mitochondrial DNA.

Exposure to high levels of environmental toxicants during pregnancy might interfere with normal *in utero* processes of brain development, such as neurogenesis, cell proliferation, cell differentiation, and apoptosis (Rice and Barone 2000; Rodier 1995). $PM_{2.5}$, and especially ultrafine particles (<0.1μm in diameter) were shown to penetrate the subcellular environment and to induce strong oxidative stress and mitochondrial damage *in vitro* (Li et al. 2002). These effects were associated with the organic carbon and polycyclic aromatic hydrocarbon contents of the particles (Li et al. 2002). *In vivo* studies in rodents have also shown that $PM_{2.5}$ activates the stress axis, involves microglial activation, and causes production of pro-inflammatory cytokines in the brain (MohanKumar et al. 2008). In one study, increased mitochondrial DNA damage, possibly caused by reactive oxygen species, was found to be more common in 67 children with ASD than in 46 typically developing children (Napoli et al. 2013).

$PM_{2.5}$ may alter the development of the neonatal immune system. In a study of 1,397 children in the Czech Republic, gestational exposures to $PM_{2.5}$ and polycyclic aromatic hydrocarbons were associated with reduction in T cells and an increase in B lymphocytes in neonatal cord blood (Hertz-Picciotto et al. 2005). Early activation of the immune system and neuroinflammation have been found to be associated with ASD in humans (Atladóttir et al. 2010; Careaga et al. 2013; Depino 2013; Gadad et al. 2013; Libbey et al. 2005; Patterson 2011) and in animal models of autism (Gadad et al. 2013; Libbey et al. 2005; Patterson 2011), and this has been proposed as a possible mechanism by which environmental toxicants could increase the risk of ASD (Hertz-Picciotto et al. 2008). Furthermore, a recent transcriptomic comparison of post-mortem brain tissues of individuals with ASD (N=19) and controls (N=17) taken from the Autism Tissue Project, the Harvard Brain Bank and the MRC London Brain bank for Neurodegenerative Disease, revealed involvement of genes related to synaptic and neuronal signaling dysfunction, and also microglial and immune dysregulation. The implicated genes related to synaptic and neuronal signaling dysfunction, compared with those related to immune changes, had more overlap with genes identified in genome-wide association studies (Voineagu et al. 2011). This may suggest that expression of immune-related genes in ASD may be driven more by environmental influences than underlying genetic differences.

These processes that could affect neurodevelopment are general in nature and so the question still would remain as to why there is an association specifically with ASD. However, the ASD phenotype is quite heterogeneous, and ASD can share features with other neurodevelopmental disorders (e.g. intellectual disability). There is some suggestion that exposures to components of air pollution can also affect neurodevelopment more generally (Perera et al. 2009; Suglia et al.

2008). Determining the range of phenotypic profiles associated with maternal PM exposure during pregnancy would be of interest in future studies. Another interesting direction for future research would be to determine whether the association between PM and ASD is different among children who have one or more siblings with ASD.

A limitation of our study is that we did not have the exact dates on which nurse mothers changed addresses. Thirty five percent of the nurses (both cases and controls) changed their residential address between the last questionnaire before pregnancy and the first questionnaire after delivery. However, we found statistically significant associations with pregnancy PM when the exposure for movers was based either on pre- or post-pregnancy address. When we reduced exposure misclassification by analyzing the smaller sample of non-movers, the association between ASD and $PM_{2.5}$ was stronger. We also did not have information on how much time the nurses actually spent at their residential address, nor did we have information about their work addresses. Error from this source, however, would not affect the estimates of PM at the residential address and so would not create an association with residential PM levels where an association with PM exposure did not exist. Information was also unavailable on indoor air pollution exposures and sources. In addition, ASD diagnoses in the study were based on parental reporting. As medically trained professionals, however, nurse mothers' reporting of ASD is likely to be reliable, a supposition supported by our validation study using the ADI-R.

Strengths of our study include the wide geographic distribution of the nurses and the nesting within a well-defined cohort, which reduces the likelihood of selection bias. In addition, the specificity of our findings for the pregnancy period places important limitations on possible residual confounding. Specifically, any factor that is not differentially related to PM during

pregnancy vs. before or after pregnancy is very unlikely to confound our results. Thus, for example, while population density, choosing to take folate supplements during pregnancy, or a host of other potential confounders (Gray et al. 2013; Kalkbrenner et al. 2012) may be related to $PM_{2.5}$ exposure, they would be expected to be equally related to $PM_{2.5}$ exposure before or after pregnancy as during it. But no association with them were seen in mutually adjusted models. In this way, $PM_{2.5}$ exposure before and after pregnancy (because no association is seen with them in mutually adjusted models) act as negative controls (Flanders et al. 2011; Lipsitch et al. 2010) and rule out confounding by many - even unmeasured - potential confounders. We cannot, however, rule out another pollutant that co-varies with $PM_{2.5}$. Nor can we determine if there is a particular component of $PM_{2.5}$ that is responsible for the associations we found. $PM_{2.5}$, however, is a complex mixture that may be correlated with other air pollution constituents. In the current study we did not have high temporal and spatial resolution data on other air pollution constituents or on specific $PM_{2.5}$ components to determine whether a specific component is associated with autism.

Conclusions

Our findings support the possibility of an effect of maternal exposure to air pollution during pregnancy, and especially during the third trimester, on the development of ASD in her child. It suggests that air pollution is a modifiable risk factor for autism, and reduced exposure during pregnancy could lead to lower incidence of ASD and reduce the substantial, increasing economic burden of ASD on families and on society (Croen et al. 2006; Leslie and Martin 2007; Mandell et al. 2006; Raz et al. 2013; Shimabukuro et al. 2008). Understanding the biological mechanism that may underlie the association by which PM exposure and ASD could provide important insight to ASD pathogenesis.

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Table 1. Study population characteristics by ASD status, Nurses' Health Study II.

Characteristics	Cases (N=245)	Controls (N=1,522)
Male sex, N (%)	209 (85)	793 (52)
Year of birth, median (IQR)	1993 (5)	1993 (5)
Maternal age at birth (years), mean ± SD	34.0 ± 4.0	33.7 ± 3.7
Paternal age at birth (years), mean ± SD	36.8 ± 5.3	36.3 ± 4.9
Median census income ($1,000), median (IQR)	63 (26)	61 (27)
Median census house value ($1,000), median (IQR)	144 (108)	136 (98)
Birth weight (lbs.), mean ± SD	7.1 ± 1.5	7.2 ± 1.3
Husband's / partner's education, N (%)		
High school	33 (13)	208 (14)
2 year college	45 (18)	218 (14)
4 year college	79 (32)	537 (35)
Graduate school	74 (30)	501 (33)
Missing	14 (6)	58 (4)
Marital Status, N (%)		
Married	186 (76)	1159 (76)
Never married	51 (21)	269 (18)
Other	8 (3)	94 (6)
Premature birth, N (%)		
Yes	44 (18)	227 (15)
No	142 (58)	1137 (75)
Missing	59 (24)	158 (10)
Gestational diabetes, N (%)		
Yes	17 (7)	87 (6)
No	189 (77)	1222 (80)
Missing	39 (16)	213 (14)
Preeclampsia, N (%)		
Yes	13 (5)	43 (3)
No	193 (79)	1266 (83)
Missing	39 (16)	213 (14)
Smoking during pregnancy, N (%)		
Yes	22 (9)	50 (3)
No	160 (65)	1099 (72)
Missing	63 (26)	373 (25)

Abbreviations: ASD, autism spectrum disorders; IQR, Interquartile range.

Table 2. Control population characteristics by pregnancy PM$_{2.5}$ quartile, Nurses' Health Study II (N = 1522 controls).

PM$_{2.5}$ Quartile (Range) µg/m^3	1st (5.24-12.3)	2nd (12.4-14.5)	3rd (14.6-16.7)	4th (16.7-30.8)
N	397	376	375	374
Male sex, N (%)	208 (52)	203 (54)	192 (51)	190 (51)
Year of birth, median (IQR)	1995 (5)	1994 (4)	1993 (4)	1992 (3)
Maternal age at birth (years), mean ± SD	34.3 ± 3.8	34.0 ± 3.8	33.5 ± 3.6	32.7 ± 3.6
Paternal age at birth (years), mean ± SD	37.4 ± 5.1	36.5 ± 4.9	36.1 ± 5.0	35.2 ± 4.5
Median census income ($1,000), median (IQR)	62 (31)	64 (28)	61 (26)	58 (24)
Median census house value ($1,000), median (IQR)	137 (107)	144 (104)	135 (96)	128 (82)
Birth weight (lbs.), mean ± SD	7.2 ± 1.3	7.2 ± 1.2	7.1 ± 1.3	7.2 ± 1.3
Premature birth, N (%)				
Yes	56 (14)	57 (15)	52 (14)	62 (17)
No	298 (75)	282 (75)	275 (73)	282 (75)
Missing	43 (11)	36 (10)	48 (13)	30 (8)
Gestational diabetes, N (%)				
Yes	18 (5)	27 (7)	21 (6)	21 (6)
No	314 (79)	303 (81)	299 (80)	306 (82)
Missing	65 (16)	46 (12)	55 (15)	47 (13)
Preeclampsia, N (%)				
Yes	12 (3)	9 (2)	8 (2)	14 (4)
No	320 (81)	321 (85)	312 (83)	313 (84)
Missing	65 (16)	46 (12)	55 (15)	47 (13)
Smoking during pregnancy, N (%)				
Yes	17 (4)	17 (4)	21 (5)	17 (4)
No	323 (73)	313 (71)	308 (70)	315 (71)
Missing	102 (23)	112 (25)	112 (25)	110 (25)

Abbreviations: IQR, Interquartile range; PM$_{2.5}$, particulate matter with an aerodynamic diameter ≤2.5 µm.

Table 3. Odds ratios (95% CI) for ASD per interquartile range increase in PM$_{2.5}$ levels in different time periods, non-movers only.[a]

Exposure Period	Odds Ratio (95% CI) per 4.40 µg/m^3 PM$_{2.5}$		
	Unadjusted	Adjusted[b]	Mutually adjusted[c]
9 months before conception	1.20 (0.98, 1.47)	1.32 (1.04, 1.69)	0.83 (0.58, 1.19)
Whole pregnancy	1.37 (1.09, 1.71)	1.50 (1.16, 1.94)	1.63 (1.08, 2.47)
9 months after birth	1.19 (0.96, 1.49)	1.29 (1.00, 1.67)	0.96 (0.65, 1.40)

Abbreviations: CI, confidence interval; IQR, interquartile range; ASD, autism spectrum disorders; PM$_{2.5}$, particulate matter with an aerodynamic diameter ≤2.5 µm.

[a]Restricted to non-movers who also have data on all exposure periods (158 cases, 977 controls).

[b]Adjusted for child sex, year of birth, month of birth, maternal age at birth, paternal age at birth, census income. [c]Mutually adjusted for other two exposure periods, as well as all other covariates listed above.

Figure legends

Figure 1. Odds ratios for autism spectrum disorder by quartile of particulate matter exposure. Odds ratios are adjusted for child sex, year of birth, month of birth, maternal age at birth, paternal age at birth, and census income. There were 245 cases and 1522 controls in analyses using pre- and post-pregnancy addresses. Pre-pregnancy address is the last known residential address before conception. Post-pregnancy address is the first known residential address after birth. Non-movers are those participants for whom pre-pregnancy and post-pregnancy addresses were the same (cases=160 (65%), controls=986 (65%)). P trend = p values from models of exposures as continuous variables. The number of cases (including movers) by quartiles from low to high: 45, 66, 66, 68; controls: 397, 376, 375, 374. $PM_{2.5}$ quartile ranges ($\mu g/m^3$): 5.24-12.3, 12.4-14.5, 14.6-16.7, 16.7-30.8; $PM_{10-2.5}$ quartile ranges ($\mu g/m^3$): 1.9-6.7, 6.8-8.9, 9-11.9, 12-49.4.

Figure 2. Odds ratios for autism spectrum disorder with exposure to particulate matter during pregnancy trimesters. Odds ratios are adjusted for child sex, year of birth, month of birth, maternal age at birth, paternal age at birth, and census income. The analyses are limited to non-movers only (i.e. those for whom pre-pregnancy and post-pregnancy addresses were the same). N cases=160, controls=986.
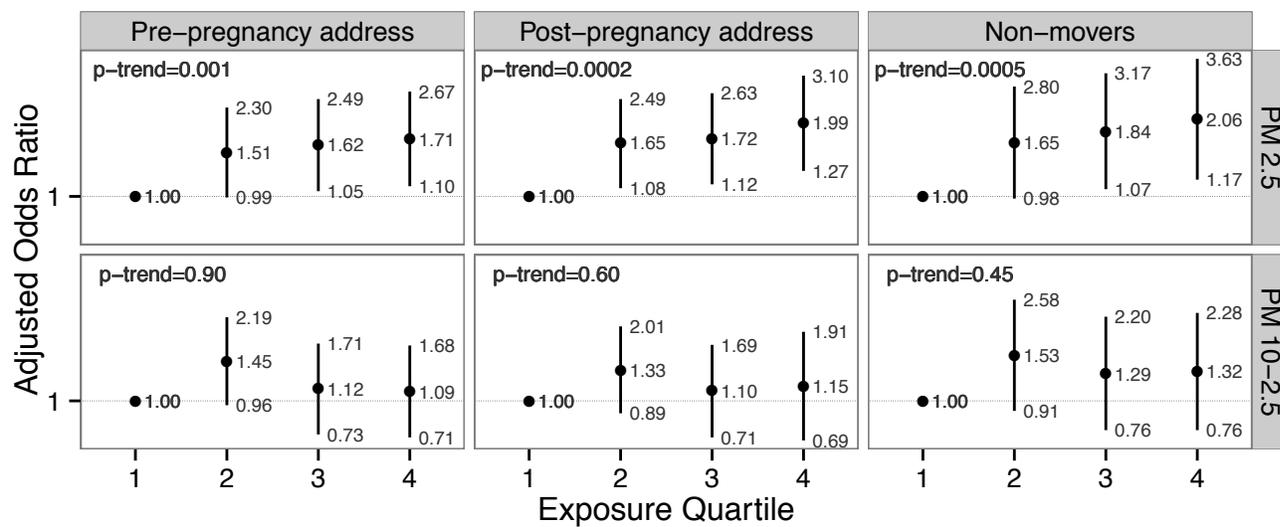
Figure 1.

Figure 2.