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Residential Proximity to Freeways and Autism in the CHARGE Study

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Abstract

Background: Little is known about environmental causes and contributing factors for autism. Basic science and epidemiological research suggest that oxidative stress and inflammation may play a role in disease development. Traffic-related air pollution, a common exposure with established effects on these pathways, contains substances found to have adverse prenatal effects.

Objectives: To examine the association between autism and residence proximity, during pregnancy and near the time of delivery, to freeways and major roadways as a surrogate for air pollution exposure.

Methods: Data were from 304 autism cases and 259 typically developing controls enrolled in the Childhood Autism Risks from Genetics and the Environment (CHARGE) Study. The mother's address recorded on the birth certificate and trimester specific addresses derived from a residential history obtained by questionnaire were geo-coded and measures of distance to freeways and major roads were calculated using ArcGIS software. Logistic regression models compared residential proximity to freeways and major roads for autism cases and typically developing controls.

Results: Adjusting for sociodemographic factors and maternal smoking, maternal residence at the time of delivery was more likely be near a freeway (≤ 309 meters) for cases, as compared to controls (odds ratio (OR), 1.86, 95% confidence interval (CI) 1.04-3.45). Autism was also associated with residential proximity to a freeway during the third trimester (OR, 2.22, CI, 1.16-4.42). After adjustment for socio-economic and demographic characteristics, these associations were unchanged. Living near other major roads at birth was not associated with autism.

Conclusions: Living near a freeway was associated with autism. Examination of associations with measured air pollutants is needed.

Introduction

Autism is a developmental disorder characterized by significant deficits in social interaction and communication, accompanied by repetitive behaviors (American Psychiatric Association 2000). Data from family and twin studies have long supported the role of genetics in autism etiology (Abrahams and Geschwind 2008; Muhle et al. 2004). Results from linkage, copy number variation, and genome-wide association studies further support the importance of genetic risk in this disease (Abrahams and Geschwind 2008; Ma et al. 2009; Wang et al. 2009). Over the last 10 years, the prevalence of diagnoses of autism, and all autism spectrum disorders (ASDs), has increased (Centers for Disease Control and Prevention (CDC) 2007a, b, 2009). While changes in diagnostic criteria and improved ascertainment have been thought to contribute to this increase, recent reports suggest that these factors may not fully explain the rising incidence of ASDs (Hertz-Picciotto and Delwiche 2009; King and Bearman 2009). Therefore, it is likely that environmental factors may augment the strong genetic risks implicated in autism etiology.

Air pollution exposure during pregnancy has been reported to have physical and developmental effects on the fetus. High levels of air pollution, including carbon monoxide (CO), nitrogen dioxide (NO₂), and ambient particulate matter (PM), have been associated with very low and low birth weight, preterm birth, and infant mortality (Currie et al. 2009; Ritz and Yu 1999). Specific pollutants, including ozone (O₃), sulfur dioxide (SO₂), PM, and CO, have also been associated with significant differences in biparietal diameter and head circumference both measured during pregnancy and at birth (Hansen et al. 2008; Vassilev et al. 2001). Maternal exposure to polycyclic aromatic hydrocarbons (PAHs) during pregnancy has been associated

with impaired cortical function and cognitive developmental delay (Bocskay et al. 2005; Perera et al. 2004; Perera et al. 2007; Perera et al. 2006; Perera et al. 2003).

Exposure to air pollution and its components, not only in the prenatal period but also in early postnatal life, has been linked to poor developmental outcomes as well. A recent epidemiologic study reported that use of gas appliances and increased NO₂ in the home during the first three months of life are associated with decreased cognitive test scores and increased inattention at four years of age (Morales et al. 2009). In a separate study, Suglia and colleagues estimated lifetime residential exposure to black carbon, a proxy for traffic-related PM, among 8-11 year old children and reported decreased performance on intelligence and memory tasks with increasing black carbon levels (Suglia et al. 2008). Additionally, autism has been associated with estimated regional concentrations of hazardous air pollutants, including arsenic and nickel, and with diesel PM exposure in early childhood (Windham et al. 2006).

Thus, an emerging literature suggests that near roadway traffic-related air pollutants, possibly influenced by specific components such as particulate matter or PAHs, affect neurodevelopment. However, the role of timing for this exposure during pregnancy or early life is not clear, nor has the relationship between traffic related air pollutants and autism been tested. In this study, we examine the relationship between autism and traffic proximity (a marker of traffic-related air pollution) during the prenatal period and at the time of birth.

Materials and Methods

We utilized data from 304 autism cases and 259 typically developing general population controls from the Childhood Autism Risks from Genetics and the Environment (CHARGE) study, a population-based case-control study of preschool children. The study design is described in detail elsewhere (Hertz-Picciotto et al. 2006). Briefly, CHARGE subjects were

between the ages of 24 and 60 months at the time of recruitment which occurred 2003-2009, lived with at least one English- or Spanish-speaking biologic parent, were born in California, and resided in one of the study catchment areas at time of enrollment. Recruitment was facilitated by the California Department of Developmental Services (DDS) and the Regional Centers with which they contract to coordinate services for persons with autism and other developmental disabilities. Population-based controls were recruited from the sampling frame of birth files from the state of California and were frequency matched by gender, age, and broad geographic area to the autism cases. All births were between 1997 and 2006.

Each participating family was evaluated in person. Children with a DDS diagnosis of autism were evaluated using the Autism Diagnostic Observation Schedules (ADOS) and parents were administered the Autism Diagnostic Interview-Revised (ADI-R) (Le Couteur et al. 2003; Lord et al. 2003). Children with a diagnosed developmental delay and general population controls were given the Social Communication Questionnaire (SCQ) to screen for the presence of autistic features (Rutter et al. 2003). If the SCQ score was 15 or higher, the ADOS was then administered to the child and the ADI-R to the parent. In our study, autism cases were children with a diagnosis of autism from both the ADOS and the ADI-R. All children were also assessed using the Mullen Scales of Early Learning (MSEL) and the Vineland Adaptive Behavior Scales (VABS) to collect information on motor skills, language, socialization, and daily living skills (Mullen 1995; Sparrow et al. 1984). Controls were children sampled from the general population with typical development, defined as having received a score less than or equal to 15 on the SCQ and who scored in the normal range on the MSEL and VABS, thereby showing no evidence of other types of delay (cognitive or adaptive).

Parents were also interviewed extensively to evaluate household exposures, demographic and medical information, and to assess reproductive, occupational, and residential histories. The residential history captured addresses and corresponding dates the mother and child lived at each location beginning 3 months before conception and extending to the most recent place of residence. Further details about the collection of clinical and exposure data have been previously reported (Hertz-Picciotto et al. 2006).

We examined associations of autism with traffic-related pollutant exposure using two broad proxies: distance to the nearest freeway and distance to the nearest major road. In accord with our previous research, a freeway was defined as a state highway or interstate highway (Gauderman et al. 2007). A major road was defined as a state highway, interstate highway, or major arterial (McConnell et al. 2006). Mother's residential address at birth, as recorded on the birth certificate, was geo-coded and distances to the nearest interstate highway, state highway, and major arterial road were estimated based on the shortest distance from the residence to the middle of the nearest side of each of the three road types using ArcGIS software (version 9.2; Environmental Systems Research Institute Inc., Redlands, CA). For each subject, freeway distance was then assigned as the shorter of the distances from the birth residence to a state or interstate highway. Similarly, major road distance was assigned as the shortest of the three distances: from a state highway, interstate highway, or major arterial. Under these definitions, it was possible for freeway and major road distances to be the same should the same road type (ex; state highway) provide the shortest distance measure for a given address. For freeway and major road distances, we examined the distribution of values among the 563 subjects in our study and determined exposure cut-points based on the top 10%, next 15%, and subsequent 25% of

distance values for freeways and for major road. The remaining 50% served as a reference category in each analysis.

Information from the residential history was used to estimate exposure to residential traffic during the first, second, and third trimesters of gestation for a subset of subjects with complete data (N=485; 257 cases and 228 controls). We determined the conception date for each child using gestational age from ultrasound measurements or the date of last menstrual period, as determined from prenatal records. Then, we calculated dates corresponding to each trimester and selected the appropriate address from the residential history. If more than 1 address fell into a trimester, we chose the address where the subjects had spent the majority of time. Addresses were geocoded and distances estimated as described above.

We used logistic regression to estimate the association between distance to the nearest freeway or major road and autism. Pertinent covariates were included in the model to adjust for potential confounding due to socio-demographic or lifestyle characteristics. Specifically, we included child's gender and ethnicity, maximum education level of the parents, maternal age, gestational age at birth, and maternal smoking during pregnancy. We obtained 95% confidence limits as measures of precision and determined statistical significance using an alpha level of 0.05.

Results

Description of Sample

The study population was 84% male and most participants were Caucasian (51%) or Hispanic (29%). No significant differences were found between cases and controls for any demographic or socioeconomic variables examined (Table 1). For the majority of participants, geo-coded birth certificate addresses (mother's residence at delivery) indicated that residences at

birth were concentrated in the areas around Sacramento, Los Angeles, and the San Francisco East & North Bay.

Distance to Freeway

We examined the distribution of distance from the nearest freeway among subjects in our study and determined exposure cut-points to define the closest 10% (<309m), subsequent 15% (309m-647m), and following 25% (647m-1419m) as exposure groups. The remaining 50% (>1419m) served as the reference group in our analysis. Living within 309m of a freeway at birth was associated with autism (odds ratio (OR) 1.86, 95% confidence interval (CI) 1.04-3.45). This association was not altered by adjustment for child gender or ethnicity, maximum education in the home, maternal age, or maternal smoking during pregnancy (Table 2). When we categorized our distance measure into deciles, only the top 10% corresponding to the <309m category above, showed evidence of an increased autism risk compared to those living farthest from the freeway (lowest decile, >5,150m) (unadjusted OR=2.48, 95%CI 1.17-5.39).

Among the subset of subjects with available residential history data, distance to the freeway measures were highly correlated across trimesters, reflecting the limited number of subjects who changed residence during pregnancy (Number moved: 17 between 1st and 2nd, 13 between 2nd and 3rd, 30 between 1st and 3rd). In each trimester living closest to the freeway (<309m versus >1419m) was associated with autism, but the OR reached statistical significance only during the third trimester (adjusted OR=1.96, 95%CI 1.01-3.93). Effect estimates for the first and second trimesters were slightly lower in magnitude (first trimester adjusted OR=1.66, 95%CI 0.91-3.10; second trimester adjusted OR=1.65, 95%CI 0.85-3.28). After restricting the sample with birth certificate addresses to those with residential history data for all three trimesters (N=485; 257 cases and 228 controls), the odds ratio for autism was more than doubled

among those living within 309m of a freeway versus >1419m (adjusted OR=2.22, 95%CI 1.16-4.42) consistent with a late-pregnancy or early life effect.

Distance to Major Road

The distribution of distance from a major road among subjects in our study was reflected in exposure cut-points corresponding to ≤ 42 m (the closest 10%), 42m-96m (subsequent 15%), and 96m-209m (following 25%) as exposure groups. The remaining 50% (>209m) served as the reference group in our analysis. There was no consistent pattern of association of autism with proximity to a major road and all were changed only slightly after adjusting for distance to the freeway (Table 3). Inclusion of child gender or ethnicity, maximum education in home, maternal age, or prenatal smoking in the model did not alter these associations. Results were similar for the three trimesters.

Discussion

We observed an increased risk of autism among the 10% of children living within 309m of a freeway around the time of birth. Our findings appeared to be limited to only this group as analysis of further distances did not demonstrate associations. Analysis of trimester-specific residential information yielded associations of roughly similar magnitude, though only the effects for the third trimester and at birth reached statistical significance. The high correlations across trimesters, and lack of analysis of postnatal residences, implies that we cannot precisely define a potentially critical window.

The association of autism with freeway, and not major road, proximity may be related to the larger volume of traffic and concentrations of pollutants observed near freeways. In Los Angeles, for example, some freeways have more than 300,000 vehicles daily, and high concentrations of traffic-related pollutants with steep gradients extending several hundred meters from the traffic corridor (Caltrans 2008; Zhu et al. 2002; Zhu et al. 2006). Specifically, studies measuring concentration and size distribution of ultrafine particles near a major California freeway demonstrate that the particles are high nearest the freeway and become closer to background levels at distances 300 meters and greater (Zhu et al., 2002). Thus, our findings are consistent with the relationship between freeway proximity and particle exposures in California. Our study did not find evidence of associations with residential proximity beyond the 300 meter range, and we currently lack adequate sample size to estimate the effect of living in even closer proximity to the freeway (<100 m) where high concentrations of particles have been detected. To examine the effects of proximity at closer distances to major roadways we estimated autism risk among subjects living within 96m (the top quartile of exposure vs. greater than 96m) and among those living within 300m (corresponding to the region of highest exposure vs. greater

than 300m) and found slightly elevated non-statistically significant risks (OR=1.17, 95%CI 0.80-1.72 and OR=1.19, 95%CI 0.84-1.68, respectively).

The traffic volumes on the classes of other major roadways used in this analysis are likely to be highly variable across California, so exposure to traffic-related pollutants on the spatial scale of interest may be less well classified by residential proximity to other major roadways than by proximity to freeways. For example, we found that the average distance to a freeway among subjects living in the second major road exposure group (42m-96m), with slightly increased risk of autism, was much shorter ((mean distance \pm SD= 1,481m \pm 1,761m) than in other major road categories (2,643m \pm 2,245m for major road <42m and 1,917m \pm 3,946m for major road 96m-209m). Residential traffic proximity has been associated with childhood asthma and lung function growth in previous studies we have conducted in Southern California and some of these associations have been restricted to freeway proximity or traffic modeled from freeway traffic volume (Gauderman et al. 2005; Gauderman et al. 2007; McConnell et al. 2006; McConnell et al. 2010).

We found little evidence of confounding by the SES and demographic characteristics included in this analysis. These factors were hypothesized *a priori* confounders based on literature reporting increased autism rates in higher SES areas, while areas of lower SES are more likely to have higher levels of air pollutants (Sexton et al. 1993). In our study, we observed no difference in level of education in the home among autism cases and controls and little effect of adjusting for these factors on the traffic and autism association suggesting that our results were not biased by such factors. In California, clusters of autism tend to have higher levels of parental education, and in countries with highly variable access to health care, diagnosed cases of autism tend to be of higher SES than the general population; at the same time, controls that

participate in studies are almost always of higher SES than non-participants (Van Meter et al 2010).

To date, little research has examined the association of air pollutants and autism. Using the Environmental Protection Agency (EPA) Hazardous Air Pollutants (HAPs) monitoring network Windham and colleagues identified an increased autism risk with modeled estimates of regional census tract ambient exposure to diesel exhaust particles, as well as metals (mercury, cadmium and nickel) and chlorinated solvents, in the San Francisco Bay Area of northern California (Windham et al. 2006). Additional research using models from HAPs program found associations between autism and air toxics at the birth residence of children from North Carolina and West Virginia (Kalkbrenner et al. 2010). Our analysis builds on this work by examining associations with individual level indicators of exposure based on traffic proximity, prenatally and at birth.

Toxicological studies suggest a biologically plausible role of air pollution in disrupting brain development and function during critical time points in gestation and early life. Diesel exhaust particles present in traffic related pollution have been shown to have endocrine disrupting activity, and to trans-placentally affect sexual differentiation and alter cognitive function in mice (Hougaard et al. 2008; Watanabe and Kurita 2001). Prenatal exposure to O₃ in rats has been seen to alter monoamine content in the cerebellum, which may then alter neural circuitry formation (Gonzalez-Pina et al. 2008). Recent work examining the effects of benzo(a)pyrene (B(a)P), a common PAH, indicates that prenatal oral exposure in mice results in decreased neuronal plasticity and behavioral deficits (Brown et al. 2007). Specifically, prenatal exposure was associated with reduced glutamate receptor development when synapses are formed. Additionally, exposure to B(a)P via breast feeding in mice during the early post-natal

period, corresponding to the rapid human brain development taking place during the third trimester, was found to affect neuromaturation as measured by classic developmental behavior tests and to reduce expression of the serotonin receptor *5HT1A* (Bouayed et al. 2009; Pan et al. 2009).

Traffic related air pollutants have been observed to induce inflammation and oxidative stress after both short term and long term exposures in toxicological and human studies, and these pathways are thought to mediate effects of air pollution on respiratory and cardiovascular disease, and perhaps on neurological outcomes (Block and Calderon-Garciduenas 2009; Calderon-Garciduenas et al. 2009; Castro-Giner et al. 2009; Gilliland et al. 2004; Kunzli et al.). The emerging evidence that oxidative stress and inflammation are also involved in the pathogenesis of autism may suggest a biologically plausible rationale for the observed associations in our study (Boso et al. 2006; A Enstrom et al. 2009; AM Enstrom et al. 2009; James et al. 2004; James et al. 2009; James et al. 2006). In particular, research examining serum biomarkers report increased levels of the pro-inflammatory cytokines tumor necrosis factor (TNF)- α , interleukin (IL)-6, IL-8, and colony stimulating factor II (GM-CSF), as well as two markers of TH-1 immune response (interferon- γ (IFN- γ) and IL-8) in postmortem brain tissue of autism cases compared to controls (Li et al. 2009). Additional research from the CHARGE Study has shown increased plasma levels of immunoglobulin (Ig)G-4 and reduced concentrations of TGF- β , related to immune response and inflammatory processes, in plasma of children with autism compared to typically developing controls and children with developmental delay (Ashwood et al. 2008; Enstrom et al. 2009). Other recent work indicates that exposure to air pollution exposure during pregnancy is associated with changes in IgE and in lymphocytes measured from cord blood supporting the idea that maternal exposure to air pollution is

associated with altered immune profiles in the fetus (Herr et al., 2010a, 2010b). Moreover, published evidence links maternal antibodies to fetal brain tissue with a subset of autism cases (Braunschweig et al 2008).

Genetic variation in oxidative stress and inflammatory pathways has also been associated with autism. Oxidative stress endophenotypes and corresponding genotypes related to metabolism of methionine transmethylation and transsulfuration were significantly decreased in children with autism compared to controls indicating increased susceptibility to oxidative stress (Boso et al. 2006; James et al. 2004; James et al. 2006). Markers of lipid peroxidation have also been associated with autism, as have increased levels of nitric oxide and mitochondrial dysfunction, which may be related to the formation of reactive oxygen species (Chauhan and Chauhan 2006; Filipek et al. 2004; Ming et al. 2005; Sogut et al. 2003; Yao et al. 2006). Polymorphisms in *glutathione S-transferase (GST) M1*, *GSTP-1*, and *glutathione peroxidase (GPX1)*, which modulate the response to oxidative stress, have been associated with increased autism risk (Buyske et al. 2006; Ming et al. 2009; Williams et al. 2007). These genetic variants have also been shown to modify the association between exposure to air oxidant pollutant associations and respiratory outcomes (Islam et al. 2009; Salam et al. 2007). Examination of the interaction between these oxidant-associated genes and environmental exposures may help to clarify susceptibilities to environmental pollutants among children with autism.

We recognize that the moderate relative risks associated with freeway proximity in our study may have been due to chance or bias. The study is currently limited by sample size and potential exposure misclassification. Analysis of larger datasets would provide additional valuable insight into these findings and the potential for replication. While we used a residential history questionnaire (available for a subset of the study participants) to choose the appropriate

address for trimester, there still may be misclassification of exposure in these data due to inaccurate date reporting on the part of the mother, or in our choice among multiple addresses in each trimester. We could not distinguish the potential effect of noise from that due to pollutant exposures, both resulting from residential location near a freeway or other road in this study. Addresses on the birth certificate could also be in error, but this would probably be less likely. We were not able to examine specific pollutant concentrations in this study, and the traffic proximity metrics were subject to misclassification of exposure because they did not account for traffic volume, prevailing wind speed and direction. However, this exposure misclassification was unlikely to have been systematically related to disease and our results may therefore have underestimated the magnitude of a true causal association.

Despite these limitations, this study has several strengths. Assessment of autism was conducted through well-validated instruments that are recognized as the gold standard in the field. We examined exposure prenatally and at birth, two pivotal times in gestational development, whereas prior work on air pollution has been limited to the birth address or a cumulative lifetime exposure measure. To our knowledge, these results are the first to show an association of autism with residential traffic proximity.

Conclusions

Little is known about potential environmental contributions to autism. The observed associations with traffic proximity merit further research to determine whether these results are reproducible in populations with improved estimates of exposure to specific ambient air pollutants. Examination of gene-pollution interactions may also help us learn about causal pathways involved in autism, identify potentially susceptible populations, and lead to prevention strategies. Our analysis is the first step in examining a hypothesized relationship between air

pollutants and autism. It has been estimated that 11% of the U.S. population lives within 100m of a four lane highway, so a causal link to autism or other neurodevelopmental disorders would have broad public health implications (Brugge et al. 2007).

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Table 1: Demographic characteristics of CHARGE cases with autism and controls with typical development (N=563)

Demographic Variable	Percent and Number of Cases (N=304)	Percent and Number of Controls (N=259)	Chi-square p-value
Male Gender	87% (263)	81% (211)	0.10
Child's Ethnicity			0.21
White	51% (154)	51% (131)	
Hispanic	29% (88)	30% (77)	
Black	3% (8)	2% (5)	
Asian	7% (21)	3% (8)	
Other*	11% (33)	15% (38)	
Maximum Education in Home			0.88
High School or Less	7% (22)	8% (22)	
Some College	31% (95)	31% (79)	
Bachelor Degree	35% (107)	36% (93)	
Graduate or Professional Degree	26% (80)	25% (65)	
Maternal Smoking During Pregnancy**	9% (27)	7% (18)	0.40
Maternal Age ≥ 35	28% (85)	25% (65)	0.44
Preterm Delivery (< 259 Days***)	11% (32)	10% (25)	0.73

*Other= mixed race, or other reported races including native American, Indian, East Indian, Cuban, or Mexican-American

**Mother reported smoking at any time during pregnancy

***Equivalent to 37 completed weeks

Table 2: Exposure odds ratios and 95% confidence intervals for autism, by category of distance from residence at time of birth to the nearest freeway (N=563). Categories represent, from top to bottom, the closest 10%, next 15%, and next 25% with the referent group comprising the furthest 50% of study households*.

Exposure Category	Number Cases / Number Controls	Crude	Adjusted*
<309m From Freeway (closest 10%)	38/19	1.86 (1.04-3.45)	1.86 (1.03-3.45)
309-647m From Freeway (10th to 25th %)	43/41	0.98 (0.60-1.59)	0.96 (0.58-1.56)
647-1419m From Freeway (25th to 50th %)	77/63	1.14 (0.76-1.71)	1.11 (0.73-1.67)
>1419m From Freeway (further 50%)	146/136	Reference	Reference

* Model adjusted for child gender (male vs. female), child ethnicity (Hispanic vs. White, Black/Asian/Other Race vs. White), maximum education of parents (parent with highest of four levels: college degree or higher vs. some high school, high school degree, or some college education), maternal age (> 35 years vs. ≤ 35 years), and maternal smoking during pregnancy (mother reported any smoking during pregnancy vs. mother reported no smoking during pregnancy)

Table 3: Exposure odds ratios and 95% confidence intervals for autism, by category of distance from birth address to the nearest major road for the address at birth (N=563). Categories represent, from top to bottom, the closest 10%, next 15%, and next 25% with the referent group comprising the furthest 50% of study households*.

Exposure Category	Number Cases / Number Controls	Crude	Adjusted*
≤42m From Major Road (closest 10%)	28/30	0.80 (0.45-1.41)	0.71 (0.39-1.26)
42-96m From Major Road (10th to 25th %)	54/32	1.44 (0.88-2.39)	1.29 (0.77-2.18)
96-209m From Major Road (25th to 50th %)	71/68	0.89 (0.59-1.34)	0.83 (0.55-1.26)
>209m From Major Road (further 50%)	151/129	Reference	Reference

* Model adjusted for child gender (male vs. female), child ethnicity (Hispanic vs. White, Black/Asian/Other Race vs. White), maximum education of parents (parent with the highest of four levels: college degree or more education vs. some high school, high school degree, or some college education), maternal age (> 35 years vs. ≤ 35 years), and maternal smoking during pregnancy (mother reported any smoking during pregnancy vs. mother reported no smoking during pregnancy), and freeway distance categories (<309m, 309m-647m, 647m-1419m vs. referent of >1419m)