

Maternal Prenatal Stress and Child Neurodevelopment

By

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Abstract

Early life experience can impact lifelong health; one of the earliest potentially adverse experiences is in-utero exposure to maternal stress, anxiety or depression. This project examined whether maternal social, economic and mental health stressors have an impact on a child's diagnosis of autism spectrum disorder (ASD) and whether these stressors can modify the risk of ASD from environmental exposures.

First, we investigated whether the maternal economic and mental health stressors have independent effects on child's neurodevelopmental outcome. Data from the CHARGE (**C**hildhood **A**utism **R**isks from **G**enetics and the **E**nvironment) Study, a population-based case-control study, were used to examine whether maternal periconceptional and prenatal exposures and experiences were associated with increased risk of ASD in the child. Specifically, maternal inability to pay for basic needs (food, housing, medical care and heating) and maternal prenatal psychological distress were explored for their association with the child's ASD diagnosis. Our data showed that both financial hardship and maternal mood disorders during pregnancy were associated with increased risk of child ASD.

Secondly, we explored the effects of maternal stressors to determine if they modified or acted synergistically with environmental chemical exposures to alter a child's developmental diagnosis. Neighborhood level environmental exposures and sociodemographic factors were obtained from the California Communities Environmental Health Screening Tool, a methodology developed by Office of Environmental Health Hazard Assessment that identifies California communities that are disproportionately burdened by multiple sources of pollution and

socioeconomic disadvantage. Geographic based relative measures of seven environmental exposures, five indicators of the effects of pollution, and seven population characteristics and socioeconomic factors, create percentile scores for all of California's census tracts. Findings suggest Air quality measures are associated with ASD risk, and that this risk varies based on maternal mental state.

Finally, we examined the interaction between the maternal prenatal experience and residential proximity to agricultural application of organophosphate pesticides for their effects on risk for ASD in the offspring. This study identified financial hardship as an amplifier of the association between organophosphate pesticide exposures during pregnancy and offspring ASD. These results add to the existing evidence highlighting the importance of studying the co-exposure of social and environmental exposures affecting children at early developmental stages.

In this investigation, we have shown that maternal mental health financial hardship are potential environmental risk factors for ASD, and that these experiences amplify the effect of air pollution and pesticide exposures. These findings have relevance for public health and provide hope for strategies that can reduce risk factors for this devastating diagnosis. Strategies may involve maternal child health interventions, poverty reduction programs and attention to environmental toxins.

Dedication

This work is dedicated to those at the frontlines; the perinatal health workers, home visitors, community outreach workers, social workers, midwives, and public health nurses working in underfunded and overburdened programs. For them these findings will come as no surprise, for they are already forging ahead with the solutions. May this work serve as a testament to the value of their efforts in support of pregnant women.

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Chapter 1: Literature Review

We have seen little improvement in the burden of health disparities borne by racial/ethnic and underserved communities. After decades of research and ever widening gaps in health and well-being, it is increasingly evident that without radical new approaches in efforts to comprehend the underlying causes of health disparities, our efforts to eliminate them are unlikely to succeed ^{1,2}. There is increasing evidence ¹⁻³ that social and environmental factors must be addressed in concert to eliminate the greater health burdens that are carried by our most vulnerable and underserved communities. Scientific progress has yielded widespread consensus that the prenatal environment is a determinant of susceptibility to poor health, and that adverse conditions in utero may have deleterious effects on the development of specific organs, and fetal homeostasis. A focus on disorders related to the central nervous system is especially warranted as the fetal brain is sensitive to changes however small, in the uterine environment during early development ⁴. We have evidence that social stress and environmental toxins may influence common physiological pathways ⁴, and increased awareness of the covariance across factors ⁵. Therefore, understanding potential synergistic effects promises to more fully inform children's environmental health risks, and our understanding of the origins of child neurodevelopmental delays.

Neurobiological disorders can be broadly divided into developmental (e.g. Autism Spectrum Disorder (ASD), Attention Deficit Hyperactivity Disorder (ADHD)), neurodegenerative (e.g. Alzheimer's) and neuropsychiatric disorders (e.g. Schizophrenia). Most of these diseases share genetic risk factors but the onset of these disorders cannot be explained solely by inheritance. This project focuses on the neurodevelopmental disorder ASD, and although ASD and ADHD

are considered distinct disorders, some researchers have called that separation into question, citing shared psychopathological, neuropsychological, brain imaging, and genetic findings that suggest that they are part of a continuum ⁶. Our current understanding recognizes that it is the interactions between genetic risk factors and the environmental that can lead to neurodevelopmental disorders ⁷. The environmental factors that may play an indirect or direct role on neurodevelopmental disorders are stress, infections, drug abuse, and environmental contaminants ⁴.

In this addition to our knowledge about neurodevelopmental disorders, this project builds on findings that emerged from the CHARGE (Childhood Autism Risks from Genetics and the Environment) study, through a re-examination of participant surveys to identify maternal stressors or measures of stress and coping during pregnancy, and explore how these social factors mitigate or modify the impacts of environmental and maternal factors that are the central exposures of interest in the CHARGE study. The CHARGE study is a large, population-based case-control investigation of the underlying causes of autism, with a focus on environmental exposures, genetic susceptibility, and the interactions between these two domains. The primary exposure information sources in the CHARGE Study are an environmental exposure questionnaire, medical charts, and chemical analyses of maternal urine and blood, baby hair, and newborn blood spots. The CHARGE study examined multiple chemical and biologic exposures, susceptibility factors, and their interactions. This project aims to expand the scope to include maternal mental state and indicators of experience of economic hardship in the perinatal period that have not previously been examined in relation to pervasive developmental disorders, bringing us closer to understanding the complex interplay between maternal mental state and

child health. It aims to add a new dimension to a field where few studies have examined maternal mental state during the perinatal period in relation to offspring neurodevelopment and environmental exposures.

Autism, ADHD and Maternal Prenatal Stress and Anxiety

Several epidemiological and animal studies^{4,8-14} have identified prenatal stress as a risk factor for neurodevelopmental disorders with diverse ages of onset and socioemotional symptoms. This review examines a few investigations of note that have widened our understanding of the impact of maternal prenatal economic, social and mental health stressors. This is followed by a discussion of why these stressors should be considered integral to the study of children's risk of neurodevelopmental delay and environmental health research.

Maternal experience of stressful life events during pregnancy was assessed in a prospective cohort of pregnant women enrolled before the 18th week of gestation¹⁵. Mothers completed the child behavior checklist when offspring were 2 years of age. Maternal stressful events during pregnancy significantly predicted ADHD behaviors in both male and female offspring, after controlling for autistic traits and multiple obstetric and sociodemographic covariates. Similarly, stressful events during pregnancy significantly predicted autistic traits in male offspring after controlling for ADHD behaviors and confounding factors¹⁵. These results suggest that typical stressful life events show a small but significant association with ASD traits and ADHD behaviors independently in offspring at age 2 years.

Studies examining the role of prenatal stress in ADHD found that stress during pregnancy contributed to ADHD diagnostic criteria, especially in the male population studied¹⁶; mothers

with an ADHD-affected child were more likely to perceive high stress during pregnancy when compared to an unaffected sibling¹¹; adolescent boys whose mothers experienced high levels of anxiety during pregnancy, had more difficulties with sustained attention/self-regulation than boys whose mothers reported low or moderate anxiety levels in the State-Trait Anxiety Inventory¹⁷; and child ADHD symptoms were significantly associated with high levels of anxiety in second trimester¹⁸.

Autism and Traumatic Weather Events

Traumatic weather events have also yielded opportunities to examine effects of maternal prenatal stress. Autism prevalence was calculated using de-identified birth dates, parishes of children diagnosed with autism in the state mental health system, and corresponding census data on all live births in Louisiana between 1980 and 1995. Prevalence increased in dose-response fashion with severity of prenatal storm exposure, especially for cohorts exposed near the middle or end of gestation ($p < 0.001$)¹⁹. Further north, investigators also took advantage of what has been called one of the worst natural disasters in Canadian history. A unique combination of weather systems formed over a huge swath of the North American continent, several million people throughout the provinces were without power, some blackouts lasting for weeks before crews were able to restore power. Soon after the storm, mothers completed questionnaires on objective exposure and subjective distress, and completed the Autism Spectrum Screening Questionnaire (ASSQ) for their children at age 6½. Investigators found that: ASSQ scores were higher among boys than girls. Greater objective and subjective stress predicted higher ASSQ scores independent of potential confounders. An objective-by-subjective interaction suggested that when subjective stress was high, objective stress had little effect; whereas when subjective stress was low, objective stress strongly affected ASSQ scores¹³.

The Nurses' Health Study facilitated an examination of the association of maternal posttraumatic stress disorder (PTSD) and ASD children. The effect of maternal PTSD on child neurodevelopment has multiple possible pathways: the link between maternal stress and child neurodevelopment; ASD in children may increase risk of PTSD in mothers; and the two disorders may share genetic factors. In the Nurses' Health Study Mother's PTSD symptoms were strongly associated with child's ASD, and greater severity was associated with increased risk. The risk ratio (RR) for mothers with 4–5 PTSD symptoms was 1.98 (95% confidence interval (CI) 1.39, 2.81), but for mothers with 6–7 symptoms, the RR increased to 2.89 (95% CI 2.00, 4.18)²⁰. PTSD has been associated with stress reactivity in both women and their children²¹, and observed in ASD cases²².

The Stockholm Youth Cohort was a large intergenerational cohort created from two population-based studies in Sweden and England with prospectively recorded data on exposures and a range of potential confounders. Although the cohorts were large; the stressful life events that were studied (occurrence of any severe life event before and during pregnancy and the child's early life) were experienced by less than 1% of the population. The Swedish cohort included 4,429 ASD cases and 43,277 controls, and the English cohort had 72 cases and 11,554 controls. Maternal exposure to multiple common and rare life events, as well as their perceived impact upon the mother during pregnancy and early life were measured. Authors found no evidence of an association between prenatal life events, or their number and perceived impact and the risk of offspring ASD. Study authors concluded that there was no evidence that exposure to stressful life events during the prenatal period is associated with an increased risk of ASD. They also

reported concerns with selective attrition; mothers with socioeconomic disadvantages and higher weighted life-events were more likely to drop out of the study ²³.

Using a population-based cohort study of all singleton pregnancies in Denmark born from 1978 to 2003 study authors hypothesized that prenatal stress exposure related to maternal bereavement was associated with an increased risk of autism later in life. 37,275 children whose mothers lost a close relative during pregnancy or up to 1 year before pregnancy were compared to an unexposed group, both were followed from birth until either a diagnosis of autism or 2006. Unadjusted odds ratio showed 37% increased risk of autism in the exposed group; however, the difference in odds ratio dissipated after adjusting for child's gender, birth year, neonatal characteristics, maternal characteristics (psychiatric history, age, education, residence, income, cohabitation status), and paternal age ²⁴.

Sandman et al, measured maternal psychosocial stress throughout pregnancy. After birth investigators measured infant cortisol, cognitive, neuromotor development, emotional regulation, and behavior responses throughout infancy. Analysis revealed that psychobiological markers of stress during pregnancy resulted in: delayed fetal maturation, disrupted emotional regulation and impaired cognitive performance during infancy, and decreased brain volume in areas associated with learning and memory in 6- to 8-year-old children ²⁵.

This review concludes with a comprehensive meta-analysis of eleven studies with important differences in study design, varied effect sizes, and prospective and retrospective investigations. Authors observed a small association between maternal perinatal stress and anxiety and child

cognitive outcomes. They also noted that the effect was moderated by study design decisions about how the study constructs were operationalized. This is a challenge in the research of stress effects; within these eleven studies as with this review, the definitions and measurements of maternal stress were not constant; some studies measured occurrences of major life events, while others utilized more subjective maternal self-reports. We would expect the defining and operationalizing of these concepts to hamper the ability to quantify the association with child cognitive development across studies. Overall, across all studies with a combined $n=5,903$, a low but significant inverse association between indices of maternal prenatal stress or anxiety and early child cognitive development was calculated. All effect sizes were in the same direction and the largest associations with child cognitive outcome were found for retrospective based indicators. There were no differences in the effect size as a function of the trimester during which maternal symptoms were measured ²⁶.

The Human Stress Response

The human stress response starts in the hypothalamus which signals the autonomic nervous system and the pituitary gland, this is followed by the release of the stress hormones, epinephrine and cortisol. Components of the nervous system: the central division (brain and spinal cord), and the peripheral division consisting of the autonomic (ANS) and somatic nervous systems (SNS) are involved. The SNS signals the adrenal glands to release adrenaline and cortisol. As the initial surge of epinephrine subsides, the hypothalamus activates the second component of the stress response system — known as the hypothalamic-pituitary-adrenal (HPA) axis. This network consists of the hypothalamus, the pituitary gland, and the adrenal glands and is a dynamic metabolic system that regulates homeostatic mechanisms, such as the ability to respond to

stressors. From early fetal development the HPA axis is highly sensitive to the impact of early adverse experience ²⁷.

If the brain continues to perceive a situation as stressful, the hypothalamus triggers the adrenals to continue to release cortisol. The system remains in a high alert state until the parasympathetic nervous system is invoked to dampen the stress response. With chronic stress and anxiety, over-activation of the HPA axis leads to release of glucocorticoids ^{28,29}. Neuroendocrine and immune systems play a major role in adaptation to stress; these systems communicate with each other through short and long communication loops. Activation of the stress system leads to suppression of the immune system, primarily via glucocorticoid-induced changes ³⁰.

Additionally, elevated SNS activity increases the release of catecholamine and activity of inflammatory cytokines ²⁸. A negative feedback system occurs between the HPA axis of the stress system and the immune/inflammatory response ³¹.

Potential Mechanisms for the impact of Maternal Prenatal Stress on Fetal

Neurodevelopment

Fetal programming has often been cited as the underlying mechanism, which occurs when the normal pattern of fetal development is disrupted by an abnormal stimulus or insult at a critical time point ⁴. Fetal adaptations to the intrauterine and maternal environments shape the structure and function of organs, leading to permanent physiological alterations in adulthood. Our understanding of how maternal prenatal stress or stress events affects fetal programming is still evolving but we do understand that the dysregulation of the two major stress response systems, the HPA axis and the sympathetic branch of the autonomic nervous system are involved ³².

Documented associations of maternal placental DNA methylation with prenatal environmental exposures suggest a mechanism involving altered gene expression as a result of prenatal exposures, and the resulting impact on child neurodevelopment³³. Epigenetic changes across multiple generations similar to those seen in environmental toxicant studies³⁴ have also been suspected. Babenko *et al.* (2015) hypothesized that epigenetic regulation may play a role in the increased risk of neurological and psychiatric disorders for children exposed in utero to maternal stress. Epigenetic mechanisms, such as miRNA expression, DNA methylation, and histone modifications are prone to changes in response to stressful experiences and hostile environmental factors. Altered epigenetic regulation could potentially influence fetal endocrine programming and brain development. Findings that support this hypothesis include evidence of methylation status of newborn genes in response to stress exposure in both human studies and animal models^{33,34}.

The placenta has also been implicated; increasing evidence suggests that miRNAs are important regulators of placental development. Their role in the placental stress response has not been sufficiently studied. Changes in DNA methylation in the placenta are associated with low infant birth weight and several pregnancy complications, such as preterm birth, fetal growth restriction, and preeclampsia³⁰. We also know that DNA methylation plays a critical role during mammalian development, with an effect on the developing hippocampus. Hippocampal synaptic plasticity in the form of long-term potentiation and long-term depression seems to be the predominant mechanism underlying certain types of learning and memory³². If long lasting hippocampal changes are induced, spatial learning and memory may be impaired³².

Animal models have also identified prenatal stress as a risk factor for neurodevelopmental disorders with varied ages and diverse social deficits³⁵. They have also aided our understanding of physiological mechanisms. Animal models have found that maternal prenatal stress: disrupts the peri-adolescent maturation of the prefrontal cortex in male rats; reduces brain-derived neurotrophic factor, an important determinant of synaptic plasticity in the prefrontal cortex of adult rats; increases corticosterone responses to mild stressors in adulthood, and is associated with a reduction in hippocampal glucocorticoid receptor (GR) expression²⁷. In primates, one of the important placental stress signals in pregnant primates is the peptide corticotropin-releasing hormone. This peptide plays a key role in the maturation of the fetal HPA axis, and coordinates processes inherent in both fetal growth and maturation before birth. Exposure to prenatal stress in animal models and to maternal stress, anxiety and depression in humans may not constitute similar adverse prenatal experiences; however, they possibly share common pathways that affect HPA axis function in offspring.

Other potential culprits are genes that moderate or exacerbate stress reactivity, specifically a maternal genetic variation in the promoter region of the serotonin transporter gene which affects serotonin transport and a heightened response to stressors²⁹. In one investigation, mothers with children diagnosed with ASD who were also carrying the stress susceptible short allele variant of 5-HTTLPR experienced a greater number of stressors and greater stress severity when compared to mothers carrying the long allele variant. Regardless of maternal genotype, the same mothers did not report increased exposure to prenatal stress during pregnancies of typically developing siblings³⁶. These findings were confirmed by animal models³⁷. Ehrlich and Rainnie (2015) exposed pregnant rats to stressful situations; their offspring, when compared to offspring of

pregnant rats who were not placed in stressful situations, had dampened socioemotional behavior and reduced amygdala neuron excitability during infancy, preadolescence, and adulthood³⁵.

Prenatal exposure to increased third trimester maternal depressed/anxious mood was associated with increased methylation of a specific gene. Increased methylation was also associated with increased salivary cortisol and stress responses at 3 months of infancy, after controlling for confounding factors²⁷.

Pathological changes in the cerebellum in autism are thought to correspond to an event before 30–32 weeks gestation. Beversdorf et al (2005) sought to determine whether there is an increased incidence of stressors in autism before this time period. Using surveys regarding incidence and timing of prenatal stressors, mothers reported the incidence of stressors during each 4-week block of their pregnancies. Control mothers with typically developing children reported fewer stressors than the ASD mothers ($p = 0.0007$). ASD mothers suffered a higher incidence of prenatal stressors at 21–32 weeks' gestation, with a peak at 25–28 weeks. Findings support the possibility of prenatal stressors as a contributor to autism, with the timing of stressors consistent with the embryological age suggested by neuroanatomical findings seen in the cerebellum of autism patients⁸.

Stress and Environment Interactions

While substantial evidence from human and animal studies suggests that psychosocial stress exerts sex-specific effects on the developing brain, mounting toxicologic and epidemiologic evidence supports a framework in which the adverse neurodevelopmental effect of neurotoxic environmental chemicals is modified by these stressors. The literature also suggests that both exposures act on common biological systems in the developing brain, with boys typically

demonstrating greater sensitivity to early life exposures than girls; these findings highlight the importance of examining the social environment as well as environmental risks which are often experienced concurrently or consecutively.

Multiple investigations have attempted to examine and quantify the strength of the interaction between stress or social disadvantage and several environmental neurotoxicants. For example, a prospective cohort study found that the effect of lead exposure on the Bayley Scales of Infant Development was affected by the child's socioeconomic status, defined by parent's educational and occupational category. Performance declined with increasing concentration of lead in blood, yet children from homes classified as upper class did not exhibit the same adverse effect unless the levels of cord blood exceeded 10 $\mu\text{g}/\text{dL}$ and children in lower socioeconomic strata were affected at even lower levels of exposure³⁸. Other investigators used tooth lead levels to assess long term exposures, they found lead-related deficits of visual-motor integration and reaction performance, but the observed lead effects were small when compared to the effects of social background³⁹. Prenatally, lead and stress disrupt similar but not completely overlapping mechanisms, and in-utero exposure to each of these insults singularly has been found to alter normal neurodevelopment⁴⁰. Additionally, prenatal stress exposure has been confirmed as a modifier of the known neurotoxic effects of prenatal lead exposure⁴⁰.

Similarly, investigators found that neurotoxic effects of prenatal polychlorinated biphenyl and dioxin on cognitive and motor abilities persist into school age, but only in children from less advantaged homes⁴¹. Children who were exposed to tobacco smoke and suffered material hardships exhibited the greatest cognitive deficits on the Bayley Scales. Material hardship was

operationally defined as “unmet basic needs in the areas of food, housing, and clothing”⁴². The main effect for traffic related black carbon exposure was not significant for any Wide Range Assessment of Memory and Learning index measures. However, in stratified analyses, among boys whose mothers reported high exposure to prenatal stress, Attention Concentration Index scores were on average 9.5 points lower for those with high compared to low prenatal black carbon exposure (p for 3-way interaction = 0.04)⁴³. These findings add to the existing evidence highlighting the imperative to study the co-exposures of stress exposure and environmental chemicals.

Conclusion

An extensive body of evidence describes the susceptibility of the vulnerable fetal brain to adverse exposures experienced in the maternal environment. The impact of maternal stress and anxiety during pregnancy on child neurodevelopment, and how this experience interacts with environmental exposures is still far from being understood. The results of this investigation, and the consideration of joint impact of prenatal stress and environmental exposures in disease outcomes are critically important to realistically improve current prevention and intervention strategies to assist a healthy life trajectory. The customary approach accepted genetic predispositions as intrinsic influences, toxic exposures as contributors to risk, and social characteristics tended to be regarded as a marginal influence, adding at most a source of confounding bias. Emerging science, including this project, contends that comprehensive understanding of neurobehavioral consequences of genetics and toxic exposures is inseparable from the sociodemographic setting of the individual. This setting creates unique vulnerabilities that are imprinted as powerfully and should be considered with the same scrutiny that we apply to genetic predispositions.

These collective findings suggest that efforts to protect expectant mothers from stress might be useful in primary prevention of ASD. Supporting expectant mothers in stressful circumstances could also provide a way to use other, less malleable ASD risk factors, such as genetic susceptibility for primary prevention. That is, these other risk factors could help identify pregnancies at particularly high risk for ASD, so that they could then be targeted by special efforts to optimize prenatal care and reduce maternal stress. Additionally, since human brain development continues postnatally, the effects of prenatal stress can be moderated by quality post-natal care²⁸. Evidence-based recommendations for treatment of maternal mood disorders, effective stress management strategies, and programs that target the reduction and prevention of sources of stress and anxiety for marginalized communities will prove to be integral to the health of both pregnant women and their offspring.

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Chapter 2: Maternal Perinatal Psychological Distress, Economic Hardship and Child ASD

Abstract

Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition with increasing prevalence, a high degree of heritability and evidence of susceptibility to environmental factors. The environmental factors that may play a direct or indirect role during pregnancy include: infections, environmental contaminants, nutrition, and maternal metabolic conditions and prenatal stress. This study examined whether self-reported maternal inability to pay for basic needs (food, housing, medical care and heating), and maternal prenatal psychological distress are associated with increased risk of ASD in the child.

Study Design/Methods: In the CHARGE (Childhood Autism Risks from Genetics and the Environment) study, a population-based, case-control study, children aged 2 to 5 years with autism were identified and frequency matched to randomly sampled controls identified from state birth files between January 2003 and September 2016. This investigation included 728 children with ASD, 482 typically developing controls, and their mothers. Children's diagnoses were confirmed using standardized assessments. Maternal periconceptional and prenatal exposures and experiences were reported through structured telephone interviews. Maternal financial hardship, and perinatal depression and anxiety and their associations with the child's ASD diagnosis were explored. Separate multivariate logistic regression models were conducted to assess the two associations, one for financial hardship and the other for maternal mental state during pregnancy. Odds ratios (ORs) were used as estimates of relative risk, and 95% confidence intervals (CIs) were calculated.

Results: 145 (22%) mothers who had children with ASD, and 79 (17%) with typically developing children reported financial hardship. 287 (59%) mothers of children with ASD and

145 (36%) mothers with typically developing children reported perinatal depression or anxiety. We found increased odds of being diagnosed with ASD among children whose mothers reported financial hardship during pregnancy (adjusted OR = 1.49; 95% CI [1.05, 2.12]) and perinatal depression or anxiety (adjusted OR = 1.93; 95% [CI 1.43, 2.66]).

Conclusions: Our findings add to the increasing evidence that maternal prenatal stress and mental state affect child neurodevelopmental outcomes and is critically important to current prevention and intervention strategies.

Introduction

A large body of evidence has identified the susceptibility of the fetal brain to an adverse maternal environment. The multifactorial model for Autism Spectrum Disorder (ASD) proposed by Goines and Ashwood (2013), starts with a genetically susceptible individual whose predisposition includes an inappropriate or ineffective response to exposures in the maternal environment. This exposure during a critical period of fetal development could disrupt development of the nervous and/or immune system, leading to ASD, as well as the neurological, behavioral, and immune dysfunctions observed in this condition. The extent to which maternal stress and anxiety during pregnancy impact child neurodevelopment is still poorly understood. Systematic reviews of maternal prenatal stress-related factors have found effects on obstetrical and developmental outcomes: these factors include racial discrimination ^{1,2}, poverty ³, and psychological distress ⁴ such as depression and anxiety.

As for ASD specifically, family discord during pregnancy ⁵, stressful life events ⁶ and maternal but not paternal history of depression, have been found to be associated with a higher risk of autism in offspring ⁷. Studies involving pregnant women who experienced natural disasters have yielded insight into the role of this type of stressful event. Kinney *et al.* (2008) used the Louisiana state birth cohort and found a significantly higher prevalence of ASD in children whose mothers experienced hurricanes or severe tropical storms during pregnancy ⁸. Walder *et al.* (2014) examined 89 children who were in utero during the 1998 Quebec Ice Storm. After the storm, mothers completed questionnaires on objective exposure and subjective distress and then completed the Autism Spectrum Screening Questionnaire (ASSQ) for their children at age 6½. Investigators determined that greater objective and subjective prenatal maternal stress predicted

higher ASSQ scores independent of potential confounders. A test of interaction suggested that when subjective stress was high, objective stress had little effect; whereas when subjective stress was low, objective stress strongly affected ASSQ scores. The timing-by-objective stress interaction suggested objective stress significantly affected ASSQ in first-trimester exposed children, though less so with later exposure⁹. In contrast, two large population studies found no association between prenatal exposure to stressful life events and ASD¹⁰ but although both cohorts were large, very few participants experienced stressful life events so these studies had limited statistical power.

Studies have also examined socioeconomic status and association with ASD risk, earlier studies found positive associations between ASD and both higher wealth and/or parental education¹¹. More recent studies have found higher rates of autism diagnosis associated with lower socioeconomic status^{10,12,13}, while others report no association¹⁴⁻¹⁷. Several investigators have measured higher ASD prevalence in census tracts and school districts with greater wealth¹⁸⁻²⁰, yet Delobel and colleagues found higher prevalence of ASD with associated intellectual disability in census tracts with indicators of low socioeconomic status¹².

The goal of this investigation was to examine the association between maternal report of financial hardship and anxiety and depression and the risk of ASD in the children in the CHARGE study.

Methods

All statistical analysis was limited to children with a confirmed diagnosis of ASD (n = 728) or typically developing (TD) children (n = 482), who participated in the CHARGE study. Study protocols have been described elsewhere²¹. Briefly, eligible children (ASD cases and controls)

were between the ages of 24 and 60 months, born in California between 2003 and 2016, living in the catchment areas of specified regional centers (RC) in California, with at least one biologic parent who speaks English or Spanish. Children with autism were identified through the California Regional Center System that coordinates services for persons with developmental disabilities. General population controls were identified from state birth files and those who met the inclusion criteria were randomly sampled with frequency-matching to the distributions among autism cases based on age, sex, and broad residential RC catchment area. CHARGE study children were assessed by research-reliable clinicians at the University of California at Davis (UCD) MIND Institute and the UCLA Neuropsychiatric Institute to either confirm their diagnosis or re-classify them for the purposes of the study using criteria described elsewhere²¹. Consent was given per protocols approved by the UCD Institutional Review Board and the State of California Committee for the Protection of Human Subjects.

Mothers participated in a telephone interview of approximately 1 hour and 40 minutes conducted by trained research staff. Questions covered demographic characteristics, medical information including reproductive history and medication use, environmental exposures, family lifestyle, and parental occupational and residential histories. Financial hardship was assessed by the question: “Was there a time between 3 months before pregnancy with [CHILD] to the present, when it was hard for you to pay for basic needs like food, housing, medical care and heating?”. Mothers also answered questions to determine if they experienced any mental health concern, including depression, anxiety, or loss of interest (“in most things like work, hobbies, and other things you usually enjoyed”) for more than 2 weeks during their pregnancy. Anxiety and depression are mental health concerns that are both highly related to one another conceptually,

and statistically similar to each other within our study population. Thus, we created a binary composite variable that measured if a CHARGE mother self-reported experiencing any mood or anxiety disorder during pregnancy; mothers diagnosed with bipolar disorder or schizophrenia were excluded.

Other potential covariates were identified and assessed for inclusion using a directed acyclic graph (DAG) - a visual representation of causal assumptions²². Use of a DAG is particularly helpful in testing for underlying relationships between social class and health, as they allow a determination of the overall causal effect of an exposure within a framework. These causal diagrams summarize underlying assumptions and identifies variables that must be measured and controlled to obtain uncomplicated effect estimates given those assumptions. When creating a DAG, an arrow connecting two variables indicates causation, the presence of a common cause or backdoor path between variables identifies the presence of confounding. To fully inform the construction of the DAG, unadjusted odds ratios and their 95% confidence intervals (CI) between sociodemographic factors were calculated. Logistic regression was used to assess the association between primary exposures and diagnosis [ASD or TD]. Several variables emerged as potentially redundant and highly correlated with the indicator of financial hardship: maximum educational level in the home, parental English language proficiency, employment status, home ownership, insurance payer, and marital status. This initial exploration of the data left six probable confounders were: child's gender and race, child's year of birth, mother's age at delivery, any metabolic condition (diabetes mellitus (DM), type 2 diabetes (T2D), gestational diabetes (GDM), hypertension, or obesity), mother's experience of any psychological distress, mother's birth place (US, Mexico or other), and mother's smoking history during pregnancy.

The DAG also highlighted that the correlation between financial hardship and perinatal mood and anxiety disorders (PMAD) and the potential causal relationship, indicated the need for examination of each of these factors in separate logistic regression models.

Model building was conducted by starting with a full model and using the step-down approach. For each exposure, the full model contained either PMAD or financial hardship, and all potential confounders previously identified. Variables were removed one at a time, and at each step the change in the estimate for the exposure, standard errors and AIC were examined. Only those variables that met the change in estimate criterion of a $\geq 10\%$ change in the estimate for financial hardship or PMAD were retained in the final models. For example, maternal age is a known risk factor for ASD but in our sample it did not differ between cases and controls, so its removal from analysis did not change the regression estimate for the exposures. The final models retained maternal metabolic conditions, child gender, child race, mother's birthplace, and year of child's birth.

All analyses were implemented using SAS version 9.4 (SAS Institute Inc. Cary, NC).

Results

As a result of frequency-matching, the ASD and TD controls in the study were similar with respect to child's age and gender; 84% were male and most participants were Caucasian (51%) or Hispanic (29%). There were no significant differences between cases and controls for the educational level in the household, child's birth weight, gestational age at birth and maternal age at delivery. During pregnancy, residences of the CHARGE study participants were distributed broadly throughout the five specific California Regional Center catchment areas, with the

greatest concentrations in Sacramento Valley, followed by the San Francisco Bay area and Los Angeles. The Southern California site, representing 7% of the sample, experienced challenges in recruiting population controls (Table 1). Exposures of interest obtained by participant interviews, are reported in Table 2. There were 224 (18%) mothers who answered yes to financial hardship, and 75 (6%) non-respondents who were not included in the analysis. There were 432 (35%) mothers who reported experiencing at least one perinatal mood and/or anxiety disorder (PMAD) and 330 non-respondents to this question 27%. To evaluate if there was an effect of missing values, we ran univariate regression models for each covariate, first using the full dataset, then without the observations with missing values for the covariate and compared the regression parameters for the main exposures of interest. We noted less than 10% change in these effect measures, indicating little chance of selection bias as a result of missing values. Adjusted Odds Ratios (ORs) are reported in Table 2. After correction for family clusters, increased risk was associated with maternal PMAD (OR = 1.93; 95% CI [1.41, 2.63]), and with financial hardship (OR = 1.48; 95% CI [1.04, 2.09]).

Discussion

In this study we observed that maternal psychological distress and financial hardship were both associated with an elevated risk for child's diagnosis of ASD, after controlling for child's gender, race, and year of birth, mother's birthplace and maternal metabolic conditions. Maternal prenatal stress, anxiety and depression are associated with low birth weight²³, preterm delivery²³, and elevated cortisol in mother and offspring²⁴. Prenatally cortisol appears to cross the placenta and be able to affect the fetus and disturb ongoing developmental processes. This is hypothesized to be mediated by the impact of prenatal experience on the developing hypothalamic-pituitary-adrenal (HPA) axis stress system²⁴. The development of the HPA axis, limbic system, and the

prefrontal cortex are likely to be affected by antenatal mood, however the mechanisms of this programming of the HPA system is unknown²⁴. Depressive symptoms have also been shown to be associated with altered immune functioning in offspring²⁵, and the silencing of a gene that moderates the production of neonatal cortisol stress responses²⁴. These explorations establish that an infant born to a prenatally stressed or depressed mother could have sustained effects on their stress response and immune systems.

Another framework from which to understand the effects of stress is the model of allostatic load, which hypothesizes that the individual does not return to physiological homeostasis after experiencing a stressor. With repeated exposures, the set points for various systems involved in the stress response, including the endocrine, metabolic, cardiovascular, and immune systems, may shift. Body systems become burdened and dysregulated by the physiological costs of adaptation to repeated and chronic stress²⁶.

Financial Hardship and ASD

Many investigators have grappled with the impact of parental social characteristics, including socioeconomic status (SES), and the results of this investigation align with more recent studies^{12,13,27}. These findings are in conflict with an early often-quoted 1979 case control study by Finegan and Quarrington that identified a positive association between parental SES and child ASD¹¹, these early findings were confirmed by other studies published between 2002 and 2012^{18,19,28}. A systematic review of 600 epidemiological studies across several countries and languages did not support evidence of the impact of socioeconomic factors²⁹. Yet, in contrast, a comprehensive review of studies published in English between 1990 and 2012 identified higher SES as one of the factors associated with earlier diagnosis²⁹. There have been several cross-sectional studies using area-based measures of socioeconomic status with conflicting findings:

prevalence increased with increasing SES in census tracts in a dose-response manner¹⁸; Thomas and colleagues found higher prevalence in wealthier census tracts¹⁹; other investigators determined that district revenue was associated with higher proportions of children identified with ASD at baseline, with increasing rates when measured longitudinally²⁰. In contrast other investigators found that prevalence of ASD with associated Intellectual Disability is more likely to be higher in areas with the highest levels of social and economic deprivation¹².

A large English birth cohort (2007-2011) did not find a statistically significant relationship between income status or neighborhood material deprivation after controlling for mothers education status¹⁴. In China, children in families with socioeconomic disadvantage, in the form of lower family income and education, had greater risk of childhood autism¹³. In Sweden, lower, not higher, socioeconomic status was associated with an increased risk of ASD¹⁰. Contradicting those findings, a population study in Japan using parental surveys only (not confirmed diagnoses), found that only low maternal education, but not paternal education or family income, were associated with having suspected ASD offspring¹⁷, while another population-based study found no relationship between ASD and SES¹⁶. A case control study that included all births in Denmark between 1971 and 1999 found no association with SES¹⁵. Yet, in the Czech Republic, Hrdicka found that an earlier diagnosis of ASD is associated with higher parental age at birth and higher parental education but not with family SES or number of family information sources³⁰.

While the literature is largely inconclusive, it does suggest that many investigations have not separated the effect of community resources from the effect of SES. Whether or not a positive,

negative or null association was found with SES, authors concluded that: there is a strong positive relationship between community resources and autism³¹; economically disadvantaged communities may need assistance to identify children with autistic spectrum disorders and other developmental delays that require attention²⁰; Lack of economic resources has been less of an obstacle to diagnosis as universal screening protocols have been established^{27,31}; differences in prevalence may be due to differential access to pediatric and developmental services^{19,27}, there is a substantial level of under diagnosis for children of lower education status mothers¹⁴ and studies finding no relationship or a positive association may be underestimating the burden of ASD in lower SES groups²⁷.

Perinatal Mood and Anxiety Disorders and ASD

A systematic review by Kingston *et al.* (2012) found evidence for an effect of prenatal psychological distress (anxiety, depression, stress, psychological distress) on behavioral, cognitive, and psychomotor development of offspring, with overall small effect sizes in the studies reviewed⁴. In a population based nested case-control study, Rai *et al* (2013, measured a 60% increase in risk of autism spectrum disorder among depressed mothers but not depressed fathers¹⁰. The sample was drawn from a Swedish cohort of 589,114 children and adolescents aged 0–17 years and included 4,429 cases of ASD. After adjusting for covariates and confounding variables, maternal depression was associated with a significantly increased risk of ASD in the offspring (OR = 1.49; 95% CI [1.08, 2.08]). In contrast to the current study, which relied on self-report of diagnoses and combined those with responses to questions about their mental state during the pregnancy, Raj and co-authors identified mother's prenatal depression by diagnosis codes in medical records.

Perinatal mood and anxiety disorders (PMAD) affect 15-25% of all pregnant and postpartum women in developed countries ³². Depression, the most common mood disorder, is approximately twice as common in women than men, and has a peak of initial onset that coincides with the reproductive years ³³. These disorders are often stigmatized and overlooked, leading to inconsistent screening of women; 50% of pregnant patients with a PMAD are not identified or treated (PMAD California Library). We can consider perinatal depression to be the most underdiagnosed obstetric complication in the United States, affecting one in seven pregnant or postpartum women ^{34,35}.

The results of this investigation have identified two potential risk factors for child ASD, yet PMAD and financial hardship are often experienced concurrently. A systematic review of studies using validated instruments to detect depression found that the incidence among pregnant women of low socioeconomic status, to be 47% in second and 39% in third trimester when obtained by self-report, and 28% and 25% when determined by structured clinical interviews. Other investigators estimate that PMAD affect up to 48% of women living in poverty ³⁶, making it likely that mothers with psychological distress are also contending with inadequate income, poor social support and stressful environments, and limited access to appropriate quality care. Both poverty and PMAD represent potential hazards to child development that are widespread, enduring yet modifiable, and in the case of maternal PMAD, often not detected. Scientific and public interest in PMAD and its underlying mechanisms have grown recently, and have moved away from a focus solely on postpartum depression to exploring the effects of prenatal anxiety and depression on a broader array of infant outcomes, including infant neurodevelopment ³⁶. This body of evidence and the current finding suggest that interventions aimed at reducing

poverty and maternal psychological distress may lower the risk of adverse neurodevelopmental outcomes.

Limitations and Strengths

This study has several strengths, it includes a representative population of cases and controls with well-defined and consistently applied diagnoses. Study protocols assured extensive information on covariates, and a thorough confounder identification and control strategy further strengthens the validity of the findings. Previous studies have examined maternal prenatal stress and family SES but none have specifically examined the association between child neurodevelopment and either the experience of material hardship during the pregnancy, or the experience of any PMAD by maternal report. Measures of maternal stressors were achieved by retrospective surveys which potentially increase the risk of measurement error and recall bias. However, several studies have measured maternal recall of prenatal events; for example a comparison of maternal report of smoking during pregnancy and maternal recall six years later found high (90%) agreement³⁷. Positive predictive value for maternal report of prenatal depression at 6 months postpartum, when compared to medical documentation was 90.4%³⁸. A systematic review of the available literature on maternal recall of hypertensive disorders in pregnancy found that while sensitivity among studies varied widely (30-100%) based on the diagnosis, specificity of maternal recall when compared to medical records was > 90% for all hypertensive disorders³⁹. Other researchers found mixed results 10-12 years after pregnancy with excellent agreement between maternal recall and medical records for some but not all prenatal factors⁴⁰. Studies that have examined the potential for recall bias among mothers with adult children with schizophrenia have found no evidence of positive recall bias for a range of perinatal factors⁴¹, reliable agreement between maternal recall and medical records for a range

of perinatal factors ⁴², and maternal recall to be an accurate source of obstetric information ⁴³.

When investigators asked mothers to assess child behavior in addition to recalling perinatal factors, they found little evidence that child behavior problems influenced the level of agreement between maternal report and medical records ⁴⁴, contradicting their original hypothesis that mothers with affected children may incorrectly exaggerate any potential exposures.

Previous studies of maternal factors contributing to ASD have examined maternal psychiatric conditions but few have included pregnant mothers with self-reported psychological distress.

Since PMAD are widely undiagnosed, the use of self-report in this study allowed a more accurate representation of the effect of these conditions on offspring neurodevelopment.

These findings have strong relevance in light of a recent survey of postpartum women in California that established the prevalence of poverty during pregnancy: over half (53%) of postpartum women in California reported low incomes during pregnancy. Nearly 34% were poor (below the federal poverty level (FPL)) and 20% were near-poor (100% - 200% FPL) ⁴⁵. These authors also found that 60% of low-income (poor or near-poor) postpartum women experienced at least one stressful event (divorce/separation, domestic violence, homelessness, spouse/partner's or respondent's involuntary job loss or incarceration, food insecurity and no social support) during their pregnancy. This confirms our understanding that poverty leads to worries and insecurities of daily life, and lack of supportive environments to handle the resulting stress. Additionally, mothers with serious psychological distress are more likely to be uninsured than those without (37).

Recommendations.

Clinical practice: Given that pregnant women frequently struggle with undiagnosed psychological distress, and that results of this study suggest that such conditions can subsequently increase risk of ASD, a serious neurodevelopmental condition, in the offspring, implementation of universal screening for PMAD during prenatal care would have a double benefit – for both the mother and the child. When indicated, treatment should include appropriate multi-disciplinary interventions, as evidence suggests that intensive therapies can improve maternal and child outcomes³⁶. Additionally, the screening for PMAD should be repeated throughout the pregnancy and into the postpartum period, with appropriate referrals to providers who can continue treatment throughout and beyond the childbearing year. Since PMAD often occur in the context of other family adversities³⁶ additional support is warranted for those pregnant women with limited financial resources.

It is still unclear whether there is a direct association between the use of SSRIs during pregnancy and increased rate of ASD in the offspring. Alongside studies which show an association with ASD^{7,46-49} there are also negative studies⁵⁰⁻⁵². Of special importance is a study by Malm *et al.* (2016) with the largest study population of children exposed prenatally to SSRI, which also adequately adjusted for confounders. It seems more reasonable that maternal depression itself, which is expressed by the use of antidepressants, might be responsible for the association. There is consensus that medical treatment during pregnancy of women with depression should continue in spite of these conflicting studies.

Policy: Strategies that include universal screening of pregnant women for PMAD, treatment, and interventions to reduce their impact are a vehicle to reduce the effect of this modifiable risk factor for pervasive neurodevelopmental disorders. The US Preventive Services Task Force recommends screening for depression in the general adult population, including pregnant and postpartum women⁵³. They also recommend that screening should be implemented with adequate systems in place to ensure accurate diagnosis, effective treatment, and appropriate follow-up. Currently there are many barriers to universal screening of pregnant women. These include limited access for uninsured women to prenatal care, limited access to preconception counseling for many childbearing women, and provider knowledge about available screening instruments. Routine assessment and referral mechanisms require community level integration of obstetrics, primary care, behavioral health, and public health. However, the current state of women's healthcare is and continues to be inaccessible, underfunded, and fragmented with little integration among clinicians, social workers, health educators and perinatal home visitation programs. The multiple risk factors affecting mothers and children, and particularly those with low income and limited resources highlights the need for policy makers, clinicians (obstetric and behavioral health), program planners and evaluators to collaborate to establish and support a model of care that addresses maternal risk factors. These findings when considered in light of the high prevalence of PMAD, and poverty among childbearing families, and the extensive body of previous research documenting the adverse consequences of these experiences, heightens our recognition of pregnancy as a critical period for health throughout the life-course. Poverty around the time of childbearing and perinatal mood disorders should be addressed as critical maternal-child health policy issues.

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Table 1. Demographic and clinical characteristics [n (%) or mean \pm SD] of the study population (n = 1218), stratified by child's diagnosis, financial hardship and maternal anxiety and depression

Covariate	Child's Diagnosis			Financial Hardship			Maternal Anxiety and Depression		
	TD ¹ (n = 485)	ASD ² (n = 733)	P-value ³	No (n = 919)	Yes (n = 224)	P-value ³	No (n = 456)	Yes (n = 432)	P-value ³
Child Male Gender, n (%)	400 (39%)	617 (61%)	0.434	757 (80%)	194 (20%)	0.128	383 (84%)	342 (79%)	0.063
Child's Race/Ethnicity - 5 categories, n (%)									
White, Non-Hispanic	255 (53%)	364 (50%)	0.007	501 (55%)	83 (14%)	<0.001	248 (55%)	198 (46%)	0.034
Black, Non-Hispanic	13 (3%)	23 (3%)		26 (3%)	9 (20%)		14 (3%)	12 (3%)	
Asian	13 (3%)	52 (7%)		52 (6%)	4 (26%)		25 (5%)	21 (5%)	
Hispanic any race	137 (28%)	218 (30%)		242 (26%)	90 (27%)		116 (25%)	152 (35%)	
Multi-Racial	64 (13%)	74 (10%)		96 (10%)	38 (14%)		52 (11%)	49 (11%)	
Regional Center catchment area group at birth, n (%)									
Alta	225 (44%)	285 (56%)	<0.001	403 (44%)	80 (36%)	0.02	212 (46%)	176 (41%)	<0.001
North Bay	80 (16%)	87 (52%)		119 (13%)	40 (18%)		65 (14%)	62 (14%)	
East Bay	77 (16%)	120 (61%)		154 (17%)	28 (13%)		92 (20%)	61 (14%)	
Valley Mountain	81 (17%)	141 (63%)		159 (17%)	51 (23%)		82 (18%)	91 (21%)	
Southern California	22 (5%)	100 (82%)		84 (9%)	25 (11%)		5 (1%)	42 (10%)	
Education level in household- 4 categories, n (%)									
HS diploma or less	42 (9%)	77 (11%)	0.581	64 (7%)	36 (16%)	<0.001	36 (8%)	47 (11%)	0.017
Some college	165 (34%)	238 (33%)		271 (30%)	107 (48%)		135 (30%)	160 (37%)	
Bachelor Degree	169 (35%)	240 (33%)		328 (36%)	63 (28%)		165 (36%)	135 (31%)	
Graduate/Professional	109 (22%)	177 (24%)		256 (27%)	18 (8%)		120 (26%)	90 (21%)	
Birth Place of Mother, n (%)									
United States	368 (82%)	525 (74%)	0.009	688 (79%)	159 (74%)	<0.001	339 (81%)	316 (77%)	0.017
Mexico	28 (6%)	58 (8%)		47 (5%)	28 (13%)		18 (4%)	38 (9%)	
Other	53 (12%)	124 (18%)		135 (16%)	27 (13%)		64 (15%)	58 (14%)	
Age of mother at time of child's birth (years)									
Mean (std dev)	31 (6)	30.9 (6)	0.903	31 (6)	29 (6)	<0.001	30.9 (6)	30.9 (6)	0.967
Child Gestational Weeks at Birth									
Mean (std dev)	39 (2)	39 (2)	0.053	39 (2)	39 (2)	0.284	39 (2)	39 (2)	0.7
Child Birth Weight (g)									
Mean (std dev)	3470 (577)	3406 (67)	0.086	3429 (649)	3471 (554)	0.375	3447 (608)	3403 (639)	0.297

¹TD, typical development; ²ASD, autism spectrum disorder; ³From ANOVA (numerical covariates), and chi-square test or Fisher's exact test (categorical covariates)

Table 2. Adjusted associations between Prenatal exposures of interest and ASD risk

<i>Exposure</i>	TD¹ (n=485) n (%)	ASD² (n=733) n (%)	Adjusted³ Odds Ratio (95% CI)
Financial Hardship	78 (16.6%)	143 (19.6%)	1.49 (1.05, 2.12)
Perinatal Mood/Anxiety Disorder	90 (19.5%)	186 (25.5%)	1.95 (1.43, 2.66)

¹TD, typical development; ²ASD, autism spectrum disorder, ³Adjusted for child gender, regional center, year of child's birth, child race, maternal place of birth and maternal metabolic conditions

Chapter 3: Effects of Neighborhood- and Individual-Level Economic and Mental Health Stressors as Susceptibility Factors for Environmental Exposures on Child ASD

Abstract

Background: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition that has demonstrated a high degree of inheritability and evidence of susceptibility to in-utero exposures.

Objective: This study examined the effects of both neighborhood- and individual-level economic and mental health stressors as susceptibility factors for the influences of exogenous environmental exposures on neurodevelopment.

Study Design/Methods: Children aged 2 to 5 years (488 with ASD and 329 controls) were enrolled in the CHARGE (Childhood Autism Risks from Genetics and the Environment) study, a population-based, case-control investigation between January 2003 and September 2012.

Eligible children were born in California, had parents who spoke English or Spanish, and were living with a biological parent in selected regions of California. Children's diagnoses were confirmed using standardized assessments. Neighborhood level environmental exposures and sociodemographic factors were obtained from the California Communities Environmental Health Screening Tool (CalEnviroScreen), developed by Office of Environmental Health Hazard Assessment. The tool ranks census tracts based on environmental exposures, socioeconomic factors, and prevalence of certain health conditions. Principal component analysis (PCA) was used to visualize the data, reduce the number of measures and derive composite variables. Odd ratios (OR's) for financial hardship, maternal mood disorders and principal components and their 95% confidence intervals (CIs) were calculated with multivariate logistic regression models.

Results: Based on PCA, principal components related to CalEnviroScreen rankings for socioeconomic status, air quality, waste disposal pollution sources, health indicators, and water quality were created. The principal component describing air quality measures included: diesel particulate matter (PM), toxic releases from facilities, traffic density and PM2.5 concentrations. This variable remained significantly associated with child ASD risk in unadjusted and adjusted logistic regression. We found increased odds of ASD among children whose mothers reported financial hardship compared with those whose mothers did not (OR = 1.5; 95% CI [1.1, 1.9]). Additionally, higher levels of CalEnviroScreen measures for air quality were associated with increased risk of ASD (OR = 1.1; 95% CI [1.01, 1.3]). Children whose mothers reported mood disorders exhibited higher risk of ASD (OR = 1.9; 95% CI [1.5, 2.3]) when compared to children whose mothers reported no mood disorders. There was also evidence of increased risk with higher indicators of poor air quality (OR = 1.2; 95% CI [1.05, 1.3]) when controlling for maternal mental health. We found increasing odds ratios at increasing levels of the AirQ principal component when adjusting for maternal mood disorders, and significant persistent risk when adjusting for financial hardship.

Conclusions: These findings are consistent with previous studies showing that prenatal exposures including air-pollutants, maternal stress and mood disorders are associated with child's ASD. We found that community-level variables impact ASD risk even when controlling for the family's SES and maternal mental health. Results add to our understanding that maternal prenatal stress and mental state are vitally important to current prevention and intervention strategies. Additionally, there is a critical need for action to reduce air pollution from multiple sources, with a focus on those communities with the highest exposure levels and socioeconomic vulnerabilities.

Introduction

Neurodevelopmental disorders include Autism spectrum disorders (ASD) and developmental delay (DD). ASDs are characterized by impairments in social interaction, communication deficits, and a pattern of restricted or repetitive interests and behaviors ¹. Approximately 1 in 59 children in the U.S. have been diagnosed with ASD ², after several years in increasing prevalence ^{3,4}. Studies linking environmental factors to ASD have proliferated in the last ten years with growing evidence for an etiologic role.

It has been accepted across many disciplines that social status and neighborhood resources affect an individual's health status ^{5,6}. Additionally, we know that place of residence is strongly determined by social position and ethnicity, and neighborhood characteristics are important contributors to inequality in health ⁷⁻⁹. Reinforcing mechanisms of this inequality include environmental pollutants, residential segregation and gradients in resources. While study of community health effects has grown exponentially over the past 15 years ⁸ the effects of both neighborhood- and individual-level economic and mental health stressors as susceptibility factors for the influences of exogenous environmental exposures on neurodevelopment, have received little attention. This project will examine how these stressors might contribute to ASD risk through the use of geographically based relative measures of community environmental exposures and socioeconomic disadvantage.

Use of geographic measures has also been examined and found to be a valid common and accessible metric for evaluation of health impacts of limited resources and community characteristics in The Public Health Disparities Geocoding Project ^{10,11}. One such metric was developed by the Office of Environmental Health Hazard Assessment (OEHHA), on behalf of

the California Environmental Protection Agency (CalEPA). In response to environmental justice legislation, OEHHA created the California Communities Environmental Health Screening Tool: CalEnviroScreen¹², a screening methodology that identifies California communities that are disproportionately burdened by multiple sources of pollution and socioeconomic disadvantage. The tool uses data from national and state sources to produce geographic based relative measures of seven environmental exposures, five indicators of the effects of pollution, and seven that describe population characteristics and socioeconomic factors for all of California's census tracts¹³. This score is an effective way to compare one geographic area to others in the state on the basis of the particular hazard or population characteristic. The primary objective of this investigation was to assess the contribution of risk of ASD from these 19 indicators while controlling for maternal factors for potential confounding.

CalEnviroScreen has previously been used to examine the health impact of living in disadvantaged communities on survival rates among women diagnosed with advanced stage epithelial ovarian cancer between 1996 and 2006 in California. An increase in community disadvantage was significantly associated with poorer ovarian cancer survival (hazard ratio, 1.16; 95% confidence interval (CI) 1.07, 1.26). Ozone levels and socioeconomic status (SES) were the most influential indicators of geographic disparities in ovarian cancer survival rates.¹⁴

CalEnviroScreen indicators were also used to compare hospitalization rates for 14 ICD-9 disease categories to measure disease burden at the zip code level. Principal component analysis was used to visualize and reduce the number of measures. When limited to the environmental variables, two PC axes, one associated with air pollution and industrial sources and the second related to ozone levels, drinking water and PM2.5 axes explained 43% of variance. When the 5

socioeconomic variables were analyzed, the first PC explained 66% of variance, indicating that more model variation was explained by the measures of community socioeconomic status than environmental pollution measures¹⁵. This analysis suggested that socioeconomic status has a greater impact on ovarian cancer survival than environmental pollution, and supported the use of the tool for screening of vulnerable communities in California. We proposed that using these spatially based relative measures of environmental impact and population vulnerability would provide a method to evaluate multiple pollution sources and stressors, and their association with risk of ASD in prenatally exposed children. Additionally, we attempted to understand if controlling for community level vulnerabilities alters the effect of individual level findings from financial and mental health stressors

Methods

This assessment of socio-economic and environmental factors in relationship to individual factors and child's neurodevelopmental diagnosis was conducted with both mapping software QGIS¹⁶ and SAS version 9.4 statistical software¹⁷.

Data are from the CHARGE study, a population-based case control investigation. Study protocols have been described elsewhere¹⁸. Our investigation included 728 children with ASD, and 482 typically developing (TD) children who served as controls. The CHARGE Study is uniquely suited for assessing environmental exposures and community level factors, with a large sample size resulting from population-based recruitment. There is a broad range of risk factors for which detailed data have been collected and standardized confirmation of all neurodevelopmental diagnoses. CHARGE participants were between the ages of 24 and 60

months, born in California, residing in the catchment areas of specified regional centers (RC) in California, living with at least one biologic parent who spoke English or Spanish.

Controls were identified from state birth files. Controls who met the inclusion criteria were randomly sampled with frequency-matching to the distributions among autism cases based on age, sex, and broad residential RC catchment area. Participation in CHARGE included assessments of the child's cognitive and social development. CHARGE study children were assessed at the University of California Davis (UCD) MIND Institute and the UCLA Neuropsychiatric Institute with standardized clinical assessments by research reliable clinicians to either confirm the child's diagnosis or re-classify them for research purposes.

Mothers completed an extensive telephone interview conducted by trained research staff that included questions regarding demographic information, environmental exposures, family lifestyle, family occupational and residential histories. Financial hardship was assessed by the question: "Was there a time between 3 months before pregnancy with [CHILD] to the present, when it was hard for you to pay for basic needs like food, housing, medical care and heating?". Mothers also answered questions to determine if they experienced any mental health concern, including depression, anxiety, or loss of interest in daily activities, for more than 2 weeks during their pregnancy. Anxiety and depression are mental health concerns that are both highly related to one another conceptually, and statistically similar to each other within our study population. We created a binary composite measure for a CHARGE mother self-reported experiencing any perinatal mood or anxiety disorder (PMAD); mothers diagnosed with bipolar disorder or schizophrenia were excluded.

All clinical assessments, surveys and questionnaires were administered in English or Spanish, depending on the language in which the parent or child felt most comfortable. Consent was given per protocols approved by the UCD Institutional Review Board and the State of California Committee for the Protection of Human Subjects.

CHARGE study participant residential addresses were standardized and geocoded to yield longitude and latitude. Unmatched or uncertain addresses were manually matched to the correct longitude and latitude using Google Maps. California Teale Albers NAD 1983 projection was used because it minimizes the distortion of the state of California. This projection is an adaptation of the Albers Conical Equal Area projection as defined by the State of California Teale Data Center GIS Solutions Group. It is a statewide projection that is optimized for area calculations, making it ideal for mapping state level data.

Community sociodemographic and pollution data were accessed and downloaded from the

Figure 1. CalEnviroScreen Indicators

Pollution Burden	Population Characteristics
Exposures Ozone concentrations Concentrations of particulate matter (PM) Diesel PM emissions Pesticide use Airborne toxic chemical releases from facilities	Sensitive Populations Asthma emergency department (ED) visits Low birth-weight infants Cardiovascular disease (ED visits for heart attacks)
Environmental effects Impaired water bodies Sites with groundwater threats Sites targeted for cleanup Hazardous waste sites	Socioeconomic vulnerability Educational attainment Linguistic isolation Poverty Unemployment

CalEnviroScreen 3.0 website maintained by OEHHA in the form of geographic information system (GIS) shapefiles, which are geospatial vector data formatted for GIS software. CalEnviroScreen 3.0 measures are available for 7929 of California's 8035 census tracts,

and are based on data collected between 2009 and 2016. The CalEnviroScreen 3.0 model uses a scoring system in which the percentiles across the state for each of the indicators are averaged and separated into four categories: Exposures, Environmental Effects, Sensitive Populations, and Socioeconomic Factors (Figure 1). Pollution Burden components are divided into 7 variables that rank Exposure (ozone concentrations, concentrations of particulate matter (PM), diesel PM emissions, pesticide use, airborne toxic chemical releases from facilities, drinking water contamination, and traffic density), and five that describe Environmental Effects (impaired water bodies, sites with groundwater threats, sites targeted for cleanup, hazardous waste sites, solid waste sites). There are 5 variables that measure socioeconomic vulnerability: educational attainment, linguistic isolation, poverty, unemployment, and the number of households severely burdened by housing costs. There are 3 specific health outcome variables that describe sensitive populations: asthma emergency department (ED) visits, low birth-weight infants, and cardiovascular disease (ED visits for heart attacks). The variables related to health and socioeconomic vulnerability describe and rank each communities' population characteristics.

CHARGE participant data were joined with the CalEnviroScreen spatial variables using QGIS ¹⁶. Differences in demographic characteristics and pollution measures between diagnostic groups were described using two tailed t-tests for continuous variables and chi-square tests for binary and categorical variables. Principal component analysis (PCA) was conducted to further explore the data and broadly examine relationships between the variables. Initially PCA was conducted to facilitate an examination of the variance explained by each of the individual variables, and the multivariate structure of the dataset, and secondly to explore the possibility of data reduction. This transformation is conducted to account for the maximum amount of variation in the data

while identifying factors that are perpendicular (or uncorrelated) to each other. We utilized the principal components rather than the 19 individual measures to facilitate a focus on the overall patterns of exposure and vulnerability while reducing the number of required analyses.

Each of the five principal components (PC) was created by averaging the relative contribution of each CalEnviroScreen indicator within each factor. These PC's were normalized to achieve more linear robust relationships from regression results. Unadjusted odds ratios (OR) of ASD for each created PC were calculated. Adjusted OR's were calculated with separate models for each PC when individual level confounders were addressed. In the third set of models, adjusted OR's were calculated for each component when maternal stressors (mother's financial hardship and mood disorders) were added to the models. These OR's and their 95% confidence intervals (CIs) were calculated with multivariate logistic regression models implemented in SAS PROC SURVEY LOGISTIC using CHARGE survey weights. Survey weights are used to adjust for the probability of study participation by diagnostic group and sociodemographic factors known to influence voluntary participation in research studies. It was possible to calculate survey weights for this investigation because data are available on the entire target population – both for ASD cases and TD controls. Within our catchment area, the participation model for ASD cases predicted participation in CHARGE from the population of all eligible ASD cases receiving services from the California Department of Developmental Services. Based on sociodemographic variables recorded in birth records; similarly, the participation model for controls predicted participation in CHARGE among all eligible births from the State Vital Statistics records based on those same sociodemographic variables. The weights represent the inverse of the probability of participation within diagnostic and demographically defined groups,

so that weighted logistic regression models can more accurately generate findings that are generalizable to the population from which participants were recruited.

Final model building was conducted by starting with full models for PMAD and financial hardship using the step-down approach. Potential confounders were identified and assessed for inclusion using a directed acyclic graph (DAG) - a visual representation of causal assumptions. Use of the DAG was informed by bivariate analysis conducted in the previous chapter, as well as exploration of the additional relationships created by the additional of CalEnviroScreen variables. Potential confounders were removed one at a time, and at each step the change in the estimate for the exposure and standard errors were examined. Only those variables that met the change in estimate criterion of $\geq 10\%$ change in the estimate for financial hardship or PMAD were retained in the final models. To further understand relationships between variables, a test for interaction was performed, and the possibility of changes in effect over differing levels of AirQ was explored.

Results

In this investigation, ASD and TD controls were similar with respect to child's age and gender as a result of frequency-matching. There were no significant differences between cases and controls for the educational level in the household, child's birth weight, gestational age at birth and maternal age at delivery; 82% were male and most participants were Caucasian (51%) or Hispanic (29%). Mothers of cases were more likely to have been born outside of the United States, and to not have used prenatal vitamins. During pregnancy, residences of the CHARGE study participants were distributed broadly throughout the five specific California Regional Center catchment areas, with the greatest concentrations in Sacramento Valley. During

interviews 155 (18.9%) mothers reported financial hardship during pregnancy and 612 (74.5%) did not, with 55 (6.7%) missing responses. For PMAD, 295 (35.9%) participants denied depression or anxiety, 291 (35.4%) suffered from a mood disorder and 236 (28.7%) were non-respondents.

Bivariate analysis of the CalEnviroScreen indicators and child's diagnosis yielded statistically significant differences between the mean percentile of the census tract between cases and controls for several of the measures. Means by diagnostic group with 95% CI and p-values for the test of means are shown in Table 2.

Pearson's pairwise correlation coefficients indicate multiple associations for the CalEnviroScreen variables (Table 3). The strongest positive pairwise association among all variables were groundwater threats versus drinking water contamination ($r = 0.85$), impaired water bodies versus diesel PM ($r = 0.8$), and ozone levels and clean-up sites ($r = 0.95$). Of the variables related to air quality measures, diesel PM was also associated with PM_{2.5} ($r = 0.40$), and the socioeconomic variables linguistic isolation ($r = 0.43$), and housing burden (0.31). PM_{2.5} was associated with drinking water, ozone, educational attainment and linguistic isolation. Multiple positive pairwise associations are noted between socioeconomic and health variables; asthma was highly correlated with all of socioeconomic variables. Several of the socioeconomic variables were moderately or highly associated with each other, linguistic isolation with education ($r = 0.67$) and housing burden ($r = 0.46$); unemployment is associated with asthma, education ($r = 0.52$) and housing burden ($r = 0.37$), and poverty ($r = 0.65$).

Principal component analysis

PCA was performed on the data set with the pollution, health and socioeconomic variables. As shown in Table 4. The first factor was comprised of most of the social/socioeconomic items, plus low birthweight, and accounted for 3% of the variance. The second factor was comprised of measures related to air quality (diesel PM, toxic releases from facilities, traffic density and PM_{2.5}) and explained 2.7% of the variance. The third factor explained 2.4% of the variance and was composed of variables describing impacts to soil and groundwater. The fourth factor, which explained 2.2% of the variance, included visits to the ED for asthma and heart attacks, ozone and unemployment. The fifth factor was composed of drinking water, pesticides and unemployment, and explained 2% of the variance. Details on these five PC's (SES, AirQ, Waste, Health and WaterQ), are in Table 4. Findings from PCA are in congruence with tests for means between groups reported in s in harmony All of the variables in the AirQ PC had statistically greater means between cases and controls (Table 2). This is similar for the variables related to WaterQ. Of the composites not included in PCA, CHARGE cases were more likely to live in communities with higher mean percentiles for their Pollution Burden: 41.4 for controls and 46.88 for cases, $p=0.007$.

The five principal components were then normalized by subtracting the mean and dividing by the standard deviation, and used as covariates in logistic regression models. Unadjusted OR's for each PC are reported in Table 5. The OR's show potential risk for mothers living in communities with elevated levels of Air Quality measures (OR = 1.1; 95% CI [1.3, 1.6]), and Water Quality (OR = 1.1; 95% CI [1.01, 1.2]). The analysis of each PC was repeated with an adjustment by individual level confounders, and the results are also reported in Table 5.

Significant risk was seen for the air and water quality composites with OR's of 1.2; 95% CI [1.1, 1.4]) and 1.1; 95% CI [1.01, 1.2] respectively. Further exploration of these community level relationships with child ASD was conducted with separate multivariate logistic regression models for each PC and inclusion of mother's financial hardship or maternal mood disorders. Adjusted OR's are reported in Table 5: increased odds of ASD were found for AirQ (OR=1.2; 95% CI [1.1, 1.4]) and WaterQ (OR=1.1; 95% CI [1.0, 1.2]) when controlling for financial hardship, and SES (OR = 1.2; 95% CI [1.04, 1.3]) and AirQ (OR = 1.2; 95% CI [1.1, 1.4]) when mothers' report of PMAD are included in the model.

The next step of this analysis utilized step-down model building starting with all of the PC community level composites and maternal stressors. The final models retained prenatal vitamin intake, maternal metabolic conditions, child gender, child race, mother's birthplace, year of child's birth and AirQ. Results are presented in Table 6. We found increased odds of ASD among mothers with financial hardship (OR = 1.5; 95% CI [1.1, 1.9]), and those who lived in communities with higher (worse) community-level air quality measures (OR = 1.1; 95% CI [1.01, 1.3]). Mothers reporting mood disorders exhibited increased odds of offspring ASD (OR = 1.9; 95% CI [1.5, 2.3]), and elevated risk with higher indicators of poor air quality (OR = 1.2; 95% CI [1.05, 1.3]) above the mean.

To evaluate if the effect of AirQ or financial hardship was modified by the presence of the other an interaction term was added to each of the models. The interaction term was not significant in either case, and model results are reported in Table 7. Then OR's for ASD with mothers with financial hardship compared to mothers without, over levels of AirQ and are reported in Table 8;

they range from 1.66 to 1.28 and are plotted in Figure 3. The effect of maternal PMAD on ASD risk with increasing levels of AirQ is shown in Table 9; odds ratios range from 1.18 to 3.02 and are graphically represented in Figure 4.

Discussion

In this examination of the effects of both neighborhood- and individual-level economic and mental health stressors as susceptibility factors for the influences of environmental exposures on neurodevelopment, we found that community-level variables impact ASD risk even when controlling for the family's SES and maternal mental health. Furthermore, community level variables are suggestive of a contribution of poor air quality to risk of child ASD. This was determined by using a PCA-derived composite variable composed of 5 CalEnviroScreen exposure measures for maternal residence during pregnancy: Diesel PM, Toxic Releases from Facilities, Traffic Density and PM_{2.5} concentrations. The results of multivariate logistic regression indicated that for each standard deviation increase in this principal component the child risk of ASD increases whether maternal mood disorders or financial hardship are included in the model. Financial hardship was not confounded by the AirQ measures of the community in which the mother resides. While AirQ measures are associated with risk this association is only statistically significant for mothers who are not already burdened by economic stressors. Worsening AirQ measures appear to contribute risk at higher levels when mothers are affected by a mood disorder. In answer to our original hypothesis, these findings suggest that consideration of community level factors aids in our understanding of maternal risk factors for ASD; a communities' air quality measures are associated with ASD risk and this risk varies based on maternal mental state.

This is not the first time that geographic measures have been explored in the search for potential risk factors of autism in the CHARGE population. A study by Volk *et al.* (2011) estimated the association between the distance from maternal prenatal residence to the nearest freeway or major road and their child's diagnosis. Maternal residence at the time of delivery was more likely to be near a freeway for cases than controls¹⁹. The current findings are in line with this previous study. These findings also augment our understanding that neighborhood context does matter; worsening indicators for air quality, or measures of sources of air pollution in a neighborhood are associated with an increased risk of ASD. Together, these CHARGE investigations add to our emerging understanding of the association between prenatal exposures to air pollution and child neurodevelopment. Thirty-one studies from multiple countries published between 2006 and 2015 were examined in a systematic review of the relationship between various air pollutants and cognitive function. Many of the studies showed weak but quantified relationships. Authors concluded that the evidence suggests that pollution generated by traffic, contributes to cognitive impairment²⁰.

A review of the epidemiologic literature on the association between outdoor air pollution and neuropsychological developmental published between 2012 and 2015 found sufficient evidence of detrimental effects of pre- or postnatal exposure to polycyclic aromatic hydrocarbons on global intelligence quotient. Authors who examined the evidence of the association between child ASD and pre-and postnatal exposure to fine particulate matter (PM_{2.5}), and nitrogen oxides found sufficient evidence for PM_{2.5} and but not for nitrogen oxides²¹. These collective findings led review authors to conclude that there was significant evidence to support their hypothesis that exposure to ambient pollution in utero and early life has a negative impact on the

neuropsychological development of children. A similar review focused on ASD risk specifically, and examined the evidence of multiple environmental exposures; authors found associations with autism, among several chemicals including traffic-related air pollutants²².

Our understanding of the mechanisms by which air pollution has an impact on children's brains, and increases risks of ASD is augmented by the use Magnetic Resonance Imaging in environmental health research. De Pardo Bert *et al.* (2018) conducted a systematic review of existing literature at the interface of neuroimaging and epidemiology. They included six studies that evaluated the impact of urban air pollution on children's brains, two studies comparing children living in Mexico City with those living in less polluted cities, a New York city cohort of mothers who were given personal air monitors in the third trimester to measure exposure to polycyclic aromatic hydrocarbons (PAH), and another that used measures of traffic related air pollution (EC, NO₂, airborne copper, PAH) in school environments in Barcelona Spain. Studies integrating epidemiology and neuroimaging revealed that long-term exposure (both pre and postnatal) are associated with adverse impacts on brain structures and functioning and that these impacts are detectable with imaging modalities. These studies suggest that white matter, cortical grey matter and basal ganglia could be the targets of traffic related air pollution, and these insults could underlie the observed association between air pollution and cognitive disorders²³.

In California specifically, data from air monitoring stations and land use regression models were used to estimate exposures, and investigators studied the influence of exposures to traffic-related air pollution during pregnancy on the development of autism. Cases were identified through the California Department of Developmental Services and linked to 1995–2006 California birth

certificates, 7,603 children with autism were matched with 10 matched controls. Investigators determined that for each interquartile range (IQR) increase, there was an increase in odds of autism for ozone (OR = 1.12; 95% CI [1.06, 1.19]; per 11.54-ppb increase), particulate matter \leq 2.5 μm (OR = 1.15; 95% CI [1.06, 1.24]; per 4.68- $\mu\text{g}/\text{m}^3$ increase) when mutually adjusting for both pollutants. Additionally, authors estimated 3–9% relative increases in odds per IQR increase for nitric oxide and nitrogen dioxide exposure estimates. These associations were strongest for children of mothers with less than a high school education. Authors concluded that an association between autism and prenatal air pollution exposure exists, mostly related to traffic sources²⁴. Conversely, 1,212 children in the Project Viva pre-birth cohort were followed to mid-childhood (median age 7.7 years) to study associations of pre- and postnatal pollution exposures with neurobehavioral outcomes. Third trimester of pregnancy exposure to black carbon (BC) and fine particulate matter (PM_{2.5}) was estimated using validated spatiotemporal models. They found that neither third trimester nor early childhood exposure to traffic-related pollution predicted greater executive function or behavior problems, or measures of metacognition and behavioral problems²⁵.

Limitations & Strengths

While this study allowed the examination of the impact of neighborhood level indicators and their associations with child neurodevelopment outcomes for exposures in the residential location, there are some unavoidable limitations. Every attempt was made to minimize error in the geocoding process, yet the potential for error remains. Moreover, spatial analysis is limited by its inherent process of creating arbitrary and modifiable boundaries. Additionally, while misclassification of the outcome is not a concern in this study due to the secondary confirmation of diagnosis conducted on all CHARGE participants, misclassification of the exposure is likely

as we have assumed that participants experienced homogeneous exposure within the spatial boundary defined by their census tracts. These boundaries can often encompass distinct and varied communities that differ in terms of population size, area socioeconomic status, and individual residential distances to sources of contaminants or polluted areas. Although this limitation is less for air quality; among CalEnviroScreen measures of environmental exposures, air quality is one that is experienced broadly across a census tract.

While air quality indicators were the only CalEnviroScreen measures with significant statistical contribution to child ASD risk in this study, it does not preclude the potential for the impacts of other exposures that did not emerge as significant in this analysis. In this study population neighborhood SES was highly correlated with several environmental exposure measures, and both financial hardship and maternal mood disorders thereby limiting our power to examine the independent effect of neighborhood level socioeconomic variables. This analytic strategy was not intended to use neighborhood context as a proxy for biomonitoring but rather to address the contribution of the community in which the pregnant woman resides. Given that environmental exposures continue to be experienced unequally across communities, further investigation is needed to understand neighborhood level exposures and stressors, and how they interact with individual vulnerabilities.

Conclusion

The value of documenting the social patterns of disease has been a core principle of the public health movement, and provides vital information about the burdens that populations face.

Additionally, emerging research continues to expand the boundaries of our understanding of the health impacts of environmental exposures. The current findings are an important addition to the

investigations that have measured and reported the effect of air quality and how they may interact with social stressors to heighten vulnerability. This interaction of environmental and social stressors has been referred to as a “double jeopardy”²⁶. Recognition of these amplified effects is critically important for decisions regarding allocation of resources and deployment of prevention efforts. This more comprehensive understanding extends our capacity to answer etiologic hypotheses about disease causation based not only on individual exposures but exposures experienced at the community level. This mounting evidence confirms that the precautionary principle should be applied to protect children, with government action through regulation and public policy to reduce air pollution, focusing on those communities with the highest exposure levels and socioeconomic vulnerabilities.

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Tables and Figures

Table 1. Demographic and clinical characteristics [n (%) or mean ± standard deviation (SD)] of the study population (n = 817), stratified by child diagnosis

<i>Covariate</i>	<i>Child's Diagnosis</i>		<i>P-value*</i>
	<i>TD¹</i> (<i>n</i> = 329)	<i>ASD²</i> (<i>n</i> = 488)	
Child Male Gender, n (%)	268 (82%)	417 (84.2%)	0.39
Father's education, n (%)			0.77
High school diploma/GED or less	33 (10.1%)	56 (11.3%)	
Some college (incl. voc., 2-yr degree)	111 (33.9%)	169 (34.2%)	
Bachelor degree	109 (33.3%)	149 (30.2%)	
Graduate or professional degree	74 (22.6%)	120 (24.3%)	
Child's Race/Ethnicity, n (%)			0.73
White	166 (51.1%)	238 (48.3%)	
Hispanic	97 (29.8%)	157 (31.8%)	
Other	62 (19.1%)	98 (19.9%)	
Regional Center Area, n (%)			<0.001
Alta	151 (46.2%)	173 (34.9%)	
North Bay	39 (11.9%)	57 (11.5%)	
East Bay	56 (17.1%)	82 (16.6%)	
Valley Mountain	63 (19.3%)	105 (21.2%)	
Southern California	18 (5.5%)	78 (15.8%)	
Birth Place of Mother, n (%)			0.02
In the USA	264 (83%)	364 (74.7%)	
In Mexico	18 (5.7%)	44 (9%)	
Outside USA or Mexico	36 (11.3%)	79 (16.2%)	
⁴ Prenatal Vitamin Use, n (%)	192 (61.3%)	236 (53.2%)	0.025
Age of mother at time of child's birth			0.08
Mean (SD)	31.1 (6)	30.8 (6)	

* The p-value is calculated from ANOVA for numerical covariates; and chi-square test or Fisher's exact for categorical covariates, where appropriate.

¹Typically Developing ², Autism Spectrum Disorder

Table 2. Bivariate analysis of CalEnviroScreen community indicators stratified by child’s diagnosis

<i>CalEnviroScreen Indicator Percentile</i>	<i>TD¹ (n = 329)</i>		<i>ASD² (n = 488)</i>		<i>p-value*</i>
	<i>Mean (95% CI)</i>		<i>Mean (95% CI)</i>		
Pollution Burden	41.4	(38, 44)	46.88	(44, 49)	0.007
Population Characteristics	48.0	(45, 51)	46.75	(44, 49)	0.5
CalEnviroScreen30	45.3	(42, 48)	46.58	(44, 49)	0.5
Asthma	56.4	(53, 59)	53.19	(51, 56)	0.1
Cardiovascular Disease	55.6	(53, 59)	54.97	(53, 57)	0.7
Cleanup Sites	29.7	(26, 33)	35.64	(33, 39)	0.001
Diesel Particulate Matter	38.7	(36, 41)	44.7	(42, 47)	0.001
Drinking Water	45.8	(42, 49)	50.29	(47, 53)	0.045
Educational Attainment	41.3	(39, 44)	42.94	(41, 45)	0.3
Groundwater Threats	46.5	(43, 50)	46.18	(43, 49)	0.9
Hazardous Waste	30.7	(27, 34)	31.45	(29, 34)	0.8
Housing Burden	41.6	(39, 44)	42.41	(40, 45)	0.7
Impaired Water Bodies	43.2	(40, 47)	37.55	(35, 40)	0.014
Linguistic Isolation	37.8	(35, 40)	39.85	(37, 42)	0.3
Low Birth Weight	46.4	(43, 49)	44.17	(42, 47)	0.3
Ozone	50.9	(48, 54)	50.61	(48, 53)	0.9
Pesticides	24.8	(21, 28)	23.78	(21, 27)	0.0006
Particulate Matter 2.5	40.1	(37, 43)	45.63	(43, 48)	0.0059
Poverty	46.0	(43, 49)	46.64	(44, 49)	0.7
Solid Waste	26.3	(23, 30)	27.7	(25, 31)	0.6
Toxic Releases from Facilities	35.7	(33, 38)	40.5	(38, 43)	0.003
Traffic Density	44.2	(41, 47)	48.13	(46, 50)	0.04
Unemployment	54.5	(51, 58)	51.55	(49, 54)	0.1

¹Typically Developing ², Autism Spectrum Disorder

* $p > |t|$ for two-sample t-test used to test the difference between population means

Table 3. Pearson Correlation Coefficients

	Cleanup Sites	Diesel PM	Drinking Water	Groundwater Threats	Hazardous Wastes	Impaired Water Bodies	Ozone Concentration	PM2.5	Pesticides	Solid Waste	Toxic Releases	Traffic Density	Asthma ED visits	Cardiovascular Disease	Educational Attainment	Housing Burden	Linguistic Isolation	Low Birth Weight	Poverty	
Diesel Particulate Matter	0.15																			
Drinking Water	0.06	-0.17																		
Groundwater Threats	0.45	0.14	0.01																	
Hazardous Waste Sites	0.43	0.24	0.04	0.39																
Impaired Bodies of Water	0.11	-0.01	0.03	0.24	0.16															
Ozone	0.00	-0.25	0.37	-0.18	-0.13	-0.12														
PM2.5	0.15	0.40	0.42	-0.03	0.11	0.38														
Pesticides	0.08	-0.38	0.44	0.15	0.02	0.14	0.16	0.12												
Solid Waste	0.32	-0.18	0.12	0.33	0.29	0.19	0.03	-0.07	0.33											
Tox Release	0.19	0.54	0.04	0.04	0.21	0.10	-0.17	0.58	-0.15	-0.02										
Traffic	0.05	0.51	0.01	0.02	0.09	0.01	-0.06	0.26	-0.32	-0.17	0.33									
Asthma	0.09	0.23	-0.11	0.10	0.06	0.13	0.10	0.15	-0.10	-0.10	0.16	0.14								
Cardiovascular Disease	0.10	0.04	0.08	0.02	0.07	0.06	0.36	0.28	0.13	0.03	0.05	0.01	0.73							
Education	0.18	0.21	0.13	0.18	0.13	0.14	0.12	0.35	0.19	0.18	0.19	0.06	0.48	0.52						
Housing Burden	0.14	0.31	0.01	0.16	0.09	0.04	0.03	0.16	-0.08	0.03	0.16	0.23	0.33	0.20	0.49					
Linguistic Isolation	0.23	0.43	0.02	0.18	0.20	0.10	-0.14	0.35	-0.05	0.07	0.37	0.25	0.26	0.22	0.68	0.46				
Low Birth Weight	0.04	0.22	-0.08	0.01	0.07	0.12	-0.04	0.18	-0.05	-0.01	0.16	0.19	0.34	0.22	0.38	0.27	0.37			
Poverty	0.19	0.18	0.19	0.22	0.11	0.09	0.25	0.27	0.11	0.12	0.06	0.11	0.50	0.49	0.74	0.71	0.53	0.29		
Unemployment	0.06	-0.06	0.18	0.08	-0.02	0.10	0.34	0.23	0.14	0.04	-0.05	0.02	0.47	0.50	0.52	0.38	0.26	0.24	0.65	

Table 4. Results of principal component analysis of CalEnviroScreen environmental hazard and socioeconomic vulnerability variables across the CHARGE population

<i>Variable</i>	<i>Factor 1</i> <i>SES</i>	<i>Factor 2</i> <i>AirQ</i>	<i>Factor 3</i> <i>Waste</i>	<i>Factor 4</i> <i>Health</i>	<i>Factor 5</i> <i>WaterQ</i>
Poverty	0.786	-0.007	0.146	0.386	0.145
Housing Burden	0.755	0.129	0.073	0.077	-0.061
Linguistic Isolation	0.731	0.361	0.185	-0.046	0.035
Low Birth Weight	0.538	0.172	-0.029	0.090	-0.098
Unemployment	0.531	-0.150	-0.024	0.549	0.196
Diesel Particulate Matter	0.232	0.801	0.108	-0.018	-0.257
Toxic Releases from Facilities	0.115	0.787	0.138	-0.067	0.126
Traffic	0.135	0.659	-0.066	0.006	-0.097
PM2.5	0.155	0.650	0.014	0.221	0.298
Groundwater Threats	0.136	-0.039	0.755	-0.018	-0.110
Cleanup Sites	-0.002	0.169	0.731	0.147	0.026
Hazardous Waste	-0.048	0.250	0.710	0.060	-0.026
Solid Waste	0.073	-0.288	0.635	-0.098	0.218
Cardiovascular Disease	0.255	0.006	0.069	0.848	0.086
Asthma	0.366	0.123	0.061	0.761	-0.245
Ozone	-0.108	-0.088	-0.183	0.567	0.553
Unemployment	0.331	-0.150	-0.024	0.549	0.553
Drinking Water	0.026	0.023	0.044	0.004	0.833
Pesticides	0.090	-0.427	0.255	-0.068	0.615

Table 5. OR and 95% CI for each principal component

<i>Composite Variable</i>	<i>Univariate Logistic regression</i>	<i>Multivariate Logistic Regression*</i>		
	<i>Model 1</i>	<i>Model 2</i>	<i>Model 3</i>	<i>Model 4</i>
	<i>Unadjusted OR</i>	<i>Composite variable plus covariates</i>	<i>Maternal Financial Hardship</i>	<i>Perinatal Mood Disorders</i>
SES	1.1 (0.9, 1.1)	1.1 (0.9, 1.2)	1.08 (0.9, 1.2)	1.18 (1.04, 1.3)
AIRQ	1.4 (1.3, 1.5)	1.2 (1.1, 1.4)	1.14 (1.01, 1.3)	1.19 (1.05, 1.3)
Waste	1.05 (0.9, 1.2)	0.99 (0.9, 1.1)	1.00 (0.9, 1.1)	1.01 (0.9, 1.1)
Health	0.87 (0.8, 0.9)	1.04 (0.9, 1.1)	1.05 (0.9, 1.1)	1.06 (.97, 1.2)
WaterQ	1.1 (0.98, 1.1)	1.1 (1.01, 1.2)	1.11 (1.0, 1.2)	1.04 (.96, 1.1)

*Adjusted for prenatal vitamin intake, maternal metabolic conditions, child gender, child race, mother's birthplace, and year of child's birth

Table 6. OR and 95% CI for Multivariate Logistic Regression for models including financial hardship and perinatal mood and anxiety disorders (PMAD)

<i>Effect</i>	<i>Financial Hardship</i>	<i>PMAD¹</i>
<i>Individual level Models*</i>		
Financial Hardship	1.64 (1.23, 2.18)	--
PMAD ¹	--	1.92 (1.55, 2.38)
<i>Individual and Community Level Models*</i>		
Financial Hardship	1.48 (1.11, 1.97)	--
PMAD ¹	--	1.86 (1.50, 2.29)
Maternal Metabolic Conditions	1.18 (1.09, 1.28)	1.14 (1.06, 1.22)
Prenatal Vitamins Intake (yes)	0.70 (0.57, 0.86)	0.67 (0.55, 0.83)
Child Gender (male)	0.95 (0.75, 1.22)	1.03 (0.81, 1.32)
Child Race Hispanic ²	0.81 (0.64, 1.03)	0.78 (0.62, 0.97)
Child Race Other ²	0.71 (0.52, 0.95)	0.68 (0.47, 0.98)
Mother's Birth Place Mexico ³	1.05 (0.68, 1.63)	0.87 (0.55, 1.39)
Mother's Birth Place Other ³	1.69 (1.26, 2.27)	1.68 (1.21, 2.33)
Child's Year of Birth ⁴ – before 2001	3.24 (1.92, 5.46)	1.48 (0.78, 2.81)
Child's Year of Birth ⁴ 2002-2009	0.88 (0.55, 1.41)	0.76 (0.47, 1.23)
Regional Center Group	1.10 (1.00, 1.21)	1.21 (1.10, 1.33)
AIRQ Principal Component	1.14 (1.01, 1.30)	1.19 (1.05, 1.34)

*Adjusted for prenatal vitamin intake, maternal metabolic conditions, child gender, child race, mother's birthplace, and year of child's birth

¹Perinatal mood and anxiety disorders

Reference categories: ²White/Caucasian race ³United States ⁴YOB category 2010 - 2012

Table 7. Multivariate logistic regression estimates for social stressors, AirQ* principal component and their interactions

<i>Financial Hardship</i>				<i>Perinatal Mood and Anxiety Disorders</i>			
<i>Parameter</i>	<i>Estimate</i>	<i>std. err.</i>	<i>Pr > t </i>	<i>Parameter</i>	<i>Estimate</i>	<i>std. err.</i>	<i>Pr > t </i>
Intercept	-0.5093	0.2594	0.0523	Intercept	-0.6945	0.2687	0.0125
Fin Hardship	0.3783	0.1377	0.0071	PMAD	0.6341	0.1055	<0.0001
AIRQ	0.1596	0.0659	0.0172	AIRQ	0.0857	0.0739	0.2516
Interaction	-0.0957	0.1428	0.5042	Interaction	0.1504	0.1118	0.1842

*Increasing levels of AirQ principal component indicate higher percentile levels of diesel particulate matter (PM), toxic releases from facilities, traffic density, and PM2.5.

Table 8. Odds Ratio with 95% CI at levels of AirQ* principal component

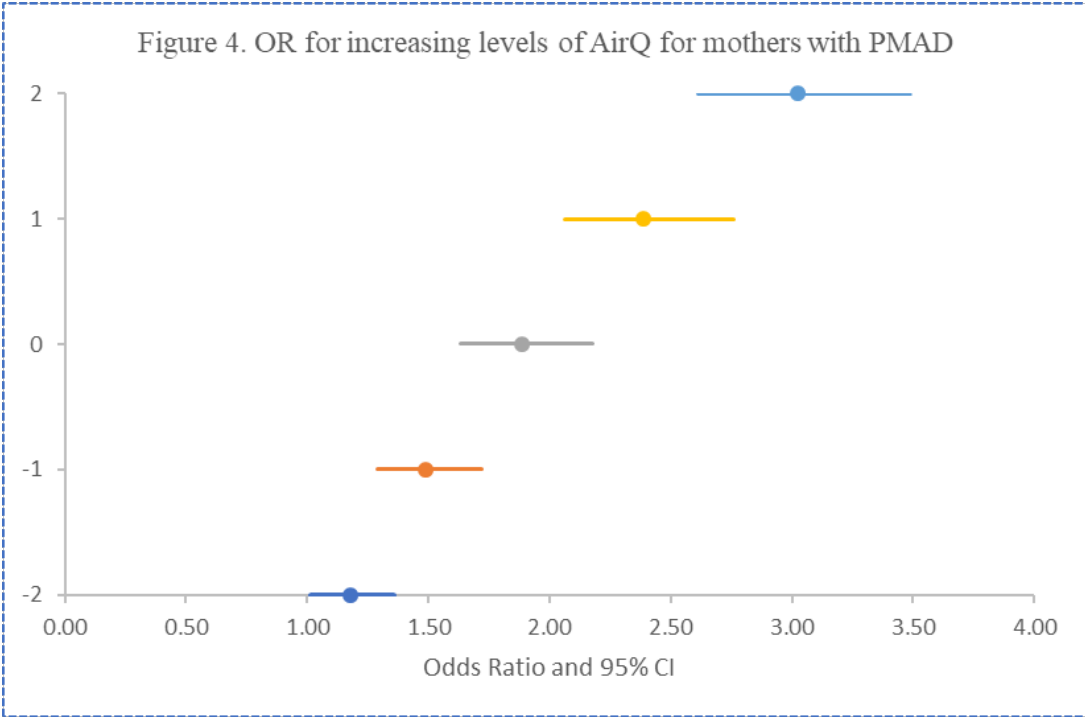
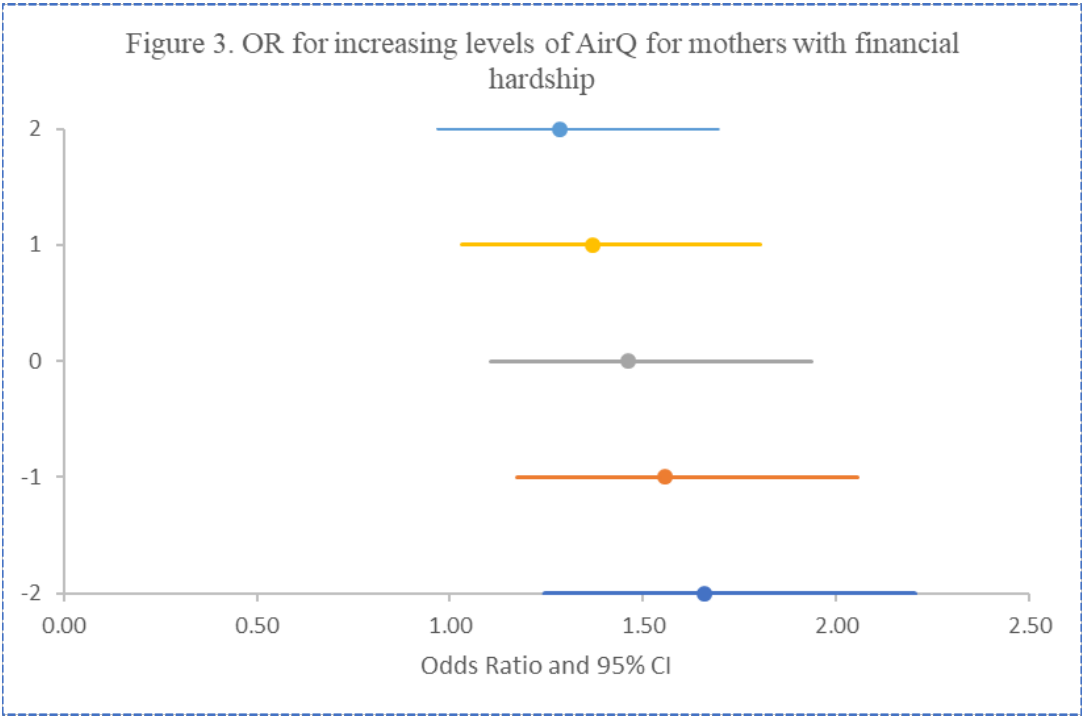
Air Quality Level	Financial Hardship Yes vs No	PMAD Yes vs No
2 std dev below the mean	1.66 (1.26, 2.20)	1.18 (1.02, 1.36)
1 std dev below the mean	1.56 (1.17, 2.06)	1.49 (1.29, 1.72)
Mean AirQ Measure	1.46 (1.10, 1.94)	1.89 (1.63, 2.18)
1 std dev above the mean	1.37 (1.03, 1.80)	2.39 (2.07, 2.76)
2 Std dev above the mean	1.28 (0.97, 1.70)	3.02 (2.61, 3.50)

*Increasing levels of AirQ principal component indicate higher percentile levels of diesel particulate matter (PM), toxic releases from facilities, traffic density, and PM2.5.

Table 9. Odds Ratio and 95% CI at highest levels of AirQ vs living at lowest levels of AirQ*

With Financial hardship	1.29 (0.98, 1.72)
Without Financial hardship	1.89 (1.43, 2.51)
With PMAD	2.57 (2.21, 2.96)
Without PMAD	1.41 (1.22, 1.62)

*Increasing levels of AirQ principal component indicate higher percentile levels of diesel particulate matter (PM), toxic releases from facilities, traffic density, and PM2.5.



Chapter 4: Organophosphate Exposures, Financial Hardship and Child

Neurodevelopmental Outcomes in the CHARGE study

Abstract

Background: In-utero exposure to organophosphate pesticides has been associated with neurodevelopmental delay. This study examined whether maternal report of inability to pay for basic needs (food, housing, medical care and heating) modified the relationship between prenatal pesticide exposure and autism spectrum disorder (ASD).

Study Design/Methods: We analyzed data from 488 cases of autism spectrum disorders and 329 typically developing controls in the CHARGE (Childhood Autism Risks from Genetics and the Environment). Children aged 2 to 5 years were enrolled in this population-based, case-control investigation between 2003 and 2008. Children's diagnoses were confirmed by standardized assessments, and information regarding maternal factors was determined from a structured interview with the mother. Residential proximity to agricultural application of organophosphate pesticides, based on using data from the California Department of Pesticide Regulation, was determined by spatial analysis of maternal residences before and during pregnancy. Multiple logistic regression was used to examine the association between exposure during several time points during the pregnancy, and effect modification by the experience of financial hardship.

Results: 213 (26%) CHARGE study mothers lived within 1.5 km of agricultural application of organophosphate compounds during their pregnancies. The adjusted odds ratio (OR) for child ASD for mothers with prenatal residences in proximity to organophosphate applications during 2nd trimester was 1.92; 95% CI [1.35, 2.76] for mothers without financial hardship. For mothers who reported financial hardship, the odds ratio for ASD increased to 3.01; 95% CI [1.34, 6.75] during this time period, and was 2.44; 95% CI [1.05, 5.63] for pesticide exposures that occurred

between the 3 months prior to conception up through delivery. For Chlorpyrifos specifically, and among women not reporting financial hardship increased odds of ASD were measured for 2nd trimester exposures (OR = 2.08; 95% [1.36, 3.18]); for those with financial hardship ORs ranged from 5 to 15 for this association at all timepoints throughout the pregnancy.

Conclusions: This study identified financial hardship as an amplifier of the association between organophosphate pesticide exposures during pregnancy and offspring ASD. It adds to the existing evidence highlighting the importance of studying the co-exposure of social and environmental exposures affecting children at early developmental stages.

Introduction

Approximately 1 in 59 children in the U.S. has Autism spectrum disorder (ASD)¹, and prevalence has been increasing^{2,3}. ASD is characterized by impairments in social interaction, communication deficits, and a pattern of restricted or repetitive interests, activities, and behaviors⁴. While the symptoms of ASD do not emerge until early childhood, current evidence is consistent with etiologic roots in fetal development⁵. Goines and Ashwood (2013) proposed a multifactorial model for ASD that posits a genetically susceptible individual whose predisposition includes an inappropriate or ineffective response to exposures in the maternal environment⁶. This exposure during a critical period of fetal development could disrupt development of the nervous and/or the immune systems, and lead to ASD. Casanova (2007) posits a triple hit hypothesis, with multifactorial disorders offering a threshold phenomenon with three factors converging: a critical period of brain development, exogenous stressors and a fetus with an underlying vulnerability⁷.

The need for understanding childhood risk from neurotoxic environmental chemicals is critical. Children while in utero and in the first months after birth have metabolic pathways that are immature when compared with adults; have greater exposures to toxic chemicals for their body weight than adults; have limited capacity to detoxify chemicals, and so are more vulnerable to their adverse effects⁸. Several examples of in-utero environmental exposures have been examined in the literature, and include pesticides and maternal prenatal stress⁹.

The CHARGE (Childhood Autism Risks from Genetics and the Environment) study has made major contributions in our understanding of the prenatal neurodevelopmental effects of in utero

exposures to pesticides ^{10,11}. These CHARGE investigations added to multiple investigations that collectively have indicated a relatively strong association between pesticide exposure during gestation and ASD ¹², with other studies with differing methodologies reporting a two- to fivefold increase in risk ¹³⁻¹⁷. One retrospective case–control study enrolled cases from the California Department of Developmental Services and examined estimates of pesticide exposure obtained from the California Department of Pesticide Regulation (CDPR). Investigators found that early exposure to organochlorine pesticides (dicofol and endosulfan), based on residential proximity to commercial applications were associated with an increased risk of ASD ¹⁶. A follow-up investigation identified two critical periods of developmental vulnerability: preconception through second trimester (23 weeks) and the immediate postpartum ¹⁸.

In 1932 German scientists first described the cholinergic nervous system effects of organophosphates (OP) after experiencing a choking sensation and a dimming of vision after exposure ¹⁹. Further development of OP compounds continued when their potential use as chemical warfare agents were explored. After World War II American companies gained access to German laboratory materials to further develop these compounds for agricultural application. Use of OP pesticides increased after many of the organochlorine insecticides were banned in the 1970's. OP samples degrade rapidly by hydrolysis on exposure to light, air and soil and were considered safer than the organochlorines which have high persistence in the environment ²⁰. Dozens of OP pesticide compounds are now registered for use in California ²¹. Commonly used organophosphates have included: parathion, malathion, methyl parathion, and chlorpyrifos. The principal mechanism of action of OP pesticides is inhibition of cholinesterase, which breaks

down the neurotransmitter Acetylcholine into choline and acetic acid to prevent over-stimulating post-synaptic nerves, muscles, and exocrine glands.

Chlorpyrifos (CPF), is one of the most widely used organophosphate insecticide compounds in the United States, and before being phased out for residential use starting in 1997, was one of the most widely used residential insecticides ²². Currently the California Department of Pesticide Regulation (CDPR) has 34 brands names registered ²¹ Approximately 10 million pounds are applied annually in agricultural settings (EPA). Like the other OP pesticides, CPF can cause cholinesterase inhibition in humans. The resulting overstimulation of the nervous system can cause nausea, dizziness, and confusion; respiratory paralysis and death are possible at very high exposures occurring as a result of accidents or major spills ²².

A systematic review of 28 studies examining prenatal OP exposures found adverse effects on neurodevelopment in 27 of the included studies ²³. The ten longitudinal studies that assessed prenatal exposure to OP pesticides also found deficits in neurodevelopment and behavior specific to the time that the children were assessed. Rauh *et al.* (2011) conducted a prospective cohort study that followed 254 New York City inner-city newborn infants who were prenatally exposed to CPF. Exposure levels were determined by umbilical cord plasma CPF concentration.

According to responses provided by mothers on the 99-item Child Behavior Checklist ²⁴, the most highly exposed newborns were five times more likely than those at lower exposure levels to exhibit symptoms of neurodevelopmental delay by age three ²⁵. CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas) investigators followed newborn infants from Latino farm-worker families in California who were exposed to OP pesticides during

pregnancy. Exposure was estimated by maternal prenatal urinary biomarkers of some OP pesticides -- dialkylphosphate (DAP) metabolites. Risk of neurodevelopmental delay doubled for each 10-fold increase in DAP metabolites prenatally²⁶. Other investigations have linked prenatal exposure to organophosphate pesticides with decreased IQ²⁷, increased psychomotor and mental development delays, attention problems, attention deficit/hyperactivity disorder (ADHD)-like problems, and symptoms consistent with pervasive developmental disorder²⁸.

In an urban cohort, inner city minority pregnant women were enrolled prospectively to determine the impact of maternal prenatal exposure of CPF on child neurodevelopment and behavior. Prenatal CPF exposure was measured using umbilical cord blood plasma, and the children were examined at 3 and 7 years of age. At 3 years of age highly exposed children (versus those with lower exposures) scored on average, 6.5 points lower on the Bayley Psychomotor Development Index²⁴, and 3.3 points lower on the Bayley Mental Development Index. Highly exposed children were 2.4 times more likely to have mental delays and 4.9 times more likely to have motor delays²⁵. Neurodevelopment was again assessed at age 7 using the Wechsler Intelligence Scale for Children (WISC-IV). On average, for each standard deviation increase in CPF exposure, full-Scale intelligence quotient among children in the cohort declined by 1.4% and working Memory declined by 2.8%¹⁵. Working memory helps kids hold on to information long enough to utilize it. It plays an important role in concentration, following instructions, and the ability acquire new information.

This cohort of children was followed to determine if neighborhood characteristics correlated with early neurodevelopment, and whether these characteristics confounded the previously

determined association between maternal prenatal CPF exposure and child's neurodevelopment. Investigators used census data to estimate measures of physical infrastructure, socioeconomic status, population density, demographic composition, and linguistic isolation for 1-kilometer network areas around each child's prenatal address. They did not find neighborhood-level confounding of the CPF-neurodevelopment association. In fact, both neighborhood context and CPF exposure were independently associated with neurodevelopment²⁹. Additional analyses did not observe a remediating effect of a high-quality home environment (either parental nurturance or environmental stimulation) on the adverse effects of prenatal CPF exposure on working memory.

While there are rigorous prospective studies that have demonstrated risks of prenatal OP pesticide exposure to neurodevelopment, few have examined these effects in the context of the experience of the stress of financial hardship. Indicating that a further investigation is warranted into how maternal economic stressors might augment the risks from OP pesticide exposures previously examined in the CHARGE population¹⁰.

Methods

This investigation was limited to children who were part of the CHARGE study for whom data regarding residential proximity to agricultural pesticide application was available. Study protocols have been described elsewhere³⁰. Briefly, eligible children (ASD cases and controls) were between the ages of 24 and 60 months, born in California between 2003 and 2008, living in the catchment areas of specified regional centers (RC) in California, with at least one biologic parent who speaks English or Spanish. Children with autism were identified through the California Regional Center System that coordinates services for persons with developmental

disabilities. General population controls were identified from state birth files and those who met the inclusion criteria were randomly sampled with frequency-matching to the distributions among autism cases based on age, sex, and broad residential RC catchment area. CHARGE study children were assessed by research-reliable clinicians at the University of California Davis (UCD) MIND Institute and the University of California Los Angeles Neuropsychiatric Institute to either confirm their diagnosis or re-classify them for the purposes of the study. Consent was given per protocols approved by the UCD Institutional Review Board, and the State of California Committee for the Protection of Human Subjects.

Mothers participated in an extensive telephone interview conducted by trained research staff. Questions covered demographic characteristics, prenatal vitamin intake, environmental exposures, family lifestyle, parental occupational and residential histories. Financial hardship was assessed by the question: “Was there a time between 3 months before pregnancy with [CHILD] to the present, when it was hard for you to pay for basic needs like food, housing, medical care and heating?”.

Estimation of pesticide exposures

Legislation passed in 1990 requires that all agricultural pesticide use in California must be reported monthly to county agricultural commissioners, who in turn report the data to the CDPR³¹. California’s reporting legislation named a broad legal definition of agricultural use applications to include parks, golf courses, cemeteries, rangeland, pastures, and along roadside and railroad rights-of-way. This regulatory program is considered by many to be a model program, and makes application data publicly available annually in the Pesticide Use Report (PUR) database. The PUR database reports compiled commercial pesticide applications

throughout the state by square mile areas called meridian-township-range-section (MTRS) that are defined by the US Geological Survey. As described elsewhere ¹⁰, mapping software was used to assign a center point or centroid for each square mile of area, i.e., MTRS. Buffers of varying radii (1.25 km, 1.5 km, and 1.75 km) were assigned for each participants' residence from 3 months before conception up to delivery. In places where the residential buffer intersected with a centroid, or multiple centroids, the amount of OP pesticides applied in that area as reported to the PUR database was assigned to the residence. This allowed the assignment of an exposure profile corresponding to applications made in the MTRS inclusive of the mother's home on the days of her pregnancy that the application occurred. Chemical exposures of interest examined in this analysis included: organophosphates - both the general category and chlorpyrifos specifically. Exposure measures for each pesticide category were created for 6 time periods: the three months prior to pregnancy, each trimester, the pregnancy – all three trimesters, and the childbearing year (inclusive of all 3 trimesters and the preconception period). For each time period each CHARGE mother was assigned a binary indicator, with 1 indicating exposure. Homes without a record of OP pesticides applied with the buffer zone received an exposure profile value of 0. multivariate logistic regression modeling with survey weights was used to estimate the association of prenatal residential proximity to applied pesticides with these binary exposure variables, using TD children as the reference group.

Potential covariates were identified and assessed for inclusion using a directed acyclic graph (DAG), which is helpful in examining causal assumptions between exposures, social factors and health, as they allow a determination of the overall effect of an exposure within a framework. Unadjusted odds ratios and their 95% confidence intervals were used to inform the construction

of the DAG and to assess the association between primary exposures and child's diagnosis. After using the DAG to examine and identify key control variables, the change in estimate criteria was used to exclude variables with minimal (<10%) effect in the β estimate. Therefore, final models included only those variables that were identified as confounders that also changed the β estimate by > 10% during a step-down approach to model building. At each step, the change in the estimate for the exposure their standard errors, and AIC were examined. Final models included: the pesticide exposure variable, financial hardship, maternal metabolic conditions, father's education level, prenatal vitamin use, child race, gender, and year of birth.

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated with multivariate logistic regression models using PROC SURVEY LOGISTIC with CHARGE survey weights, in SAS version 9.4³². Survey weights are used to adjust for the probability of study participation by diagnostic group and sociodemographic factors known to influence voluntary participation in research studies. It is possible to calculate survey weights for this investigation because data on every ASD case in California is available from the California Regional Center System. The weights represent the inverse of the probability of participation within diagnostic and demographically defined groups, so weighted logistic regression models can more accurately generate findings that are generalizable to the population from which participants were recruited.

After obtaining the estimate of the main effect of proximity to pesticide application for each time period, we tested to see if this measure differed according to whether or not the mother reported experiencing financial hardship. This allowed a test the hypothesis that financial hardship would magnify the association between prenatal pesticide exposure and child ASD.

Results

As a result of frequency-matching, the ASD and TD controls in the study were similar with respect to child's age and gender; 84% were male and most participants were Caucasian (52%) or Hispanic (18%). Table 1 describes the characteristics of the sample, stratified according to child's diagnosis and whether or not the mother reported financial hardship. There were no significant differences between cases and controls for the educational level in the household, child's birth weight, gestational age at birth and maternal age at delivery. During pregnancy, residences of the CHARGE study participants were distributed broadly throughout the five specific California Regional Center catchment areas, with the greatest concentrations in Sacramento Valley, followed by the San Francisco Bay area and Los Angeles. The Southern California site, representing 12% of the sample, experienced challenges in recruiting population controls.

Table 2 describes the exposure to OP pesticides stratified by child's diagnosis. There were 213 homes within 1.5 km buffers of organophosphate application during pregnancy. Of these, 87 (41%) children were typically developing and 126 (59%) were diagnosed with ASD. For CPF specifically, 108 mothers lived in homes with proximity to applications of these compounds. Of these mothers, 46 (42.6%) delivered typically developing children, and 62 (57.4%) had children with ASD. Figure 1 shows odds ratios and 95% confidence intervals for child's ASD with prenatal proximity to pesticide application, stratifying on mother's report of financial hardship. These results indicate elevated risk for 2nd trimester exposure to any OP (OR = 1.92; 95% CI [1.35, 2.76]), and CPF in particular (OR = 2.08; 95% [1.36, 3.18]). For mothers reporting financial hardship, the ORs for ASD for children with in-utero exposure to any OP or CPF s rise

dramatically from 2.4 to 15.6 depending on the time period being evaluated. ORs graphically represented in Figure 1, are reported in Table 3.

Discussion

Our work expands on the findings of the previous chapter, and established financial hardship as a modifier of the association between in-utero exposure to organophosphate pesticides and ASD. This adds to existing evidence highlighting the importance of studying the co-exposure of social and chemical exposures affecting children at early developmental stages of life. These findings of exacerbated risk are in harmony with other epidemiological studies of pre- and postnatal exposures to environmental neurotoxins that measured greater risk from exposure in the presence of social disadvantages. For example, investigators have found that the strength of the association between lead exposure and early neurodevelopment and performance deficit increased when exposed children were from low income homes when compared to children from middle-class backgrounds^{33,34}, and maternal stress modified the effect of exposure to lead during pregnancy and child neurodevelopment at age 2³⁵. An investigation into neurodevelopmental toxicity of prenatal PCB and dioxin exposure on cognitive and motor abilities found that effects were only seen in children from less advantaged homes³⁶. Rauh *et al.* (2014) found that children who were exposed to tobacco smoke and had families that suffered material hardships exhibited the greatest cognitive deficits on the Bayley Scales³⁷ when compared to children who were only exposed to tobacco smoke.

Limitations and Strengths

This study has several strengths, as with the previous chapters it is a population-based case control study with well-defined and consistently applied diagnoses, and extensive information on covariates. Additionally, many studies of children's exposure to OP pesticides have focused on

special populations, like urban children or children of agricultural workers who are expected to have higher rates of exposure. By using residential proximity this investigation allows measure of exposures experienced by a diverse population, that includes large suburban and urban areas. Exposure measures are further strengthened by the fact that the PUR database report of pesticide program is recognized as the most comprehensive in the world and follows California's expanded definition of agricultural use includes all commercial applications. The database is rigorously maintained with up to 50 different validity checks made on entered reports of pesticide use.

Despite these strengths, it important to remember that this study is only examining one class of pesticide compounds and only agricultural and commercial applications. It is limited by potential misclassification produced by the pesticide exposure assessment, the PUR database though comprehensive cannot measure all potential sources of exposure to pesticides, for example dietary sources. As a result of widespread use of pesticides in the United States, mothers may have been exposed to unmeasured non-commercial sources, such as residential indoor use, professional or personal pesticide application in or around their residence. Additionally, there are no systems in place to capture data on residential use of pesticides that includes improper usage and the frequency of accidents. This unavoidable misclassification would likely be non-differential and would have shifted exposure risk estimates towards the null. Potential confounders of the association between pesticide exposure and neurodevelopment which were not addressed include neighborhood conditions such as the level of disrepair of maternal residences. Deterioration of homes or neighborhoods has been associated with

increased pest levels and the resulting increases in use of pest control measures including pesticides^{38,39}.

The risk assessment process is complex and challenged by several factors. While the goal may be total exposure it is necessary to consider the aggregate as well as the cumulative. Aggregate exposure is the total exposure to one chemical via all pathways and routes, while the cumulative measures the combined aggregate of all pesticides with the same mechanism of toxicity.

Adding to the complexity of the challenge, we know pregnant women are not exposed to one type of chemical but absorb, respire, and ingest mixtures with potential for complex interactions that further daunts our ability to understand toxic effects. The challenge of risk assessment, combined with the mounting evidence to date, amplifies the need for action to protect children. We may never completely and accurately measure the magnitude of the risk, but we do know that early life OP pesticide exposures are associated with neurodevelopmental delay.

There has been limited progress in reducing exposures, a few countries have instituted bans on some OP compounds. In the US, California regulates agricultural usage, and has limited agricultural applications near schools and childcare facilities, but only when children are present. Despite these actions, and because they do not adequately protect vulnerable children, the American Academy of pediatricians has called for more comprehensive action, and for governments to acknowledge and reduce pesticide exposures through multiple mechanisms¹². Recently, a group of concerned scientists from multiple disciplines, children's environmental health advocates and health professionals have issued recommendations for policy to eliminate human exposures to OP pesticides including action by: legislative bodies and agencies at the

federal local and state level, public health programs, medical schools, healthcare associations, and agriculture ⁴⁰. The evidence of risk to child neurodevelopment is mounting, the body of scientific literature is consistent, and also clarifies that the risk exists at low levels of exposure. Recognizing that the risk is disproportionately experienced is a critical addition to our understanding of the need for comprehensive action to protect children's health.

There is a growing understanding that a limited focus on individual based explanations of the causes of disease are insufficient to capture important disease determinants ⁴¹. Investigations that are examining the social ecology of children's vulnerability to environmental pollutants are based on the understanding that the effect of exposure to neurotoxic chemicals early in life are shaped by the nature of a child's social environment, including before birth. This research area is guided by the hypothesis that toxicity is not only an inherent property of the toxicant but arises from an assortment of mutually interacting variables bound inherently to the individual ⁴², and recognizes that the effects of environmental neurotoxins are inseparable from the child's social environment. A child's social ecology creates unique vulnerabilities that are imprinted as powerfully as genetic predispositions, and therefore should be examined with similar rigor. The evidence that social stress and environmental toxins may influence common physiological pathways suggests that understanding the potential synergistic effects promises to more completely inform children's environmental health risk. This more comprehensive understanding of how maternal prenatal social factors could interact and exacerbate known and suspected risk factors for neurodevelopmental delay should give greater impetus for reducing exposures particularly in disadvantaged, vulnerable and underserved communities and further guide current prevention and intervention strategies.

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Tables and Figures

Table 1. Demographic and clinical characteristics [n (%) or mean ± standard deviation (SD)] of the study population (n = 817), stratified by child diagnosis and financial hardship

Covariate	Child's Diagnosis			Financial Hardship			
	TD ¹ (n = 329)	ASD ² (n = 488)	P- value*	No (n = 684)	Yes (n = 115)	P- value*	
Child Male Gender, n (%)	272 (82.7%)	414 (84.8%)	0.41	571 (83.5%)	115 (86.5%)	0.39	
Father's education, n (%)							
High school diploma/GED or less	80 (24.3%)	108 (22.1%)	0.47	136 (19.9%)	52 (45.2%)	<0.001	
Some college (incl. vocational, 2-yr degree)	96 (29.2%)	151 (30.9%)		194 (78.5%)	53 (46.1%)		
Bachelor degree	105 (31.9%)	140 (28.7%)		224 (32.7%)	21 (18.3%)		
Graduate or professional degree	48 (13.3%)	87 (17.8%)		128 (18.7%)	7 (6.1%)		
Child's Race/Ethnicity, n (%)							
White	174 (52.9%)	254 (52.0%)	0.88	385 (56.3%)	43 (37.4%)	<0.001	
Hispanic	61 (18.5%)	87 (17.8%)		118 (30.7%)	30 (26.1%)		
Other	94 (28.6%)	147 (30.1%)		181 (20.5%)	60 (52.2%)		
Regional Center Area, n (%)							
Alta	138 (41.9%)	174 (55.8%)	<0.001	268 (39.2%)	44 (38.3%)	0.02	
North Bay	56 (17.0%)	66 (54.1%)		95 (13.9%)	27 (23.5%)		
East Bay	66 (20.0%)	81 (55.1%)		132 (19.3%)	15 (13.0%)		
Valley Mountain	51 (15.5%)	86 (62.8%)		113 (16.5%)	24 (20.9%)		
Southern California	18 (5.4%)	81 (81.8%)		76 (11.1%)	23 (20.0%)		
Birth Place of Mother, n (%)							
In the USA	271 (82.3%)	371 (76.0%)	0.07	543 (79.4%)	99 (86.1%)	0.002	
In Mexico	22 (6.7%)	37 (7.6%)		40 (7.2%)	19 (16.5%)		
Outside USA or Mexico	36 (10.9%)	80 (16.4%)		101 (14.8%)	15 (13.0%)		
⁴ Prenatal Vitamin Use, n (%)	196 (43.5%)	255 (56.5%)	0.02	397 (58.0%)	54 (40.7%)	<0.001	
Age of mother at time of child's birth	Mean (SD)	31.1 (5.7)	31.3 (5.4)	0.6	31.5 (5.4)	29.4 (6.1)	<0.001

¹ASD, autism spectrum disorder; ²TD, typical development; ³The p-value is calculated by ANOVA (numerical covariates); and chi-square test or Fisher's exact test (categorical); ⁴ Prenatal vitamin use (during 3 months before pregnancy through the first month)

Table 2. Exposure to organophosphateP pesticide applications (any vs. none) within 1.5 km of the home during the 3 months before conception through delivery according to outcome

Covariate	TD n = 329	ASD n = 488	P-value
Organophosphate, n (%)	87 (40.8%)	126 (59.1%)	0.842
Chlorpyrifos, n (%)	46 (42.6%)	62 (57.4%)	0.597

¹ASD, autism spectrum disorder; ²TD, typical development; ³The p-values are calculated from chi-square tests

Figure 1. Odds Ratios and 95% confidence intervals for child autism with prenatal proximity to pesticide application, with and without financial hardship

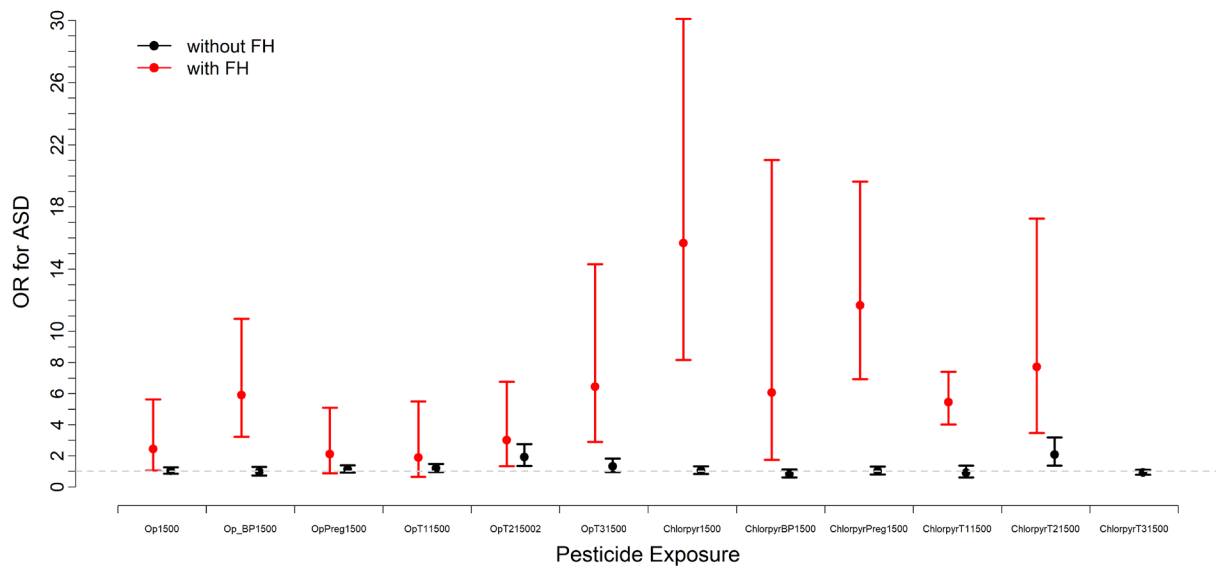


Table 3. Odds Ratios and 95% confidence intervals for child’s autism with prenatal proximity to organophosphate application, with and without financial hardship (FH)

Exposure Time Point	OR (95% CI) for Organophosphate		OR (95% CI) for Chlorpyrifos	
	Without FH	With FH	Without FH	With FH
Childbearing Year	1.04 (0.86, 1.26)	2.44 (1.05, 5.63)	1.05 (0.84, 1.32)	15.67 (8.16, 30.09)
Before Pregnancy	0.97 (0.74, 1.29)	5.90 (3.22, 10.81)	0.83 (0.61, 1.12)	6.07 (1.75, 21.01)
During Pregnancy	1.13 (0.93, 1.38)	2.11 (0.88, 5.09)	1.02 (0.79, 1.31)	11.67 (6.94, 19.62)
First Trimester	1.19 (0.96, 1.48)	1.89 (0.65, 5.50)	0.92 (0.61, 1.37)	5.45 (4.02, 7.41)
Second Trimester	1.93 (1.35, 2.76)	3.01 (1.34, 6.75)	2.08 (1.36, 3.18)	7.72 (3.46, 17.25)
Third Trimester	1.32 (0.95, 1.83)	6.44 (2.89, 14.32)	0.93 (0.78, 1.11)	Too few exposed

Chapter 5: Conclusion and Future Directions

In these investigations, we have shown that maternal mental health and financial hardship are among the potential environmental factors of Autism Spectrum Disorder (ASD); these experiences persist when community factors are examined, and they amplify the effect of organophosphate pesticide exposures.

Poverty is now recognized to be one of the most enduring risk factors for morbidity and mortality throughout the life course, with inter-related mechanisms causing disproportionate exposure to stressors. These mechanisms include the maternal and family characteristics arising from the economic, political, social and environmental factors associated with low income. Contextual factors like race, ethnicity, immigration status or community setting, combine with these mechanisms to have direct and indirect effects on perinatal and mental health. Poor mothers are likely to face a bombardment of stressful life events, including teenage pregnancies, unemployment, more crowded and polluted homes and communities, and minimal support and resources to handle these stress exposures.

Pregnant women suffering with mood disorders are unlikely to receive appropriate and timely treatment. A 2012 nationally representative study found that more than half of pregnant (65.9%) and non-pregnant women (58.6%) experiencing depression went undiagnosed ¹. This is in large part due to inadequate screening. According to a randomized cross-sectional survey of OB/GYNs who completed residency training during the previous 5 years, only 9% to 12% reported that they routinely asked patients about depression or used a screening questionnaire to identify major or minor depression ². However, patients who receive screening for postpartum

depression show improved outcomes when compared with patients who receive no screening³. According to a new review from AHRQ's Effective Health Care Program the potential effectiveness of screening appears to be related to the availability of systems to ensure adequate follow-up of women with positive results³.

Additionally, after the perinatal period, families with limited resources and education, and Black and Hispanic children with ASD, are more likely to receive a delayed diagnosis of ASD⁴⁻⁶. They are also more likely to live in communities and circumstances that limit their ability to maximize their use of appropriate interventions. Low socioeconomic position may confer limited ability to navigate the medical system, and magnify the impact of the diagnosis on a family already burdened with multiple stressors and stress and poor mental health. Poor families may be additionally burdened by the substantial economic burden associated with neurodevelopmental disorders. For ASD, in the United States lifetime cost is estimated to be \$1.36 million for ASD without Intellectual Disability (ID), and \$1.43 million for an individual with both ASD and ID⁷. This estimate of total costs includes the costs from services needed (79%), and productivity loss by both the affected individual (12%) and caregiver (9%)⁷.

Maternity care clinicians and pediatric providers should be aware of these intersections of social factors. For example many black parents living with the fear of racism, and heightened terror for their male children especially underreport some autism symptoms like social deficits, and restricted and repetitive behaviors, and emphasize general developmental and disruptive behavior concerns⁸. Hispanic families reported difficulties receiving an appropriate diagnosis for their child and accessing care as a result of poor language proficiency, maternal education

and fear of legal reprisals for seeking care ^{6,9}. Without clinician awareness and vigilance about these contextual factors, addressing perinatal stress and depression, referral for appropriate diagnostic evaluation, and subsequent interventions may not be appropriately addressed, with lifelong implications for the health of the affected child.

Future Directions

There are several areas for further study identified by these findings. These include further examination of the link between maternal psychological stress during pregnancy and: indicators of dysregulation of inflammatory processes in the newborn, exposures to mixtures and pesticides other than the organophosphate compounds. Mothers and their children could be aided by greater understanding of the protective possibilities of maternal nutrition, when economic and psychological stressors are present.

Indicators of Dysregulation of Inflammatory Processes

The human immune response is regulated by a complex network of control factors, with an intricate balance of anti-inflammatory cytokines and cytokine inhibitors. Cytokines have been described as the common language between the immune and nervous systems, and facilitators of cross-systemic communication. They are complex proteins that control the nature, duration, and intensity of an immune response ¹⁰. Anti-inflammatory cytokines interact with cytokine inhibitors and soluble cytokine receptors to regulate the human immune response. Evidence of immune dysfunction has been observed in many children with ASD, including activation of microglia, increased levels of pro-inflammatory cytokines in brain tissue, cerebrospinal fluid and plasma ¹¹. A potential role for immune dysfunction has been suggested in ASD ¹²⁻¹⁹.

Additionally, research supports altered immune activity in both children with autism and their

mothers²⁰ and children with autism have higher incidence of autoantibody production, and skewed cytokine profiles compared to those developing typically¹³.

Several studies have examined potential biological mechanisms between in-utero stress exposure and ASD and hypothesized inflammatory processes that explain these associations²¹⁻²⁵.

Psychological stress or depressive symptoms during pregnancy has been associated with elevated serum inflammatory markers as well as exaggerated inflammatory responses to both biological and psychosocial challenges^{23,24}. Research that examines prenatal stressors and their impact on inflammatory processes in the newborn is needed to fully examine the implications of stress-induced immune dysregulation in pregnancy for pervasive developmental disorders in the child.

Prenatal Dietary intake and Maternal Psychological Stress

Higher maternal intake of multivitamins, especially periconception folic acid supplementation has been associated with reduction in ASD risk^{26,27}, emerging evidence also suggest that the microbiome plays an important role in neurodevelopmental disorders²⁸. More work is needed to examine the preventative possibilities and mechanisms of maternal prenatal dietary intake in the face of maternal psychological stress.

Multiple Exposures and Neurodevelopment

While this dissertation has focused on community level exposures and residential proximity to one type of pesticide, we have not addressed the impact of multiple environmental exposures.

We already know that pregnant women are exposed to multiple environmental chemicals²⁹.

Biomonitoring data from a nationally representative sample of the U.S. population found detectable levels of 62 chemicals in 90% of pregnant women³⁰. Almost all women (>99%) had detectable levels of: certain polychlorinated biphenyls, organochlorine pesticides, PFCs, phenols,

PBDEs, phthalates, polycyclic aromatic hydrocarbons, and perchlorate, many known to cross the placenta and be detected in fetal serum or cord blood. Additional research needs to examine the interaction between social factors and the complex exposures that may impact the neurodevelopmental trajectory of our future generations.

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