ENVIRONMENTAL RISK FACTORS FOR AUTISM SPECTRUM DISORDER (ASD)

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ABSTRACT

Background: Autism Spectrum Disorder (ASD) is a significant public health concern. The prevalence rate of this heterogeneous group of neurological conditions has more than doubled in the US during the last twenty years. The amount and cost of care needed to provide support and services for families impacted by ASD are increasing as well. Current research suggests that the etiology of ASD is not wholly genetic. The objective of this research is to investigate environmental contributors to the disease. Parental, prenatal, and obstetric factors and exposure to air pollutants during the prenatal period were examined for potential associations with ASD status.

Methods: Risk factors were extracted from the birth certificates of singleton cases (n=198) and controls (n=4,801) from the Case-Control Study of Personal and Environmental Risk Factors for Childhood Autism in Southwestern Pennsylvania. Cases and controls were born between 2005 and 2009. The 2005 National Air Toxics Assessment (NATA) and the Toxic Release Inventory (TRI) for 2004 – 2009 were used to estimate air pollutant exposure during pregnancy. Logistic regression models estimated the odds of being an ASD case in all investigations.

Results: Multivariable logistic regression modelling showed that increased maternal age, greater maternal education, gestational hypertension, cesarean delivery, and maternal infection were independent risk factors for ASD. In the analysis comparing higher quartiles of NATA exposure

estimates of metal compounds to the lowest quartile of metal exposure at mother's residence at the time of her child's birth, arsenic, chromium, and lead were significantly associated with being an ASD case. However, an analysis of proximity to chromium emitting industrial sites, as reported in the TRI, uncovered no elevated or statistically significant association with ASD status.

Conclusions: Maternal and birth characteristics as well as exposure to metal compounds during the prenatal period were shown to have an association with ASD in this case-control study. This research adds to a growing body of literature suggesting that environmental risk factors contribute to the ASD burden. However, improved exposure assessment methods and a prospective study design would aid in establishing a causal association.

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PREFACE

I would like to thank my dissertation committee for teaching and guiding me through this process. Without their help, and especially that of Dr. Evelyn Talbott, my success would not have been possible. My love and respect for the public health field, and epidemiology in particular, is a reflection of their scientific devotion and integrity.

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1.0 INTRODUCTION

1.1 SPECIFIC AIMS

Autism Spectrum Disorder (ASD) is a heterogeneous neurological disease that has been increasing in prevalence for the past few decades.[1] Although it is usually diagnosed in early childhood, current research indicates that ASD originates during the prenatal period.[2] This considerable and rapid increase, as well as results of recent twin studies,[3] suggests that ASD etiology has environmental contributions. Thus investigations into environmental risk factors for ASD have steadily emerged in recent years. Prior studies have found associations with parental, prenatal, obstetric, and neonatal risk factors as well as associations with extrinsic exposures such as maternal smoking and proximity to pesticide application.

Many of the studies on parental, prenatal, obstetric, and neonatal risk factors were conducted with large, nationwide cohorts.[4, 5, 6, 7, 8, 9, 10] Although these types of studies have the benefit of large sample size, they often are conducted in countries with homogenous populations such as Sweden[8] and Denmark,[7] which may limit their generalizability. The administrative records stretch over many years, so the possibility of changing ASD diagnostic criteria over time exists. Additionally, the cases are identified from hospitalization records, so they may over represent the more severe ASD cases. Of the studies that have been conducted in the US, the vast majority of them were in California.[11, 12, 13, 14] As rates of ASD prevalence vary by place, it is

important to add to the geographic regions in which ASD environmental risk factor studies are conducted.

Much of the current body of research on the association between air pollutants and autism have been conducted in the US and parts of Asia. There is little research on environmental associations with autism in the United States outside of California and Texas. However, Western Pennsylvania has been the setting for many health studies on air pollution – in no small part because of the history of industrial air pollution in this area. Although air quality has improved over the years, Allegheny County repeatedly makes the American Lung Association's annual list of "Most Polluted Cities" for both particulate and ozone pollution. Recent studies conducted in Allegheny County have found associations with current levels of PM_{10} exposure and term low birth weight[15] and cardiopulmonary hospital admissions.[16]

The Case-Control Study of Personal and Environmental Risk Factors for Childhood Autism in Southwestern Pennsylvania was recently conducted at the University of Pittsburgh, Graduate School of Public Health. The study recruited autism cases that were born between the years of 2005 and 2009 in one of six counties – Allegheny, Armstrong, Beaver, Butler, Washington, and Westmoreland. Cases (n=217) were recruited from autism clinics, educational services, and physicians. The birth certificates of these cases and controls (n=4,991), which were frequency matched on birth year, race, sex, and county born, were available for this analysis. The cases and a subset of the controls (n=226), who responded to a mailed request for participation, were asked detailed questions on demographics and exposures during pregnancy until the second year of life. This current research will use both control groups.

The primary objective of this research is to examine environmental risk factors in this set of Southwestern Pennsylvania ASD cases and controls born between 2005 and 2009. The following aims will be investigated:

1. To determine if parental characteristics, prenatal conditions, obstetric complications, and neonatal characteristics found on the Pennsylvania birth certificate are associated with the risk of ASD.
2. To determine if environmental air exposure to metals (arsenic, cadmium, chromium, lead, manganese, mercury, and nickel), as modeled by the National Air Toxics Assessment (NATA), is associated with the risk of ASD.
3. To determine if air chromium exposure as modeled by spatial interpolation of annual emissions documented in the Toxic Release Inventory (TRI) between industrial sites is associated with the risk of ASD.

1.2 BACKGROUND AND SIGNIFICANCE

Autistic Disorder (AD) was first described in medical literature by Kanner in 1943.[17] It is a neurodevelopmental disorder which is characterized by abnormalities in two categories - social interactions, including verbal and non-verbal communication and relationships, and repetitive actions or behaviors.[18] People with ASD often have one or more comorbid conditions including epileptic seizures, severely impaired speech, increased brain size, and intellectual disability.[19] Additionally, the spectrum can include highly functioning cases as well as those that require life-long care. Besides causing hardships for the families involved, these latter cases are also a significant financial burden.

Case definition of autism has changed, which makes ascertainment of changes in prevalence over time very difficult. The American Psychiatric Association published the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM*) for the first time in 1952.[20] Over the years, they have revised and updated this widely used diagnostic and classification tool. The term "autism" did not appear in the DSM until its third revision in 1980. For years, under the DSM-IV released in 1987, autism fell under the category of Pervasive Developmental Disorder (PDD) along with Asperger's disease and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS). Recently, DSM-5 introduced the term Autism Spectrum Disorder (ASD) and has redefined the diagnosis to address the heterogeneity of the disorder.[18] ASD incorporates what was known as autism disorder (AD), Asperger's disorder, and pervasive developmental disorder not otherwise specified into one overarching diagnosis. One of the most significant changes to the diagnostic criteria is that a child must have repetitive actions or behaviors to be diagnosed with ASD. These nuances may make it difficult to compare studies using earlier criteria (DSM-IV) with those using DSM-5 criteria.

Although there is some dispute on the subject, most research points to the growing prevalence of autism in the last few decades. Surveillance data from the CDC funded Autism and Developmental Disabilities Monitoring (ADDM) Network indicates that autism prevalence increased from 6.7 per 10,000 (1 in 150) children born in 1992 to 14.7 per 10,000 (1 in 68) children born in 2002.[1] The dramatic increase in prevalence can be due to many factors, including changes in diagnostic procedures, increased awareness, and actual increases in incidence. Many researchers have concluded that autism is increasing in prevalence not just due to changes in ascertainment,[21] but some researchers are less certain.[22] Whether there is a true increase in autism cases or not, it is clear that ASD is a significant public health burden.

Autism is recognized as having strong genetic origins. Studies of twins show that concordance among monozygotic twins is significantly higher than concordance among dizygotic twins. However, as monozygotic twins do not have a 100% concordance rate, it is believed that environmental factors have some role in the etiology of ASD. After conducting their rigorous twin study, Hallmayer et al. concluded that environmental factors account for 55% of the autism burden.[3] Because the origins of ASD appear to most likely be a complicated mix of genetics and environment and research into environmental contributions to the etiology of autism is only a few decades old, definitive risk factors are still contentious. Table 1 includes many of the most studied characteristics and strength of significant associations with ASD found in studies referenced in this document.

Table 1. Strength of association of non-genetic risk factors for ASD

RISK FACTOR	STRENGTH OF SIGNIFICANT ASSOCIATION: OR, RR OR PR (95% CI)	
PARENTAL CHARACTERISTICS		
MATERNAL AGE	1.31 (1.07-1.62)[12] 1.9 (1.3-2.8)[11]	
PATERNAL AGE	1.28 (1.09-1.51)[12] 2.04 (p=0.023)[24] 2.63 (1.38-5.00)[23]	↑
MATERNAL COLLEGE EDUCATION	1.33 (1.24-1.44)[25] 1.36 (1.08-1.69)[12]	
BLACK RACE	1.67 (1.57-1.78)[25]	
MATERNAL BORN ABROAD (FROM OUTSIDE OF NORTH AMERICA OR WESTERN EUROPE)	3.0 (1.7-5.2)[4]	↑↑
MATERNAL AUTOIMMUNE DISORDER	1.37 (1.17-1.60)[5] 2.7 (1.3-5.8)[13]	↑
MATERNAL ASTHMA OR ALLERGIES	1.6 (1.2-2.2)[13] 1.8 (1.0-3.4)[13]	
MATERNAL PSYCHIATRIC HISTORY	2.52 (1.43-4.12)[6] 3.44 (1.48-7.95)[7]	↑↑
PRENATAL CONDITIONS		
BLEEDING	2.5* (p=0.001)[26]	↑
PREECLAMPSIA	1.64 (1.08-2.49)[8] 1.69 (1.26-2.28)[27]	
GESTATIONAL DIABETES	1.76 (1.34-2.32)[5]	
THREATENED ABORTION	2.41 (1.56-3.73)[28]	↑
OBSTETRIC COMPLICATIONS		
INDUCED AND/OR AUGMENTED LABOR	1.21 (1.01-1.46)[25] 1.22 (1.03-1.44)[6] 1.43 (1.12-1.83)[28]	
FETAL DISTRESS	1.59 (1.20-2.11)[28] 5.13 (3.03-8.69)[29]	↑↑
CAESAREAN DELIVERY	1.6 (1.1-2.3)[4] 2.05 (1.49-2.82)[28]	↑
BREECH PRESENTATION	1.80 (1.36-2.38)[7]	
NEONATAL CHARACTERISTICS		
MALE	4.3 (3.9-4.6)[14] 6.1 (5.0-7.6)[11]	↑↑
ASD DIAGNOSED SIBLING	11.04 (8.29-14.69)[6]	↑↑
FIRST BORN VS. 3RD BORN	1.67 (1.25-2.00)[30] 1.94 (1.29-3.33)[24]	
LOW APGAR SCORE	1.89 (1.10-3.27)[7] 3.2 (1.2-8.2)[4]	↑↑
LOW BIRTH WEIGHT	1.79 (1.28-2.51)[7]	
PRETERM BIRTH	1.4 (1.2-1.7)[30] 2.5 (1.6-3.9)[30] 3.32 (2.35-4.71)[7]	↑↑
SMALL FOR GESTATIONAL AGE	1.32 (1.04-1.68)[7] 2.1 (1.1-3.9)[4]	↑
EXTRINSIC EXPOSURES		
ORGANOCHLORINE PESTICIDES (DURING CNS DEVELOPMENT)	4.2 (1.7-10.9)[34]	↑↑
VIRAL INFECTION (IN FIRST TRIMESTER)	2.98 (1.29-7.15)[9]	↑
FEVER	2.12 (1.17-3.84)[36]	↑
CIGARETTE SMOKING	1.4 (1.10-1.80)[4]	
ANTI-DEPRESSANT USE	3.34 (1.50-7.47)[39]	↑↑

* = ratio of unadjusted rates, calculated by Marshall

↑ = any association of at least 2.0

↑↑ = any association of at least 3.0

1.2.1 Parental characteristics

Some of the parental characteristics that have been investigated for association with ASD are parental age, education, race, maternal birth country, and maternal factors such as autoimmunity, allergies, asthma, and psychiatric disorder. A case-control study was conducted in China with 95 confirmed ASD cases and 95 population controls frequency matched on sex and birth year.[23] Positive significant associations were found for paternal age over 29 (OR=2.63; 95% CI, 1.38-5.00), but not for maternal age. However, in an adjusted prevalence rate model, Windham et al.[11] found that mothers who were at least 40 years old who gave birth in either 1994 and 1996 in the San Francisco Bay area were 1.9 times more likely (95% CI, 1.3-2.8) to have an ASD child than women under 40. The 1994 cohort consisted of 82,153 live births and the 1996 cohort consisted of 80,249 live births. These investigators did not find a significant association with paternal age, parental education, or maternal race.[11] An Iranian case-control study with 179 cases and 1,611 controls found that fathers who were at least 40 had 2.04 times the odds of having an educational system identified ASD case than matched controls (p=0.023).[24] Croen et al. found that maternal age (RR=1.31; 95% CI, 1.07-1.62), paternal age (RR=1.28; 95% CI, 1.09-1.51), and maternal college education (RR=1.36; 95% CI, 1.08-1.69) were associated with ASD risk in a Northern California cohort of children born between 1995 and 1999 (n=132,844).[12] All live births between 1990 and 1998 in North Carolina were linked to educational records for the 1997-1998 and 2007-2008 school years.[25] This cohort of 625,042 births was assessed for characteristics hypothesized to influence ASD diagnosis. Adjusting for maternal level factors, women with a college education had 1.33 the odds (95% CI, 1.24-1.44) of having an ASD diagnosed child than women with a high school education. Additionally, non-Hispanic blacks had 1.67 times the odds (95% CI, 1.57-1.78) of having an ASD diagnosis than non-Hispanic whites.[25] In a Swedish case-

cohort study, Hultman et al.[4] found that in children born between 1974 and 1993, foreign born mothers (outside of Europe or North America) had 3.0 times the odds (95% CI, 1.7-5.2) of having an autistic child compared to mothers born in Europe or North America. Their study included 408 autism cases and 2,040 matched controls selected from the entire birth cohort.[4]

A nested case-control study with 420 ASD cases and 2,100 matched controls was conducted in California with infants born between January 1995 and June 1999.[13] The investigators found that mothers with an autoimmune disease (psoriasis) was associated with 2.7 odds of having a child diagnosed with ASD as compared to having a child without ASD (95% CI, 1.3-5.8).[13] Additionally, Croen et al. found that maternal asthma (OR=1.6; 95% CI, 1.2-2.2) and allergies (OR=1.8; 95% CI, 1.0-3.4) are associated with ASD.[13] Lyall et al. studied ASD risk factors in the offspring of women in the Nurses' Health Study II.[5] The mothers with an autoimmune disease had 1.37 times the odds (95% CI, 1.17-1.60) of having an ASD child compared to mothers without an autoimmune disease.[5]

Mother's psychiatric history was studied as a risk factor for ASD. Medical records were examined in a cohort of 129,733 children born between 1990 and 2002 in Nova Scotia, Canada.[6] The investigators found that mothers with psychiatric disorders had 2.52 times the risk of having a child with ASD than mothers without a psychiatric disorder (95% CI, 1.43-4.12). Larsson et al. found that schizophrenia-like psychosis in parents was associated with ASD risk (OR=3.44; 95% CI, 1.48-7.95) in a nested case-control study conducted in Denmark.[7] This study did not, however, address the impact or presence of maternal use of psychotropic drugs, which could confound the relationship between maternal psychosis and development of ASD in their offspring.

1.2.2 Prenatal conditions

The prenatal conditions of bleeding, preeclampsia, gestational diabetes, and threatened abortion have been associated with ASD risk. Researchers in New Jersey calculated prevalence rates of prenatal and birth complications in a cohort of 164 children who had been diagnosed with autism, ASD, or PDD.[26] Children who presented to The Autism Center at New Jersey Medical School between October 2001 and August 2003 were diagnosed and parents completed a survey. The prevalence of vaginal bleeding during pregnancy in their research cohort compared to both New Jersey and US rates was 16.7% versus 6.6% (2000 rate for both New Jersey and the US).[26] This 2.5 fold increase is statistically significant (p=0.001). In a case-control study conducted in South Carolina including women who were pregnant between 1996 and 2002, women with preeclampsia had 1.69 times the odds (95% CI, 1.26-2.28) of having a child diagnosed with ASD than women without preeclampsia.[27] This analysis was conducted by linking Medicaid billing records to birth certificates; 472 ASD cases were compared to the rest of the birth cohort (n=87,205).[27] Buchmayer et al. conducted a population based case-control study in Sweden.[8] The ASD cases (n=1,216) and gender, birth year, and birth hospital matched controls (n=6,080) were born between 1987 and 2002. After adjusting for maternal age, smoking, maternal country of origin, mother and father living together, and maternal schizophrenia, preeclampsia increased the odds of having an ASD child by 64% (95% CI, 8%-149%).[8] A cohort of 66,445 mothers who gave birth between 1989 and 2003 from the Nurses' Health Study II was surveyed for pregnancy and obstetric complications.[5] In a model adjusted for parental characteristics, women with gestational diabetes had 1.76 times the odds (95% CI, 1.34-2.32) of having a child with ASD compared to women without gestational diabetes. Researchers investigated risk factors for ASD in children born in Western Australia between the years of 1980 and 1995.[28] Cases (n=465) were

diagnosed at one of 5 diagnostic centers and controls (n=1,313) were randomly chosen from the same birth cohort, but matched on sex. Women who experienced threatened abortion at less than 20 weeks of pregnancy had 2.41 times the odds (95% CI, 1.56-3.73) of having a child with ASD than women who had not experienced threatened abortion.

1.2.3 Obstetric complications

Obstetric interventions and complications including induced and/or augmented labor, fetal distress, Caesarean delivery, and breech presentation have been associated with ASD risk. Induced labor increased the risk of ASD 1.22 (95% CI, 1.03-1.44) times over spontaneous labor in a cohort in Nova Scotia.[6] In a North Carolina population-based cohort, Gregory et al. found that women with induced and augmented labor had 1.21 times the odds (95% CI, 1.01-1.46) of having a child with ASD than women who were not induced or augmented during labor.[25] Glasson et al. found that mothers of ASD children in Western Australia had higher odds of having induced labor compared to both a population control (OR=1.43; 95% CI, 1.12-1.83) and to a sibling control (OR=1.40; 95% CI, 1.03-1.90) than women without induced labor.[28] Fetal distress also emerged as a significant risk factor in this study.[28] Infants that endured fetal distress had 1.59 times the odds (95% CI, 1.20-2.11) of being an ASD case compared to the population control and 1.64 times the odds (95% CI, 1.15-2.34) of being an ASD case compared to the sibling control.[28] Questionnaires were used to assess risk factors in a randomly sampled case-control study in India.[29] Recruitment of 2 – 10 year old cases (n=471) and controls (n=471) was completed between 2010 and 2012. In a logistic regression model adjusted for maternal age, gender of child, and birth year, fetal distress was associated with ASD diagnosis (OR=5.13, 95% CI, 3.03-8.69).[29]

In the Glasson et al. study, elective Caesarean birth was significant when compared to the population control.[28] Caesarian deliveries had 2.05 times the odds (95% CI, 1.49-2.82) of having an ASD diagnosis when compared to the population control group. Hultman et al. found that infants born via cesarean delivery had 1.6 times the odds (95% CI, 1.1-2.3) of having ASD as compared to those born via vaginal delivery.[4] A large Danish nested case-cohort study was conducted to investigate multiple risk factors for autism.[7] Researchers identified 698 autism cases born between 1973 and 1994 through health records. Twenty-five gender and age matched population based controls were selected for each case. Infants with breech presentation had 1.80 times the odds (95% CI, 1.36-2.38) of being a case as compared to infants with cephalic presentation.[7]

1.2.4 Neonatal characteristics

Autism affects more males than females.[12, 6, 30] The ratio of boys to girls varies between different studies, but Croen et al. found the ratio to be 4.3:1 (95% CI, 3.9:1-4.6:1).[14] Their study population consisted of all live births in California from 1989 to 1994 (n=3,551,306). Within this population there were 4,381 autistic children who were identified from the California Department of Developmental Services (DDS).[14] Windham et al. found a 6.1:1 (95% CI, 5.0:1-7.6:1) male to female ratio in their multi-source surveillance prevalence study in the San Francisco Bay Area.[11] It is more likely for parents to have a child with autism if they already have a child diagnosed with autism. The overall risk of having an autistic child is currently 1 in 68 (or about 1.5%) whereas parents who already have one child on the autism spectrum have a 2-18% risk of having another.[1] In their population based cohort of births in Nova Scotia, Dodds et al. found that infants having a sibling with ASD were 11.04 times more likely to have ASD (95%

CI, 8.29-14.69) than those not having a sibling with ASD.[6] Durkin et al. analyzed a multi-site cohort to find that compared to the first born child, the second born (OR=0.8; 95% CI, 0.7-0.9), third born (OR=0.6; 95% CI, 0.5-0.8), and fourth or higher born (OR=0.5; 95% CI, 0.4-0.6) were statistically less likely to be diagnosed with ASD.[30] This cohort of 254,598 births contained 1,251 ASD cases.[30] Being born as third child or later was associated with a 48.5% decrease in odds (95% CI, 22.6-70.0%) of ASD diagnosis compared to first born in an Iranian population.[24] The reduced risk for the third birth has been attributed to self-selection factors related to a "stoppage rule" in families with one or more autistic children associated with the burden as well as the fear of a genetic predisposition.[31]

Although the American Academy of Pediatrics state that the Apgar score at 5 minutes is not indicative of lifetime neurological risk,[32] there has been research that suggests an association with ASD diagnosis. Larsson et al. found an 89% (95% CI, 10-227%) increase in ASD risk for children with low Apgar score at 5 minutes.[7] Hultman et al. found that infants with a low Apgar score had 3.2 times the odds (95% CI, 1.2-8.2) of having ASD compared to infants with an Apgar score of 7 or greater.[4]

Low birth weight, preterm birth, and small for gestational age have also been studied as risk factors for ASD. A population based case-control study conducted by Durkin et al. used data from a 10 site cohort to determine that infants of low gestational age (28-36 weeks) had 1.4 times the odds of having ASD (95% CI, 1.2-1.7) than infants of typical gestational age (37-41 weeks).[30] This association was even more pronounced in infants with less than 28 weeks of gestation (OR=2.5; 95% CI, 1.6-3.9).[30] Hultman et al. found that infants that were small for gestational age had 2.1 times the odds (95% CI, 1.1-3.9) of having ASD than average for gestational age in a Swedish cohort.[4] Birth weight, gestational age, and small for gestational age

were all associated with autism risk in a Danish population study.[7] Weighing 2,001-2,500 grams at birth increased autism risk by 79% (95% CI, 28%-151%), when compared to infants weighing 3,001-3,500 grams at birth.[7] Infants who were born before 35 weeks of gestation had 3.32 times the risk (95% CI, 2.35-4.71) of having an autism diagnosis than infants who were born with 37 to 42 weeks of gestation. Additionally, Larsson et al. found that infants in the lowest decile of size for gestational age had 1.32 times the risk (95% CI, 1.04-1.68) of having an autism diagnosis than infants who were appropriately sized for gestational age.[7]

Multiple births have not been extensively studied as a risk factor for ASD. Many studies restricted their population to all twin pairs or all singleton births. However, a few studies have investigated multiple births, reporting disparate results. Croen et al. found an association between multiple births and ASD status.[14] In a model adjusted for maternal characteristics and birth weight, multiple births had 1.7 time the risk (95% CI, 1.4-2.0) of being an ASD case compared to singletons. Larsson et al. found an elevated, but not statistically significant, ASD risk associated with being a multiple birth (OR=1.11; 95% CI, 0.70-1.77) in an unadjusted model.[7]

1.2.5 Extrinsic exposures

There is a diverse literature on environmental exposures hypothesized to be associated with ASD. Most of the these exposures such as dietary factors, exposure to environmental toxins, such as pesticides or metals, exposure to a virus or infection, traditional risk factors such as smoking and alcohol, and maternal medication use have not yet been extensively studied in human populations. Many of the epidemiological studies that have been conducted produced equivocal results. Kočovská et al. wrote an in-depth review on the possibility of an association

with Vitamin D intake and autism.[33] Roberts et al. conducted a case-control study in the Central Valley area of California.[34] In their population of term births (465 cases and 6,975 controls) from 1996 to 1998, mothers living near organochlorine pesticide use had a significantly increased risk of having an autistic child. Children exposed to the highest quartile of pounds of pesticide during CNS development had 4.2 times the odds (95% CI, 1.7-10.9) of having an ASD diagnosis than children with no exposure in this time period.[34] Libbey et al. conducted a review of the literature on maternal exposure to viral infections in 2005.[35] They reviewed 23 studies, many of which were case studies, and found mixed results for measles, rubella, herpes, cytomegalovirus, mumps, and varicella viruses.[35] A population cohort of all children born between 1980 and 2005 in Denmark (n=1,612,342) found a significant association between mothers who were hospitalized for a viral infection in their first trimester and ASD risk (HR=2.98; 95% CI, 1.29-7.15).[9] Zerbo et al. investigated the role of maternal influenza and fever in the CHildhood Autism Risks from Genetics and Environment (CHARGE) Study.[36] Their study population consisted of 528 confirmed cases of ASD, 163 cases of children with developmental delays, and 421 typically developing controls in California. They did not find a significant association with influenza virus, but did find that women who reported having a fever during pregnancy had 2.12 times the odds (95% CI, 1.17-3.84) of having a child with ASD than women who did not report a fever during pregnancy.[36] When this exposure was investigated by trimester, maternal fever during the second trimester (but not first or third) remained significant (OR=2.60; 95% CI, 1.14-5.95).[36]

There have been a few studies with inconsistent results on the risk of autism associated with maternal smoking, alcohol consumption, and illicit drug use.[37] However, Hultman et al. found that daily smoking was associated with 40% higher odds (95% CI, 10-80%) of having a

child with ASD.[4] The associations between autism and prenatal exposure to thalidomide, valproic acid, and misoprostol have been relatively consistent.[19, 37, 38] Rai et al. recently studied the association between anti-depressant use in mothers in a Swedish case-cohort study.[39] They analyzed 4,429 ASD cases and 43,277 controls. Mothers who reported using anti-depressants in their first antenatal doctor's appointment had 3.34 times the odds (95% CI, 1.50-7.47) of having an ASD diagnosed child than women who did not report anti-depressant use.[39]

1.2.6 Environmental air exposures

In the mid-2000s, research turned to air pollution for possible answers to the autism question. Air pollutants had previously been associated with adverse birth outcomes such as low birth weight.[40, 41] Additionally, many gaseous substances that are released through industrial processes, transportation, and natural activities are known to be harmful to human health. Table 2 contains a list of such substances noted for neurologic, developmental, or immune system toxicity as well as possible endocrine disruption properties as identified by the EPA's National Air Toxics Assessment (NATA), Integrated Risk Information System (IRIS), Technology Transfer Network, or Agency for Toxic Substances & Disease Registry (ATSDR).[42-45]

Table 2. Environmental air exposures classified by risk type

Environmental Pollutant	Neurological Risk as classified by NATA	Neurologic Toxicant	Developmental Toxicant	Suspected Endocrine Disruptor	Immune System Toxicant	Studied association with ASD
METALS						2↑[46, 54]
Antimony						1↑[54]
Arsenic		X	X			3NS[46. 49. 54]
Cadmium		X	X			1↑[46] 2NS[50, 54]
Chromium					X	4NS[46, 49, 54, 55]
Lead	X	X	X		X	2↑[54, 55] 2NS[46, 49]
Manganese	X	X	X			1↑[54] 3NS[46, 49, 55]
Mercury	X	X	X			3↑[46, 47/48, 54] 1NS[49]
Nickel					X	2↑[46, 54] 2NS[49, 55]
AROMATIC SOLVENTS						1NS[46]
Benzene		X	X		X	1↑[55] 1NS[49]
Cumene		*		X		
Ethyl benzene		X	X			1↑[55] 1NS[49]
Styrene	X	X		X		2NS[49, 54]
Toluene	X	X	X			1↑[55] 1NS[49]
Xylene	X	X	X			1↑[55] 1NS[49]
CHLORINATED SOLVENTS						1↑[46]
1,1,1-Trichloroethane	X	X				
Allyl chloride	X	X				
Methyl chloride	X	X				
Methylene chloride		X				4NS[46, 49, 54, 55]
Perchloroethylene	X	X				1↑[55] 2NS[46, 54]
Trichloroethylene	X	X	X			3↑[46, 54, 55] 1NS[49]
Vinyl chloride			X		X	1↑[46] 2NS[49, 54]
OTHER HAZARDOUS AIR POLLUTANTS						
2,4-Dinitrotoluene	X	X				
Acrylamide	X	X				
Benzidine	X	X				
Calcium Cyanamide	X	X				
Carbon disulfide	X	X				
Carbon tetrachloride		X				
Chloroform		X				2NS[49, 55]
Cresol (cresylic acid)	X	X				
Cyanide	X	X				
Dichlorvos	X	X				
Diesel PM						2NS[49, 54]
Ethylene dibromide		X	X			
Ethylene dichloride		X				
Ethylene oxide	X	X	X			1NS[49]
Hexachlorobenzene		X	X			
Hexachloroethane	X	X				
Hexane	X	X				1NS[49]
Hydrazine						1NS[49]
Isophorone			X			
Methanol			*			
Methyl isobutyl ketone		*	X			
Methylene		X				
PAH					X	2NS[49, 55]
PCBs		X	X	X	X	
Quinoline						2NS[49, 54]
Selenium	X	X	X			1NS[55]
CRITERIA AIR POLLUTANTS						
Carbon Monoxide			X			1↑[53] 1NS[52]
Nitrogen Dioxide						3↑[51, 52, 53]
Nitrogen Monoxide						1NS[52]
Ozone						2↑[52, 53] 1NS[51]
$PM_{2.5}$						1↑[51] 1NS[52]
PM_{10}						1↑[51] 2NS[52, 53]
Sulfur Dioxide					X	1↑[53]

* = animal studies only ↑ = significant association NS = not significant

In 2006, Windham et al. published one of the first studies to consider an association between air pollutants and autism.[46] The investigators used a case-control design with 284 autism cases and 657 randomly chosen matched controls from the same birth cohort of infants born in the San Francisco Bay Area in 1994. Autism cases were identified through active surveillance of existing medical records. A 2 to 1 ratio of controls to cases was used, and controls were matched on sex and month of birth. Cases and controls were linked to their birth certificate to ascertain the residence of birth. Using modeled concentrations of air toxics by census tract as their exposure measure (NATA, 1996), Windham et al. found that women who lived in census tracts with the highest quartile of exposure to metals (OR=1.50; 95% CI, 1.05-2.12) and chlorinated solvents (OR=1.55; 95% CI, 1.08-2.23) had higher odds of having an autistic child when compared to women who lived in census tracts with the lowest quartile of exposure to these substances. When the researchers further investigated these groups of pollutants, they found that cadmium (OR=1.54; 95% CI, 1.08-2.20), mercury (OR=1.92; 95% CI, 1.36-2.71), and nickel (OR=1.48; 95% CI, 1.04-2.06) in the metals group, were significantly associated with autism diagnosis in adjusted logistic regression models. In the chlorinated solvents group, trichloroethylene (OR=1.47; 95% CI, 1.03-2.08) and vinyl chloride (OR=1.75; 95% CI, 1.25-2.43) were significantly associated with autism diagnosis in adjusted models. Additionally, diesel particulate matter (DPM) was significantly associated with autism diagnosis (OR=1.44; 95% CI, 1.03-2.02). The logistic regression models were adjusted for mother's age, education, and race.

That same year, Palmer et al. published a study investigating the association between autism and industrial mercury releases.[47] Counts of autism cases in Texas by school district were obtained for the school years 2000-2001. Although autism diagnosis was not standardized, the cases were identified by special education professionals. County-level industrial mercury

releases (measured in pounds) as captured in the EPA's Toxic Release Inventory (TRI) were used in a multilevel Poisson regression to estimate counts of autism cases by school district. After adjusting for percent European American, percent poverty, district wealth, and measures of urbanicity, Palmer et al. found that for every 1000 pounds of mercury released from industry, autism rates increased by 61% (95% CI, 48.7%-75.2%). After adding the number of children enrolled in special education programs to this model, the association was attenuated, but still statistically significant (RR=1.174; 95% CI, 1.103-1.249). Conversely, when the investigators designed a Poisson regression model to predict special education counts, the association became insignificant after adjusting for autism cases (RR=0.940; 95% CI, 0.882-1.002). This result suggests that mercury exposure is associated with autism, but not to the more general diagnostic group of learning disabilities.

In 2009, Palmer et al. conducted another study on the association of industrial mercury releases and autism counts in Texas school districts.[48] They identified autism cases for the 2002 school year in each school district through educational records. Palmer et al. mapped 95 industrial facilities, including 39 coal-fired power plants, which reported mercury emissions to the EPA for the 1998 TRI. Within each school district, the investigators calculated the distance from the centroid to each mercury emitting industrial facility to determine a weighted distance. Using this method, each child in a given school district was assigned the same proximity to sources and pounds of emission exposure level. Palmer et al. found that pounds of mercury released were no longer significant in this adjusted model. However, the Poisson model adjusted for 1997 autism rates, district wealth, measures of urbanicity, percent white, and pounds of mercury released, resulted in a 2% decrease in the rate of autism for every 10 miles farther away

from the source ($p<0.05$). This study suggests that distance from a mercury source is more indicative of autism risk than the amount of mercury emitted from the source.

Kalkbrenner et al. conducted a case-control study in North Carolina and West Virginia in 2010.[49] The 383 autism cases and 2,829 randomly sampled language and speech impaired controls were both identified through the Autism and Developmental Disabilities Monitoring (ADDM) Network. Each child was linked to their birth certificate, and each birth residence was linked to census tract level NATA exposures (NATA, 1996). The investigators screened 35 hazardous air pollutants for possible association with autism as compared to language and speech impairment. After adjusting the Semi-Bayes models for race, maternal education, maternal age, maternal smoking during pregnancy, marital status, and census tract level median income and urbanicity, quinoline (OR=1.4; 95% CI, 1.0-2.2) and styrene (OR=1.8; 95% CI, 1.0-3.1) were associated with increased odds of autism as compared to language and speech impaired controls but did not reach statistical significance.

Volk et al. have published two manuscripts from the Childhood Autism Risks from Genetics and the Environment (CHARGE) study on the relationship between traffic-related air pollution and autism in California.[50, 51] The 2011 study consisted of 304 autism cases and 259 typically developing controls.[50] Pre-school aged cases and controls were recruited from Southern California between 2003 and 2009. Controls were matched on age, sex, and general geographical area. The researchers met with both cases and controls to ascertain autism diagnosis or typical development of the controls. Additionally, a detailed residential history was recorded from three months before conception until the current address. Volk et al. calculated the distance from each residence to the nearest freeway or major road. After adjusting for sex, ethnicity, education of parent, maternal age, and maternal smoking during pregnancy, children who lived within 309 m

of a freeway had 1.86 the odds (95% CI, 1.04-3.45) of having autism as children who lived further from a freeway. Investigating the proximity to freeway or major road by trimester, the investigators found only the third trimester association to be statistically significant (OR=1.96; 95% CI, 1.01-3.93).

Volk et al. used a slightly smaller subset of their 2011 CHARGE population to further refine and classify traffic exposure in their 2013 investigation.[51] In this case-control study, the investigators applied the CALINE4 line-source air quality dispersion model to estimate exposure to traffic related air pollutants, $PM_{2.5}$, PM_{10}, ozone, and nitrogen dioxide.[51] After adjusting for sex, ethnicity, education of parent, maternal age, and maternal smoking during pregnancy, a 2 standard deviation increase from the mean level of $PM_{2.5}$ (OR=2.08; 95% CI, 1.93-2.25), PM_{10} (OR=2.17; 95% CI, 1.49-3.16), and nitrogen dioxide (OR=1.81; 1.23-2.65) were all significantly associated with autism risk for the entire gestational period. Although the odds were slightly elevated with increased ozone exposure, they did not reach statistical significance.

Becerra et al. investigated the association between traffic related air pollution and autism risk in Los Angeles County, California.[52] Their population of 7,603 autism cases, identified through the California DDS, and 75,782 controls matched on birth year, sex, and gestational age were linked to birth certificates for identification of birth residence and individual risk factors. Case and control children were born between 1995 and 2006. The investigators used two methods of exposure assessment, nearest Criteria Air Pollutant (CAP) monitoring station and land use regression (LUR) modeled nitrogen monoxide and nitrogen dioxide exposures. Each model was adjusted for maternal age, education, race/ethnicity, maternal place of birth, type of birth, parity, insurance type, and gestational age at birth. Using CAP monitors as the exposure assessment method, Becerra et al. found that each interquartile range (IQR) increase in ozone

(OR=1.06; 95% CI, 1.01-1.12) was significantly associated with the odds of autism. Using LUR models as the exposure assessment method, they found that each IQR increase in nitrogen dioxide (OR=1.07; 95% CI, 1.03-1.12) was significantly associated with increased odds of autism.

A population based cohort study was conducted in Taiwan in which 49,833 children were followed for 11 years to assess the risk of autism incidence.[53] The investigators selected children under the age of three from an insurance database who were not currently diagnosed with ASD. Jung et al. evaluated exposure to ozone, carbon monoxide, nitrogen dioxide, PM_{10}, and sulfur dioxide from the time of study entry until an ASD diagnosis. Therefore, all exposure assessment was postnatal. Exposure was estimated by an annual inverse distance weighted surface created from pollutant monitors in Taiwan. The largest associations were seen in 1 and 2 years prior to ASD diagnosis. Cox models were adjusted for age, anxiety, gender, intellectual disabilities, preterm and socioeconomic status. In the year prior to ASD diagnosis, each 10 ppb increase in ozone (HR=1.59; 95% CI, 1.42-1.78), each 100 ppb increase in carbon monoxide (HR=1.37; 95% CI, 1.31-1.44), each 10 ppb increase in nitrogen dioxide (HR=4.43; 95% CI, 3.33-5.90), and each 1 ppb increase in sulfur dioxide (HR=1.18; 95% CI, 1.09-1.28) was associated with increased autism risk. The sulfur dioxide model was also adjusted for obsessive compulsive disorder and phobia.

Roberts et al. investigated the association of air pollutant exposures in a cohort of women from the Nurses' Health Study II.[54] This population containing 325 autism cases and 22,098 controls, comprised the first study on the association between air pollution and ASD to have a national scope in the US. The children were born to mothers in the Nurses' Health Study II between the years of 1987 and 2002. Mothers reported ASD diagnosis via survey. Depending on

birth year, Roberts et al. assigned quintile levels of air pollution concentration by census tract from the 1990, 1996, 1999, or 2002 NATA assessments. Comparing the highest quintile of exposure to the lowest for both sexes, the investigators found significant associations between ASD and exposure to overall metals (OR=1.5; 95% CI, 1.0-2.3), lead (OR=1.6; 95% CI, 1.1-2.3), manganese (OR=1.5; 95% CI, 1.1-2.2), mercury (OR=2.0; 95% CI, 1.2-3.3), and nickel (OR=1.7; 95% CI, 1.1-2.5). These models were adjusted for individual and census tract level covariates as well as an indicator for NATA year model used. Interestingly, the authors also tested each pollutant for sex specific associations. Each of the significant associations was also significant for boys, but was attenuated and not significant for girls. Additionally, two more pollutants had significant associations with ASD in boys only (antimony: OR=1.7; 95% CI, 1.1-2.7 and trichloroethylene: OR=1.4; 95% CI, 1.1-2.3).

Von Ehrenstein et al. investigated the relationship between exposure to air toxics and ASD in children born between 1995 and 2006 in Los Angeles County, California.[55] Their cohort included children with birth residences that were within 5 km of an air monitor (n=148,722). Using varimax rotation factor analysis to create factor groupings and multi-pollutant models, they found exposure to benzene (OR=1.46; 95% CI, 1.12-1.89), perchloroethylene (OR=1.40; 95% CI 1.09-1.80), 1-3-butadiene (OR=1.59; 95% CI, 1.18-2.15), toluene (OR=1.37; 95% CI, 1.12-1.67), o-xylene (OR=1.42; 95% CI, 1.19-1.70), m/p-xylene (OR=1.51; 95% CI, 1.26-1.82), ethylbenzene (OR=1.48; 95% CI, 1.25-1.75), lead (OR=1.49; 95% CI, 1.23-1.81), acetaldehyde (OR=1.20; 95% CI, 1.07-1.34), formaldehyde (OR=1.34; 95% CI, 1.17-1.52), trichloroethylene (OR=1.14; 95% CI, 1.03-1.27), and copper (OR=1.09; 95% CI, 1.02-1.16) to be significantly associated with ASD diagnosis.

1.2.7 Possible mechanisms

Just as the causes of autism are unclear, the mechanisms involved in autism pathogenesis are predominantly still in the hypothesis stage. One of the few mechanisms that would explain the higher prevalence of autism in older parents is de novo mutations.[56] De novo mutations occur more frequently in older people, and especially men. Advanced paternal age is one of the most consistent associations found in the literature.[57] In their medical hypothesis manuscript, Kinney et. al.[58] added to this mechanistic hypothesis by noting that an increase in de novo mutations paired with deficiencies in Vitamin D may be a plausible two-hit theory. One of the functions of Vitamin D is DNA repair. Thus, lack of DNA repair pathways would allow mutations to replicate. Lack of Vitamin D may explain the higher autism prevalence in higher altitude, higher precipitation areas found in some studies. Toxics that act on the neurological system, most likely contribute to ASD etiology by causing atypical neural development during pregnancy. Exposure to developmental toxics can lead to abnormal fetal brain development. There is literature that points to abnormalities in the lateral ventricle and hippocampus regions of the brain that would seem to support this mechanistic theory.[59] Toxics which are endocrine disruptors can cause altered Central Nervous System (CNS) development observed in people with ASD. Some environmental toxins can cause immune system dysregulation which can also lead to atypical neural development. Additionally, associations have been found between prenatal infection and autism and it has been hypothesized that it is the immune response, rather than the infection itself, that has a role in ASD etiology.[60] Kinney et al. noted that mutagenic toxics, such as mercury, cadmium, chromium, and nickel, may contribute to autism pathogenesis in two ways – through oxidative stress causing oxidative DNA damage by free radicals, and by inhibiting DNA repair.[58]

1.2.8 Conclusions

Of the parental, prenatal, obstetric, neonatal factors, and extrinsic factors addressed here, nine of them have been found to have a strong significant association (OR, RR, or HR ≥ 3.0) with ASD diagnosis. Two of these factors are maternal characteristics or behaviors: mother from a country outside of Europe, North America, or Australia, and maternal psychiatric disorder. One of these factors, fetal distress, is an obstetric complication. Two factors are extrinsic environmental exposures: pesticides and maternal anti-depressant use. The remaining four factors are neonatal characteristics. Being male, having a sibling with ASD, having a low Apgar score at 5 minutes, and preterm birth are the neonatal factors that have stronger associations with ASD in this review.

Significant associations with air pollutants tended not to be as strong. Only three air pollutants had an OR ≥ 2.0 in any study – $PM_{2.5,}$[51] $PM_{10,}$[51] and $NO_{2.}$[53] It is interesting to note that these studies used air monitor data (or models created from monitor data) and not NATA modeled concentrations or TRI reported releases. Using air monitor data allows for investigation into critical time periods of fetal development. Studies that looked at exposures by trimester[36] or during a critical time period[34] found associations that were different from the association found during the entire pregnancy.

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2.0 ARTICLE ONE: MATERNAL AND BIRTH CHARACTERISTICS AND THE RISK OF AUTISM SPECTRUM DISORDER (ASD) IN A SOUTHWESTERN PENNSYLVANIA CASE CONTROL STUDY

2.1 ABSTRACT

Background: Autism Spectrum Disorder (ASD) is a heterogeneous neurological disease that now affects one in 68 children born in the US. As ASDs increase in prevalence, more research is being conducted on possible non-genetic contributions to this disease.

Methods: Independent variables were extracted from the birth certificates of 198 singleton ASD cases from the Case-Control Study of Risk Factors for Childhood Autism in Southwest Pennsylvania and 4,801 gender and year of birth matched singleton controls randomly chosen from births in the same six counties occurring between 2005 and 2009. Logistic regression modelling was used to analyze the association between these independent variables and ASD status.

Results: Multivariable logistic regression modelling showed that increased maternal age (OR=1.05; 95% CI, 1.02-1.08), greater maternal educational attainment (OR=1.42; 95% CI, 1.03-1.95), gestational hypertension (OR=2.02; 95% CI, 1.20-3.41), cesarean delivery (OR=1.45; 95% CI, 1.08-1.96), and maternal infection (OR=2.07; 95% CI, 1.06-4.06) were independent significant risk factors for ASD. Smoking was not found to be related to the risk of

autism after adjustment for mother's age, education, and race. Although the analysis was limited to singleton births, it was noted that proportionally, more ASD cases were multiple births than controls (8.3% versus 3.8%, $p<.05$).

Conclusions: Previous studies have found maternal age and education, gestational hypertension, and cesarean delivery to be associated with ASD. Although an association with maternal infection has also been observed, this finding is more uncommon. Over time, consistency in results of studies evaluating risk factors for ASD will continue to strengthen the likelihood of these findings being true associations.

2.2 INTRODUCTION

Autism Spectrum Disorder currently affects 1 in 68 children born in the US.[1] Although this disease has a strong genetic component, there have been recent studies that suggest that there is also an environmental contribution.[2] Parental characteristics, prenatal conditions, obstetric complications, and neonatal characteristics have been studied for associations with ASD, both in the US and internationally. Extrinsic exposures, such as maternal tobacco, alcohol, medication use, pesticides, and air pollution have also been explored.

Parental characteristics are among the most consistently studied risk factors for ASD. Maternal age,[3, 4] paternal age,[4, 5, 6] race,[7] and country of origin[8] have all been shown to be independently associated with ASD. Prenatal conditions of preeclampsia[9, 10] and gestational diabetes[11] have been found to be associated with ASD. Breech presentation and cesarean delivery can lead to adverse birth outcomes.[12, 13] Induced and augmented labor can cause, or be indicative of, other problems with the infant or the birth process.[14] All of these obstetric

complications have also been associated with ASD.[7, 15, 16, 17] Low birth weight and preterm birth are associated with many subsequent health problems including ASD.[16, 18] Being first born has also been shown to have an association with ASD status.[5, 18] Maternal exposures of smoking[8], medication use[19], and viral infections[10] during pregnancy have been associated with ASD.

The etiology of ASD is also unknown at this time. Much of the existing research suggests that ASD begins during the prenatal period. Differentiation in the neocortex of children with ASD compared to typically developing children is the latest of such indications.[21] De novo mutations have been suggested as an etiological pathway as they could explain the association with increased parental age.[22] The association between prenatal infection and autism has been hypothesized to be caused by the immune response, rather than the infection itself.[23] In addition, high levels of intrauterine testosterone may explain many of the suggested risk factors for ASD. Smoking and stress are associated with high levels of testosterone in women.[24] Although many risk factors have been considered, there are also plausible etiologic theories to explain them.

As ASD prevalence is not constant over space and time, it is important to analyze risk factors for ASD in varied geographical areas, populations, and birth cohorts. To our knowledge, this is the first such study conducted in southwestern Pennsylvania. The objective of this case-control study is to investigate the association of risk factors documented on the birth certificate and ASD in a six county birth cohort in Pennsylvania from 2005 to 2009.

2.3 METHODS

2.3.1 Study population

Cases (n=198) and controls (n=4,801) were singleton births collected from the Case-Control Study of Risk Factors for Childhood Autism in Southwest Pennsylvania. Cases and controls were born between 2005 and 2009 in one of six southwestern Pennsylvania counties: Allegheny, Armstrong, Beaver, Butler, Washington, or Westmoreland. Cases had physician diagnosed ASD and were recruited from autism clinics and institutions and through early education and intervention programs. Of the participant cases, 81.6% provided a diagnosis report. Controls were randomly selected from the Pennsylvania Department of Health birth registry and frequency matched on birth year and gender to cases. Although 199 singleton cases were interviewed for the study, only 198 cases could be linked to their birth certificate for the purposes of this analysis.

2.3.2 Independent variables

All independent variables were extracted from the PA birth certificate. Mother's age and father's age were examined as continuous variables. Low birth weight (< 2500 g), preterm birth (< 37 weeks of gestation), cesarean delivery, induced labor, augmented labor, fetal intolerance to labor, breech presentation, gestational diabetes, gestational hypertension, first birth, maternal infections, maternal antibiotic use, and anesthesia were included as dichotomous variables. First birth is defined as a mother not having any prior births (dead or alive). Maternal infections are defined as the presence of at least one of the following during this pregnancy: gonorrhea,

syphilis, herpes simplex virus, chlamydia, hepatitis B, hepatitis C. Smoking was examined in three categories: no smoking in the three months prior to pregnancy through the entire pregnancy, smoking in the three months prior to pregnancy and/or the first trimester only, and smoking at least into the second trimester of pregnancy. Race was defined from the mother's self-selected race as white, black, or other. Mother's education was categorized as less than a high school education, a high school diploma with or without some years of college, and an undergraduate degree or more.

2.3.3 Statistical analysis

Logistic regression modelling was used to investigate the association between the birth certificate risk factors and ASD status. Each risk factor was examined in a univariable regression and was then entered into an adjusted logistic model. Any variable found to be statistically different between cases and controls was entered into the adjusted model. A multivariable model was then constructed by placing any variable which was significant at the $p < 0.2$ level in the adjusted analysis into a logistic regression. Each variable that did not reach statistical significance ($p<0.05$) in this model was subsequently removed using a backward stepwise approach. StataSE 12 statistical software was used for all analyses.

2.4 RESULTS

By design, cases did not differ from the control group by gender, year of birth, or county residence at birth (Table 3). However, mothers of ASD cases were significantly more likely to be

white, have at least an undergraduate degree, be older, be non-smokers, have a cesarean delivery, and have gestational hypertension than the mothers of control children. Additionally, fathers of ASD cases were more likely to be older than the fathers of control children. Singleton cases and controls had similar incidence of low birth weight, preterm birth, induced labor, augmented labor, fetal intolerance to labor, breech presentation, gestational diabetes, maternal infections, maternal antibiotic use, and maternal anesthesia during birth. ASD cases and controls also had a similar likelihood of being the first born child.

Table 3. Comparison of birth certificate risk factors between cases and controls

Variables	Case (n=198)	Control (n=4,801)	p-value	Number missing (cases/controls)
Male gender	154 (77.8)	3,852 (80.2)	0.396	0/0
Year of birth 2005 2006 2007 2008 2009	 40 (20.2) 52 (26.3) 45 (22.7) 33 (16.7) 28 (14.1)	 1,123 (23.4) 1,108 (23.1) 1,034 (21.5) 853 (17.8) 683 (14.2)	0.751	0/0
County at birth Allegheny Armstrong Beaver Butler Washington Westmoreland	 120 (60.6) 7 (3.5) 7 (3.5) 14 (7.1) 19 (9.6) 31 (15.7)	 2,783 (58.0) 147 (3.1) 379 (7.9) 388 (8.1) 435 (9.1) 669 (13.9)	0.331	0/0
Mother White Black Other	 178 (89.9) 16 (8.1) 4 (2.0)	 3,912 (81.8) 668 (14.0) 203 (4.2)	0.014	0/18
Mother's education < High school graduate High school graduate and some college ≥ College graduate	 6 (3.0) 84 (42.4) 108 (54.5)	 461 (9.6) 2,458 (51.4) 1,860 (38.9)	< 0.001	0/22
Mean (SD) maternal age	30.4 (5.3)	28.4 (6.0)	< 0.001	0/1
Mean (SD) paternal age	32.7 (5.9)	31.4 (6.6)	0.006	13/617
Low birth weight (< 2500g)	12 (6.1)	250 (5.2)	0.604	0/12
Preterm birth (< 37 weeks)	20 (10.3)	413 (8.8)	0.473	4/115
Cesarean Delivery	75 (38.1)	1,329 (27.7)	0.002	1/3
Induced Labor	42 (21.2)	911 (19.0)	0.432	0/0
Augmented Labor	28 (14.1)	801 (16.7)	0.346	0/0
Fetal Intolerance to Labor	28 (14.1)	576 (12.0)	0.364	0/0
Smoking None Pre and 1st trimester only At least into 2nd trimester	 170 (86.3) 10 (5.1) 17 (8.6)	 3,574 (75.8) 324 (6.9) 814 (17.3)	0.003	1/89
Breech Presentation	9 (4.5)	119 (2.5)	0.071	0/0
Gestational Diabetes	11 (5.6)	167 (3.5)	0.122	0/0
First Born	96 (48.5)	2,057 (42.8)	0.116	0/14
Gestational Hypertension	17 (8.6)	203 (4.2)	0.003	0/0
Maternal Infections	10 (5.1)	173 (3.6)	0.288	0/0
Maternal Antibiotic Use	48 (24.2)	1,168 (24.3)	0.978	0/0
Maternal Anesthesia	114 (57.6)	2,760 (57.5)	0.980	0/0

Table 4 contains univariable and adjusted odds ratios of birth certificate risk factors for ASD cases compared to controls. Mother's age, father's age, cesarean delivery, mother's smoking status, gestational hypertension, mother's race, and mother's education were all significantly associated with ASD status in the univariable analyses. However, father's age was not used as an adjustment variable because there were many missing values in this category (n=630, or 12.6%). Father's age, mother's smoking status, race, and education were no longer statistically significant in the models also containing mother's age, smoking status, race, education, cesarean delivery, and gestational hypertension. Conversely, although being first born and maternal infections during pregnancy were not significantly associated with ASD status in univariable models, they were significant in the adjusted models. After adjusting for mother's race, education, and smoking status, cesarean delivery, and gestational hypertension, each additional year of mother's age resulted in a 3% increase in odds (95% CI, 1.001-1.060) of having a child with ASD. Mothers who delivered via cesarean had 1.50 the odds (95% CI, 1.12-2.02) of having a child with ASD compared to mothers who delivered via any other method, after adjusting for mother's age, race, education, and smoking status, and gestational hypertension. Mothers who had gestational hypertension had 2.14 the odds (95% CI, 1.27-3.62) of having a child with ASD compared to mothers who did not have gestational hypertension in the adjusted model. ASD cases had 1.38 times the odds (95% CI, 1.01-1.88) of being the first born compared to controls after adjusting for mother's age, race, education, smoking status, cesarean delivery, and gestational hypertension. Mothers of ASD cases had 2.17 the odds (95% CI, 1.11-4.27) of having an infection during pregnancy than mothers of controls. This finding was independent of mother's age, race, education, and smoking status, cesarean delivery, and gestational hypertension.

Table 4. Univariable and adjusted odds ratios of birth certificate risk factors

Risk Factor	Odds Ratio (95% CI)*	p-value*	Odds Ratio (95% CI)**	p-value**
Age of mother	1.06 (1.03, 1.08)	< 0.001	1.03 (1.001, 1.060)	0.043
Age of father	1.03 (1.01, 1.05)	0.006	1.00 (0.97, 1.04)	0.813
Low birth weight	1.17 (0.64, 2.12)	0.604	1.21 (0.65, 2.24)	0.545
Preterm birth	1.19 (0.74, 1.91)	0.473	1.10 (0.67, 1.81)	0.699
Cesarean delivery	1.60 (1.20, 2.15)	0.002	1.50 (1.12, 2.02)	0.007
Induced labor	1.15 (0.81, 1.63)	0.433	1.24 (0.86, 1.77)	0.247
Augmented labor	0.82 (0.55, 1.24)	0.347	1.00 (0.66, 1.52)	0.989
Fetal Intolerance to Labor	1.21 (0.80, 1.82)	0.365	1.12 (0.73, 1.70)	0.609
Smoking				
Pre and 1st trimester only vs none	0.65 (0.34, 1.24)		0.77 (0.40, 1.48)	
At least into 2nd trimester vs none	0.44 (0.27, 0.73)	0.003	0.61 (0.36, 1.05)	0.171
Breech Presentation	1.87 (0.94, 3.75)	0.076	1.44 (0.70, 2.98)	0.321
Gestational Diabetes	1.63 (0.87, 3.06)	0.126	1.30 (0.67, 2.53)	0.433
Gestational Hypertension	2.13 (1.27, 3.57)	0.004	2.14 (1.27, 3.62)	0.004
First born	1.25 (0.94, 1.66)	0.125	1.38 (1.01, 1.88)	0.044
Maternal Infections	1.43 (0.74, 2.74)	0.290	2.17 (1.11, 4.27)	0.024
Maternal Antibiotic Use	1.00 (0.71, 1.39)	0.978	1.00 (0.71, 1.40)	0.987
Anesthesia	1.00 (0.75, 1.34)	0.980	1.01 (0.75, 1.36)	0.947
Race				
Black vs white	0.53 (0.31, 0.889)	0.016	0.78 (0.45, 1.33)	0.134
Other vs white	0.43 (0.16, 1.18)		0.39 (0.14, 1.08)	
Education				
< 12 vs 12-15	0.38 (0.17, 0.88)	< 0.001	0.56 (0.24, 1.32)	0.067
16+ vs 12-15	1.70 (1.27, 2.27)		1.35 (0.97, 1.87)	

* = univariable analysis

** = adjusted for age, race, smoking status, and education of mother, cesarean delivery, and gestational hypertension

Table 5 contains the odds ratios for variables in the final multivariate model. For every additional year of mother's age, there is a 5% increase in odds (95% CI, 1.02-1.08) of having a child with an ASD diagnosis. This finding is independent of all other variables in the model (mother's education, gestational hypertension, cesarean delivery, the baby being the first born, and maternal infection during pregnancy). Mother's education is also an independent risk factor for being an autism case. After adjusting for the other variables in the final model, women with at least a college degree have 1.42 the odds (95% CI, 1.03-1.95) of having an ASD child compared to women with a high school degree. Women who have gestational hypertension have 2.02 the odds (95% CI, 1.20-3.41) of having an ASD child compared to women without gestational hypertension after adjusting for mother's age, mother's education, cesarean delivery,

having their first baby, and maternal infection. Cesarean delivery was associated with ASD status (OR=1.45; 95% CI, 1.08-1.96) after adjusting for all other variables in the final multivariate model. ASD cases had 1.40 the odds (95% CI, 1.03-1.90) of being first born compared to controls. Mothers of ASD cases had 2.07 the odds (95% CI, 1.06-4.06) of having at least one of the following infections during pregnancy: gonorrhea, syphilis, herpes simplex virus, chlamydia, hepatitis B, hepatitis C, than mothers of controls.

Table 5. Final multivariable model: the association of included variables with ASD status*

Variable	Odds Ratio (95% CI)	p-value
Mother's age	1.05 (1.02, 1.08)	0.002
Mother's education		
< 12 vs 12-15	0.48 (0.20, 1.13)	0.015
16+ vs 12-15	1.42 (1.03, 1.95)	
Gestational hypertension	2.02 (1.20, 3.41)	0.009
Cesarean delivery	1.45 (1.08, 1.96)	0.014
First birth	1.40 (1.03, 1.90)	0.034
Maternal infection	2.07 (1.06, 4.06)	0.033

* = associations are adjusted for all other variables in the model

2.5 DISCUSSION

Mother's age, mother's education, gestational hypertension, cesarean delivery, first born, and maternal infection were found to be independent risk factors in this case control study conducted with singleton births in southwestern Pennsylvania. There were no significant interactions between mother's age and each other variable in the model (mother's education, gestational hypertension, cesarean delivery, first birth, and maternal infection). Additionally, there was not a significant interaction between gestational hypertension and any other variable in the model or cesarean delivery and any other variable in the model. Although the analysis was limited to

singleton births, 8.3% of ASD cases (18 of 216) were multiple births compared to 3.8% of controls (190 of 4,991; p=0.001). In a model adjusted for maternal characteristics and birth weight, Croen et al. found that multiple births had 1.7 times the risk (95% CI, 1.4-2.0) of being an ASD case compared to singletons.[25] Multiple births are often premature and are at increased risk for birth complications; moreover, as twins and triplets are considered "high risk" they are often under the scrutiny of pediatrician and neonatologists and therefore there may be a selection bias for this diagnosis. There is mounting evidence that premature delivery can increase the risk of adverse consequences to a child's nervous system development.[26]

Although mother's race and smoking status and father's age were associated with ASD in unadjusted models, the relationships were attenuated and not significant in adjusted models. Unsurprisingly, maternal age and paternal age were highly correlated (ρ=0.7718, p<0.001). Cesarean delivery was explored in our singleton cases and controls; 28.1% of the women in our study who delivered via cesarean had at least one previous cesarean delivery. Additionally, 8.3% of the women in our study who delivered via cesarean were carrying a baby with breech presentation. Of the 183 mothers who were diagnosed with an infection during their pregnancy, 84 (45.9%) of them had herpes simplex virus, 71 (38.8%) had chlamydia, and 26 (14.2%) had more than one type of infection. Mother's education is a difficult risk factor to interpret. Higher education is highly correlated with age of mother. Additionally, it has been suggested that women with higher educations are more likely have children with an ASD diagnosis because they are more likely to recognize and test for ASD symptoms in their children.[4] Smoking was not found to be significant after adjustment for other covariates. This also held true in an analysis stratified by race (see Appendix A). Although whites and blacks have a similar non-smoking rate (75.6% versus 76.0%, respectively), more whites quit smoking after the first trimester of

pregnancy (7.5% versus 4.1%). An exploratory analysis of alternate birth weight and gestational age categories can also be found in Appendix A.

All independent risk factors were ascertained by birth certificate records. Indeed there are strengths and weaknesses associated with this method. As data is collected at, or close to the time of birth, ASD status has not yet been discerned. Therefore, all limitations can be assumed to be non-differential. Studies have shown varying agreement with distinct birth certificate variables. A recent study found high agreement for birth weight within 500 grams, cesarean delivery, and cephalic presentation.[27] Most of the factors with low agreement in this study (total number of prenatal visits, previous preterm birth, and meconium staining) were not used for the current analysis. Additionally since these control mothers were not contacted, it is possible that some of the controls have been diagnosed with ASD or have behaviors on the autism spectrum.

Croen et al. conducted an historical birth cohort study in northern California.[4] Their analyses of singleton births compared 593 ASD cases and 132,251 controls born between 1995 and 1999. In a model containing maternal and paternal age, birth order, date of birth, sex of the child, maternal and paternal education level, and maternal and paternal race, they found significant associations between maternal age (OR=1.31; 95% CI, 1.07-1.62) and paternal age (OR=1.28; 95% CI, 1.09-1.51), both examined as continuous variables, with ASD status. They also found that women with an undergraduate degree (OR=1.36; 95% CI, 1.08-1.69) or a graduate degree (OR=1.44; 95% CI, 1.06-1.95) were more likely to have ASD diagnosed children compared to women who were high school graduates. Their adjusted models did not find a significant association with mother's race, identified as white, black, Asian, and other.

In a case-control study nested within a Swedish 1974-1993 birth cohort, Hultman et al. found a significant association between ASD status and cesarean delivery, but not maternal age,

birth order, or gestational hypertension.[8] Mothers who had a cesarean delivery had 1.6 times the odds (95% CI, 1.1-2.3) of having an ASD diagnosed child, compared to mothers who delivered vaginally. This association was independent of maternal age, parity, smoking, mother's country of birth, gestational hypertension, diabetes, bleeding during pregnancy, season of birth, gestational age, birth weight for gestational age, Apgar score at 5 minutes, and congenital malformations. There were 316 cases and 1,436 controls included in this analysis.

Although preeclampsia is included within the broader term of gestational hypertension, they are not the same condition. Preeclampsia is the most severe type of gestational hypertension which includes proteinuria.[28] As this study had access to birth certificate variables only, we were unable to ascertain the more serious condition of preeclampsia in either cases or controls. However, there was a strong association between ASD and this broader diagnosis of gestational hypertension (OR=2.05; 95% CI, 1.21-3.46). Mann et al. conducted a study on preeclampsia and ASDs.[9] In their South Carolina birth cohort (n=87,677), women who had preeclampsia had 1.69 (95% CI, 1.26, 2.28) the odds of having a child with ASD compared to women without preeclampsia, after adjusting for birth weight. Their analysis was restricted to singleton births from 1996 through 2002.

To our knowledge, only one prior study looked at the association of sexually transmitted diseases (STDs) and ASD.[20] Atladóttir et al. used a cohort of all Danish births from 1980 through 2005 to analyze the association of maternal infection requiring hospitalization and ASD. Although genital infections (including STDs) in the second trimester had an elevated hazard ratio (HR=1.43; 95% CI, 0.86-2.37), this association failed to reach statistical significance. However, when the authors divided infections into microorganism-specific infection categories of viral and bacterial, both subsets reached statistical significance at some point in the pregnancy. Mothers

who had viral infections requiring hospitalization in the first trimester (HR=2.98; 95% CI, 1.24-7.15) and mothers who had bacterial infections requiring hospitalization in the second trimester (HR=1.42; 95% CI, 1.08-1.87) were significantly more likely to deliver children who were subsequently diagnosed with ASD than women without infections requiring hospitalizations in those time periods. These associations are likely underestimated as, only the worst cases of infection were included in the analysis. Of the STDs included in our analysis, three are viral (herpes simplex virus, hepatitis B, and hepatitis C) and three are bacterial (chlamydia, gonorrhea, and syphilis). Additionally, Libbey et al. conducted a review of the literature and found four reports of case studies[29-32] linking herpes simplex virus to autism.[33]

2.6 CONCLUSIONS

This study adds to the literature on maternal and birth characteristics as potential risk factors for ASD. Although independent associations were found with maternal age, education, gestational hypertension, cesarean delivery, maternal infections, and first born, additional research must be conducted to replicate these findings. It would be advantageous to conduct a prospective study to assess more risk factors than are noted on a birth certificate.

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3.0 ARTICLE TWO: PRENATAL EXPOSURE TO METALS AND THE RISK OF AUTISM SPECTRUM DISORDER (ASD) IN A SOUTHWESTERN PENNSYLVANIA CASE CONTROL STUDY

3.1 ABSTRACT

Background: For the past few decades, Autism Spectrum Disorder (ASD) has been increasing in prevalence. Studies of environmental risk factors are emerging to determine if air pollution is a contributor to this disease.

Methods: The 2005 National Air Toxics Assessment (NATA) estimates of the concentration of seven metals by census tract of birth residence were linked to 198 singleton case births and 4,782 gender and year of birth matched singleton controls in southwestern Pennsylvania. Statistical analyses comparing women living in census tracts of the three upper quartiles of exposure to the lowest quartile of exposure for seven individual metals and a total metals score were examined. Factor analysis was used to investigate the correlation structure between NATA estimated metal concentrations.

Results: Arsenic, chromium, and lead were significantly associated with being an ASD case in logistic regression models using quartiles of exposure after adjusting for age, race, education, and smoking status of mother, cesarean delivery, and gestational hypertension. Women living in census tracts with the highest quartile of arsenic and chromium exposure at the birth of their

child had significantly higher odds (OR=1.72; 95% CI, 1.10-2.71 and OR=1.78; 95% CI, 1.17-2.72 respectively) of having a child diagnosed with ASD than women living in the census tracts with the lowest arsenic and chromium exposure at the birth of their child. Women living in census tracts with the third quartile of lead exposure at birth had significantly higher odds (OR=1.55; 95% CI, 1.00-2.38) of having a child diagnosed with ASD than women living in the census tracts with the lowest lead exposure at birth. Factor analysis revealed that the seven metals studied loaded onto two factors.

Conclusion: This research is one of a small, but growing, list of studies to find an association between air pollution and ASD status. More research must be conducted to corroborate these findings.

3.2 INTRODUCTION

The rapid increase in the incidence of Autism Spectrum Disorders (ASD) in the US and internationally, suggests that ASD is not wholly a disorder of genetics. Surveillance data from the CDC funded Autism and Developmental Disabilities Monitoring (ADDM) Network indicates that autism prevalence increased from 6.7 per 10,000 children born in 1992 to 14.7 per 10,000 children born in 2002.[1] Additionally, studies reporting discordance of ASD in twin pairs suggest an environmental contribution to the disease. Studies of twins show that concordance among monozygotic twins is significantly higher than concordance among dizygotic twins.[2] However, since monozygotic twins do not have a 100% concordance rate, it is believed that environmental factors have some role in the etiology of ASD. After conducting their rigorous twin study, Hallmayer et al.[3] concluded that environmental factors account for 55% of the autism burden.

Although there is an environmental contribution to the etiology of ASD, few risk factors have been established. Two of the more consistent findings are male gender[4, 5, 6, 7, 8] and siblings of ASD cases.[6] It is more likely for parents to have a child with autism if they already have another child diagnosed with autism.[1, 6] A recent study conducted in California found that the sibling recurrence risk in families with an ASD diagnosed child was 10.1% compared to a prevalence rate of 0.52% of ASD in control families (no prior children with an ASD diagnosis).[9] Parental factors and pregnancy and obstetric complications have been studied for association with ASD with inconsistent results. Advanced maternal and paternal age has been repeatedly found to be associated with ASD.[4, 5, 10, 11] Studies have also found that mothers that attain higher education are more likely to have children with ASD.[5, 12] However, this factor is often correlated with mother's age. Some studies have shown that first born children are more likely to be diagnosed with ASD.[7, 11] Previous studies have found associations with ASD and cesarean delivery,[13, 14] low birth weight,[15] preterm birth,[7, 15] gestational diabetes,[16] and the more severe form of gestational hypertension, preeclampsia.[17, 18] Additionally, maternal smoking[13] is just one of the extrinsic exposures that have been linked to ASD.

In the mid-2000s, research shifted to the area of air toxics for possible answers to the autism question. Air pollutants had previously been associated with adverse birth outcomes such as low birth weight.[19, 20] Additionally, many substances that are released through industrial processes, transportation, and natural activities are known to be harmful to human health. Various criteria air pollutants (CAPs) and hazardous air pollutants (HAPs) have been shown to have an association with ASD.[21-29] Three previous studies have used the same EPA National Air Toxics Assessment (NATA) estimated exposures for HAPs in their research on the association of ASD and air pollution.[21, 24, 29]

Much of the current body of research on the association between air pollutants and autism had been conducted in the US and parts of Asia. There is little research on environmental associations with autism in the United States outside of California and Texas. However, Western Pennsylvania has been the setting for many health studies on air pollutants – in no small part because of the history of air pollution in this area. Although air quality has improved over the years, Allegheny County repeatedly makes the American Lung Association's annual list of "Most Polluted Cities" for both particulate and ozone pollution. More recent studies conducted in Allegheny County have found associations with current lower levels of PM_{10} exposure and term low birth weight[30] and cardiopulmonary hospital admissions.[31] The objective of this current study is to determine if metal compound air pollutants, as modeled by the NATA, are associated with ASD in a case-control study conducted within a southwestern Pennsylvania 2005 – 2009 birth cohort.

3.3 METHODS

3.3.1 Study population

Cases and controls were from the Case-Control Study of Risk Factors for Childhood Autism in Southwest Pennsylvania. Cases were born between 2005 and 2009 and resided since birth in one of six southwest Pennsylvania counties: Allegheny, Armstrong, Beaver, Butler, Washington, or Westmoreland. Cases had physician diagnosed ASD and were recruited from autism clinics and institutions and through early education and intervention programs. Although 217 case mothers consented and were interviewed for the study, only 216 cases could be linked to their birth

certificate for the purposes of this analysis. Controls (n=4,991) were randomly selected from the Pennsylvania Department of Health (PA DOH) birth registry and frequency matched on year of birth and gender to cases. Each of the controls was born in one of the same six southwestern PA counties.

Multiple births are more likely to result in adverse birth outcomes, including low birth weight and preterm birth, which may be considered risk factors for ASD. Therefore, this analysis was restricted to singleton births only: 198 cases and 4,801 controls.

3.3.2 Birth certificate variables

All independent variables were extracted from the PA birth certificate for both cases and controls. Mother's age and father's age were examined as continuous variables. Low birth weight (< 2500 g), preterm birth (< 37 weeks of gestation), cesarean delivery, breech presentation, gestational diabetes, gestational hypertension, and first birth were included as dichotomous variables. Smoking was examined in three categories: no smoking in the three months prior to pregnancy through the entire pregnancy, smoking in the three months prior to pregnancy and/or the first trimester only, and smoking at least into the second trimester of pregnancy. Race was defined from the mother's self-selected race as white, black, or other. Mother's education was categorized as less than a high school education, a high school diploma with or without some years of college, and an undergraduate degree or more.

3.3.3 Exposure assessment

All NATA estimates by census tract for the 2005 Assessment were downloaded from the US EPA website.[32] The concentrations were linked to census tract of residence at birth as documented on the birth certificate. PA DOH birth certificates contained census tract of birth residence for 4,201 births in the population: 172 (86.9%) of the cases and 4,029 (83.9%) of the controls. 796 births in the population did not contain census tract or address of birth. However, they did contain a zip code of birth. The 2010 ZCTA shapefile was used to calculate the geographical center of each zip code in ArcGIS 10.2. Each ZCTA centroid was spatially linked to the 2000 census tract that contains it. 26 cases (13.1%) and 753 controls (15.7%) were linked to a census tract using this estimate. 17 births (all of which were controls) could not be linked to a census tract using this method because the documented zip code was not in the 2010 ZCTA shapefile. Additionally, 2 birth census tracts (both control births) could not be linked to a 2005 NATA census tract. The final population for this analysis was 198 cases and 4,782 controls.

3.3.4 Statistical analysis

Logistic regression modelling was used to investigate the association between NATA concentrations of metals and ASD status. Concentrations were examined as quartiles of exposure; NATA concentrations were divided into quartiles based on the distribution of the controls. The three highest quartiles were individually compared to the lowest quartile. Additionally, an aggregated metal index, with a score ranging from 7 to 28, was created with the seven metals in this analysis (arsenic, cadmium, chromium, lead, manganese, mercury, and nickel). The sum of all seven metal quartiles was used to create quartiles of the total metal

exposure based on the distribution of the controls. Each metal was examined in an unadjusted single pollutant logistic regression and then additionally adjusted for all variables that were significantly different between cases and controls.

As it was hypothesized that the concentrations of these metals were most likely highly correlated, we conducted a principal components factor analysis to investigate the relationship between them. StataSE 12 statistical software was used for all analyses.

3.4 RESULTS

Cases did not differ from the control group by the geographic and demographic variables of source of census tract, gender, year of birth, or county residence at birth (Table 6). Cases and controls also had similar incidence of low birth weight, preterm birth, breech presentation, and gestational diabetes. The percentage of mothers having their first birth was also not statistically different between ASD cases and controls. However, mothers of ASD cases were significantly more likely to be white, have at least an undergraduate degree, be older, be non-smokers, and have gestational hypertension than the mothers of control children. Additionally, fathers of ASD cases were more likely to be older than the fathers of control children. However, father's age was not used as an adjustment variable because there were many missing values in this category (n=611, or 12.3%). ASD cases were significantly more likely to be delivered via cesarean than ASD controls.

Table 6. Demographic and risk factor comparison between ASD cases and controls

Characteristic	Cases (n=198)	Controls (n=4,782)	p-value
Census tract found on birth certificate – n(%)	172 (86.9)	4,029 (84.3)	0.321
Gender: Male child – n(%)	154 (77.8)	3,839 (80.3)	0.387
County at birth – n(%) Allegheny Armstrong Beaver Butler Washington Westmoreland	 120 (60.6) 7 (3.5) 7 (3.5) 14 (7.1) 19 (9.6) 31 (15.7)	 2,777 (58.1) 145 (3.0) 378 (7.9) 387 (8.1) 433 (9.1) 662 (13.8)	0.324
Year of birth – n(%) 2005 2006 2007 2008 2009	 40 (20.2) 52 (26.3) 45 (22.7) 33 (16.7) 28 (14.1)	 1,117 (23.4) 1,105 (23.1) 1,030 (21.5) 847 (17.7) 683 (14.3)	0.759
Mother – n(%) White Black Other	 178 (89.9) 16 (8.1) 4 (2.0)	 3,894 (81.7) 667 (14.0) 203 (4.3) 18 missing	0.013
Mother's education – n(%) < High school graduate High school graduate and some college ≥ College graduate	 6 (3.0) 84 (42.4) 108 (54.5)	 461 (9.7) 2,446 (51.4) 1,853 (38.9) 22 missing	< 0.001
Mean (SD) maternal age	30.4 (5.3)	28.4 (6.0) 1 missing	< 0.001
Mean (SD) paternal age	32.7 (5.9) 13 missing	31.4 (6.6) 614 missing	0.006
Low birth weight – n(%) (< 2500g)	12 (6.1)	250 (5.2) 12 missing	0.613
Preterm birth – n(%) (< 37 weeks)	20 (10.3) 4 missing	413 (8.8) 115 missing	0.484
Cesarean Delivery – n(%)	75 (38.1) 1 missing	1,323 (27.7) 3 missing	0.001
Smoking – n(%) None Pre and 1st trimester only At least into 2nd trimester	 170 (86.3) 10 (5.1) 17 (8.6) 1 missing	 3,564 (76.0) 322 (6.9) 803 (17.1) 93 missing	0.003
Breech Presentation – n(%)	9 (4.5)	119 (2.5)	0.073
Gestational Diabetes – n(%)	11 (5.6)	166 (3.5)	0.121
Gestational Hypertension – n(%)	17 (8.6)	203 (4.2)	0.004
First Birth – n(%)	96 (48.5)	2,051 (42.9) 14 missing	0.128

Table 7 contains the 5th, 25th, 50th, 75th, and 95th percentiles of the selected NATA concentrations for both cases and controls. The median distribution for arsenic, cadmium, chromium, lead, manganese, mercury, and nickel does not differ between cases and controls (Mann-Whitney test).

Table 7. Single pollutant percentiles of concentration for ASD cases and controls

Pollutant Concentration in ng/m^3		Percentiles					p-value*
		5	25	50	75	95	
Arsenic	Case	0.649	1.043	1.145	1.354	1.959	0.258
	Control	0.595	1.001	1.132	1.351	2.005	
Cadmium	Case	0.090	0.129	0.153	0.184	0.351	0.893
	Control	0.086	0.124	0.154	0.186	0.340	
Chromium	Case	1.161	1.578	1.787	2.279	4.473	0.174
	Control	0.997	1.554	1.790	2.183	4.200	
Lead	Case	1.954	3.400	3.871	4.396	9.572	0.240
	Control	1.922	3.273	3.827	4.421	7.966	
Manganese	Case	1.221	1.745	2.092	2.385	4.429	0.276
	Control	1.144	1.711	2.076	2.306	4.389	
Mercury	Case	0.027	0.037	0.055	0.080	0.158	0.831
	Control	0.024	0.037	0.056	0.080	0.163	
Nickel	Case	0.466	0.796	0.912	1.077	3.254	0.752
	Control	0.424	0.790	0.924	1.072	3.077	

* = Mann-Whitney test

Table 8 contains the results of the upper three quartiles of exposure compared to the lowest quartile of exposure for individual 7 NATA metals and total metals score. In the logistic models adjusted for age, race, education and smoking status of mothers, as well as cesarean delivery and gestational hypertension, exposure to arsenic, chromium, and lead showed an association with ASD status. Women living in census tracts with the highest quartile of arsenic exposure at the birth of their child had significantly higher odds (OR=1.72; 95% CI, 1.10-2.71) of having a child diagnosed with ASD than women living in the census tracts with the lowest

arsenic exposure at the birth of their child. Women living in areas of the highest quartile of chromium exposure at the birth of their child had 1.78 times the odds (95% CI, 1.17-2.72) of having an ASD diagnosed child than women living in areas of the lowest quartile of chromium at the birth of their child after adjusting for age, race, education, and smoking status of mother, cesarean delivery, and gestational hypertension. Women living in census tracts with the third quartile of lead exposure at birth had significantly higher odds (OR=1.55; 95% CI, 1.00-2.38) of having a child diagnosed with ASD than women living in the census tracts with the lowest lead exposure at birth. Although women living in census tracts with the highest quartile of lead exposure had 1.51 times the odds (95% CI, 0.97-2.35) of having a child with an ASD diagnosis compared to women living in census tracts with the lowest quartile of lead exposure, it failed to reach statistical significance (p=0.071). Additionally, in the adjusted model, the total metals score had an elevated odds ratio (OR=1.47; 95% CI, 0.97-2.22) for the highest quartile compared to the lowest in the adjusted logistic model, but this finding did not reach significance (p=0.070).

Table 8. Unadjusted* and adjusted odds ratios: quartiles of exposure during pregnancy**

Risk Factor	Odds Ratio (95% CI)*	p-value*	Odds Ratio (95% CI)**	p-value**
Arsenic				
Q2 vs Q1	**1.58 (1.03, 2.42)**	**0.034**	1.50 (0.98, 2.30)	0.065
Q3 vs Q1	**1.56 (1.02, 2.39)**	**0.042**	1.50 (0.97, 2.32)	0.066
Q4 vs Q1	1.40 (0.91, 2.17)	0.129	**1.72 (1.10, 2.71)**	**0.018**
Cadmium				
Q2 vs Q1	1.31 (0.87, 1.95)	0.192	1.19 (0.79, 1.79)	0.410
Q3 vs Q1	1.09 (0.72, 1.65)	0.687	1.06 (0.70, 1.63)	0.772
Q4 vs Q1	1.12 (0.74, 1.69)	0.596	1.34 (0.88, 2.05)	0.175
Chromium				
Q2 vs Q1	1.45 (0.96, 2.18)	0.074	1.52 (1.00, 2.26)	0.050
Q3 vs Q1	1.00 (0.64, 1.55)	0.997	1.15 (0.73, 1.81)	0.548
Q4 vs Q1	1.40 (0.93, 2.11)	0.105	**1.78 (1.17, 2.72)**	**0.007**
Lead				
Q2 vs Q1	**1.65 (1.09, 2.51)**	**0.018**	1.52 (0.99, 2.32)	0.053
Q3 vs Q1	1.45 (0.95, 2.23)	0.085	**1.55 (1.00, 2.38)**	**0.048**
Q4 vs Q1	1.30 (0.84, 2.02)	0.235	1.51 (0.97, 2.35)	0.071
Manganese				
Q2 vs Q1	0.95 (0.63, 1.43)	0.813	0.88 (0.58, 1.33)	0.545
Q3 vs Q1	0.92 (0.61, 1.39)	0.689	0.95 (0.63, 1.45)	0.820
Q4 vs Q1	1.17 (0.79, 1.73)	0.423	1.36 (0.92, 2.03)	0.127
Mercury				
Q2 vs Q1	1.09 (0.73, 1.62)	0.680	1.08 (0.72, 1.62)	0.719
Q3 vs Q1	1.15 (0.77, 1.70)	0.497	1.02 (0.68, 1.53)	0.915
Q4 vs Q1	0.90 (0.59, 1.37)	0.629	0.92 (0.60, 1.40)	0.691
Nickel				
Q2 vs Q1	1.23 (0.84, 1.82)	0.284	1.16 (0.78, 1.72)	0.457
Q3 vs Q1	0.80 (0.52, 1.22)	0.300	0.89 (0.57, 1.39)	0.614
Q4 vs Q1	1.03 (0.69, 1.54)	0.889	1.19 (0.79, 1.80)	0.406
Total Metals Score				
Q2 vs Q1	1.11 (0.75, 1.65)	0.595	1.08 (0.72, 1.62)	0.706
Q3 vs Q1	1.05 (0.70, 1.58)	0.807	1.15 (0.76, 1.74)	0.504
Q4 vs Q1	1.16 (0.78, 1.73)	0.470	1.47 (0.97, 2.22)	0.070

** Adjusted for age, race, education, and smoking status of mother, cesarean delivery, and gestational hypertension

Table 9 reveals the results of the principal component factor analysis. The seven metals loaded onto two factors; chromium, lead, manganese, and nickel represent one factor while arsenic, cadmium, and mercury comprise the other. Table 10 contains the Pearson correlation coefficients for all pairwise comparisons between the seven metals by census tract.

Table 9. Factor loadings from principal components factor analysis

Air Toxic	Factor 1	Factor 2
Arsenic	0.4001	0.7202
Cadmium	0.4476	0.8037
Chromium	0.8439	-0.3512
Lead	0.7185	-0.1480
Manganese	0.6386	-0.1830
Mercury	0.2810	0.6263
Nickel	0.7763	-0.3918

Table 10. Pearson correlation coefficients between seven metals

	Factor 1				***Factor 2***		
	Chromium	Lead	Manganese	Nickel	Arsenic	Cadmium	Mercury
Chromium	1.0000						
Lead	0.4550*	1.0000					
Manganese	0.3923*	0.5919*	1.0000				
Nickel	0.9443*	0.3728*	0.2933*	1.0000			
Arsenic	0.0996*	0.1821*	0.0383	0.0560*	1.0000		
Cadmium	0.1210*	0.1874*	0.0618*	0.0674*	0.7010*	1.0000	
Mercury	0.0446	0.0012	0.1601*	-0.0038	0.2678*	0.5072*	1.0000

* = significant at the p<0.01 level

3.5 DISCUSSION

This study of cases and controls in southwestern Pennsylvania revealed significant associations between arsenic, lead, and chromium exposure and ASD status. Factor groups were investigated using factor analysis and evaluating the correlation matrix between NATA concentrations of these metals at the census tract level. All seven of these metals are associated with coal combustion. However, the metals in Factor 1 (chromium, lead, manganese, and nickel) are indicative of steel and metal manufacturing. Additionally, a combined metals score was marginally associated with ASD status (p=0.070).

There are six prior studies that have investigated the association of the air exposure to metals and ASD. Metals were chosen as the exposure for this southwestern Pennsylvania case-control study in part because they had been found to be significantly associated with ASD in five of them.[21, 22, 23, 29, 34] Three of the six studies[21, 24, 29] used the same exposure assessment method (NATA) as this study. However, the results of this study contrasted with these previous findings.

Windham et al.[21] used a case-control design with 284 autism cases and 657 randomly chosen matched controls from the same birth cohort of infants born in the San Francisco Bay Area in 1994. Autism cases were identified through active surveillance of existing medical records. A 2 to 1 ratio of controls to cases was used, and controls were matched on sex and month of birth. Cases and controls were linked to their birth certificate to ascertain the residence of birth. Using modeled concentrations of air toxics by census tract as their exposure measure (NATA, 1996), Windham et al. found that women who were exposed to the highest interquartile level of exposure to metals and chlorinated solvents had higher odds of having an autistic child when compared to women who gave birth in census tracts with the lowest interquartile levels of these substances. When the researchers further investigated the aggregated metal group, they found that cadmium, mercury, and nickel were significantly associated with autism diagnosis in adjusted logistic regression models. The current study found no association with cadmium, mercury, and nickel and a marginal association with the metal group.

The research team of Palmer et al. conducted two investigations using the industrial release of mercury, as reported in the EPA's Toxic Release Inventory (TRI), as the exposure for possible association with autism.[22, 23] In their 2006 manuscript, counts of autism cases in Texas by school district were obtained for the school years 2000-2001. Although autism diagnosis was not standardized, the cases were identified by special education professionals. County-level

industrial mercury releases (measured in pounds) were used in a multilevel Poisson regression to estimate counts of autism cases by school district. After adjusting for percent European American, percent poverty, district wealth, and measures of urbanicity, and number of children enrolled in a special education program, Palmer et al. found a significant association between pounds of mercury released from industry and autism rates. Then, in 2009, Palmer et al. identified autism cases from the 2002 school year in each school district through educational records. The investigators mapped 95 industrial facilities, including 39 coal-fired power plants, which reported mercury emissions to the EPA for the 1998 TRI. Within each school district, the investigators calculated the distance from the centroid to each mercury emitting industrial facility to determine a weighted distance. Using this method, each child in a given school district was assigned the same proximity to sources/pounds of emission exposure level. Palmer et al. found that pounds of mercury released were no longer significant in this adjusted model, but distance to a mercury emitting source was associated with autism status.

Kalkbrenner et al. conducted a case-control study in North Carolina and West Virginia in 2010.[24] The 383 autism cases and 2,829 randomly sampled language and speech impaired controls were both identified through the Autism and Developmental Disabilities Monitoring (ADDM) Network. Each child was linked to their birth certificate, and each birth residence was linked to census tract level NATA exposures (NATA, 1996). The investigators screened 35 hazardous air pollutants (HAPs) for possible association with autism as compared to language and speech impairment. After adjusting the Semi-Bayes models for race, maternal education, maternal age, maternal smoking during pregnancy, marital status, and census tract level median income and urbanicity, none of the HAPs, including the same 7 metals included in this study, reached statistical significance.

Roberts et al. investigated the association of air pollutant exposures in a cohort of women from the Nurses' Health Study II.[29] Mothers reported ASD diagnosis status for selection of the 325 autism cases and 22,098 controls to comprise the first study on the association between air pollution and ASD to have a national scope in the US. The study population was selected from children born to mothers in the Nurses' Health Study II between the years of 1987 and 2002. Depending on birth year, Roberts et al. assigned quintile levels of air pollution concentration by census tract from the 1990, 1996, 1999, or 2002 NATA assessments. Comparing the highest quintile of exposure to the lowest for both sexes, the investigators found significant associations between ASD and exposure to overall metals, lead, manganese, mercury, and nickel. These models were adjusted for individual and census tract level covariates as well as an indicator for NATA year model used. Robert's lead finding had a similar strength of association as the current study. Women living in census tracts with the third quintile (OR=1.6; 95% CI, 1.1-2.3), fourth quintile (OR=1.7; 95% CI, 1.1-2.4), and highest quintile (OR=1.6; 95% CI, 1.1-2.3) of lead exposure and birth all had significantly higher odds of having a child diagnosed with ASD than women living in the census tracts with the lowest lead exposure at birth.

In a recently published study, von Ehrenstein et al. found lead, but not chromium, to be associated with ASD in a cohort of children born in southern California between 1995 and 2006.[33] They built multi-pollutant models based on factor analysis of air toxics measured in Los Angeles County. The primary analysis was restricted to women living within 5 km of an air monitor. Lead was associated with ASD status (OR=1.49; 95% CI, 1.23-1.81) in a model containing 10 other pollutants as well as birth year, maternal race/ethnicity, maternal age and education, type of insurance, place of birth of mother, child sex, and parity.

There are limitations to this type of study. Although NATA is a sophisticated model that uses data from the National Emissions Inventory, traffic density, and meteorological information, it is not as accurate as measuring each woman's individual exposure to air pollutants. There was limited temporality to our exposure assessment as well. NATA 2005 estimates were used for all five years of birth (2005-2009) included in this study. Additionally, emissions inventories used for the modeling of NATA are self-reported by industrial sources. However, studies have found that the relative exposures of the HAPs reported are appropriate,[34] and this may be of greater relevance to this type of analysis. Birth certificate data is an unbiased method of exposure ascertainment. However, as there is no follow-up with the control group, we cannot be sure that all of the controls have no ASD symptoms or diagnosis. Additionally, birth certificates are not uniformly completed.[35] Although this would be a non-differential bias as mothers and doctors would have no way of knowing if the newborn will be diagnosed with ASD in the future. Another limitation in exposure assessment was that it was conducted at the residence of birth only. This method does not take into account any moving of residence or indeed, any traveling outside of the census tract while pregnant. Thus, although individual level variables were used to adjust each logistic model, the chemical exposures were ecological in nature as every woman who gave birth in a particular census tract was assigned the same exposure value.

3.6 CONCLUSIONS

This study adds to the growing body of work on the investigation into the association of ASD with air pollutants. Arsenic, chromium, and lead were found to have a significant association with ASD in this first analysis for the southwestern PA region. While this is the first study to

find a significant association between ASD and both arsenic and chromium, it is the third study to find a significant association between ASD and lead exposure. Two prior studies[21, 29] found a significant association between an additive metals score and ASD status, while we found an elevated but marginally significant association. Additional studies in varying geographical areas, with more precise exposure methods, must be conducted to verify and expand upon all previous findings.

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4.0 ARTICLE THREE: PROXIMITY TO CHROMIUM EMITTING INDUSTRIAL SITES AND THE RISK OF AUTISM SPECTRUM DISORDER (ASD) IN A SOUTHWESTERN PENNSYLVANIA CASE CONTROL STUDY

4.1 ABSTRACT

Background: Autism Spectrum Disorder (ASD) is a heterogeneous neurologic disorder. Several factors, including an increasing prevalence and an unknown etiology, make it a significant public health concern. Current studies suggest that ASD originates in the prenatal or early postnatal period. Emerging research suggests that air toxics that affect the immune system, such as chromium, are implicated in atypical neurologic development.

Methods: Yearly emissions of chromium as reported in the Toxic Release Inventory (TRI) were downloaded from the US EPA. Inverse Distance Weighting (IDW) was used to spatially interpolate yearly emissions of chromium in a six county area in southwestern Pennsylvania. Interpolated surfaces were then used to predict exposures for 198 singleton ASD cases and three nested control groups (n=217; n=4,060; n=4,785). Logistic regression was used to investigate the predicted exposure in univariable models, and in models adjusted for mother's age, race, education, and smoking status.

Results: There was no elevated or statistically significant association between ASD status and proximity to chromium emitting industrial sites. Cases did not have elevated estimated exposure to chromium when compared to any of the three control groups.

Conclusion: Although this analysis did not reveal and association between ASD and chromium exposure, exposure assessment is always a limitation in investigations into environmental contributions to disease. Since heavy metals such as chromium are biologically plausible to be a factor in the etiology of ASD, methodologies to more accurately assess exposure are warranted.

4.2 INTRODUCTION

Chromium is a heavy metal that most often occurs in one of three valence states: elemental chromium (oxidation state of 0), trivalent chromium (Cr(III)), or hexavalent chromium (Cr(VI)). Cr(III) is, in small amounts, an essential part of the human diet, however, Cr(VI) is more toxic to humans.[1] Although elemental chromium and Cr(III) are found naturally in the environment, most human exposure to chromium, and all exposure to Cr(VI), is a result of industrial emissions.[2] Chromium is widely used in metallurgical processes. Elemental chromium is used in steel making and in the manufacturing process of other metal alloys.[1] Cr(III) and Cr(VI) are used for most other industrial processes that involve chromium, including chrome plating, manufacture of dyes and pigments, and treatment of cooling tower water.[1] Chromium is also emitted into the air when fossil fuel is burned.[2] Therefore, chromium is released during electrical generation from fossil fuels and from traditionally powered vehicles.[3] Cigarette smoke is also a significant source of chromium exposure in the population.[2]

There has not been much research on the neurological effects of exposure to chromium, however, it is classified as a human carcinogen, affects the immune system, and has some indication of being detrimental to fetal development when exposed during pregnancy.[2] When Cr(VI) is inhaled, it is reduced to Cr(III) in the body. Immunological reactions to chromium are most likely induced by the reduction process.[4] The oxygen free radicals that are generated during reduction, activate genes involved in inflammation and immunity through activation of the transcription factor, NF-κB.[4] Environmental toxins that cause immune system dysregulation can lead to atypical neural development.[5] The immune system and the neurological system are highly inter-related, especially during the prenatal period.[6] This relationship means that a dysfunction in the immune system during critical time periods of pregnancy can lead to abnormalities in neurologic development.[6] Immune dysfunction in many ASD cases adds strength to this hypothesis.[6] There is emerging research that indicates a relationship between prenatal air pollution exposure, immune system responses and development, and nervous system development.[7]

Autism Spectrum Disorder (ASD) is a heterogeneous group of neurologic conditions that affect the way those with the disorder socialize and communicate with others. ASD was first recognized and described in medical literature in 1943,[8] but it has only more recently been widely studied. Once thought to be a disease of almost solely genetic origins, most recent studies attribute a significant portion of cases to environmental factors.[9, 10] Although many environmental risk factors have been investigated, including parental characteristics,[11, 12] prenatal conditions,[13, 14] obstetric complications,[15, 16] and extrinsic exposures,[17] causal factors for the disease are still unknown.

A preliminary study was conducted using data collected for the Case-Control Study of Personal and Environmental Risk Factors for Childhood Autism in Southwestern Pennsylvania.[18] This study found a significant association between ASD status and exposure to chromium, arsenic, lead, and styrene as estimated by the National Air Toxics Assessment (NATA). The NATA is conducted every three years. To attempt a better temporal estimate of exposure, a yearly inventory of industrial emissions will be used in this analysis. Chromium not only had a strong association with ASD status in this preliminary study (OR=1.65; 95% CI, 1.10-2.47), it also has the highest contribution to air exposure from industrial emissions. Therefore, this current investigation considers whether the association noted in our preliminary study is additionally associated with industrial sources of chromium pollution.

The US EPA Toxic Release Inventory (TRI) is a reporting program that requires certain industries to report chemicals that they use, produce, or emit on a yearly basis.[19] Industries in the manufacturing, utilities, mining, hazardous waste, and publishing sectors as well as some wholesalers are required to report their use and processing of 594 individual toxic chemicals. These reports differentiate between anything emitted to the air, nearby waterways, or shipped off of the site as hazardous waste. Facilities processing at least 25,000 pounds of any combination of these nearly 600 chemicals or using at least 10,000 pounds of the chemicals on site are required to submit yearly reports that are publicized on the EPA's website.

It is common in the epidemiological literature to assess health outcomes in relation to proximity of environmental exposures such as highways and industrial sites. In fact, ASD has been associated with proximity to highways,[20] pesticide application,[21, 22] and industrial emissions of mercury.[23, 24] The objective of this study is to explore the relationship between ASD status and proximity to industrial sources of chromium and chromium compounds. TRI reports for the years

2004 through 2009 will be used to estimate exposure to chromium air emissions for ASD cases and controls born between 2005 and 2009.

4.3 METHODS

4.3.1 Study population

Cases

Cases were singleton births from the Case-Control Study of Risk Factors for Childhood Autism in Southwest Pennsylvania. These children were born between 2005 and 2009 and resided since birth in one of six southwest Pennsylvania counties: Allegheny, Armstrong, Beaver, Butler, Washington, or Westmoreland. Cases had physician diagnosed ASD and were recruited from autism clinics and institutions and through early education and intervention programs. Although 199 singleton case mothers consented and were interviewed for the study, only 198 cases could be linked to their birth certificate for the purposes of this analysis. Twenty-six of these cases did not have latitude and longitude on their birth certificate, so address of birth was obtained from the interview. Three control groups were used in this analysis; Control #1 and Control #3 are subsets of Control #2.

Control #1

Controls (n=4,801) were randomly selected from the Pennsylvania Department of Health (PA DOH) birth registry and frequency matched on year of birth and gender to cases. Each of the controls was born in one of the same six southwestern PA counties during the same time period as the cases (2005-2009). Control births with missing latitude and longitude of birth on the birth

certificate were excluded from this control group (n=771). However, as thirty of these 771 was an interviewed control (from Control #3), data obtained from the interview was used for address of birth. Therefore, the final population for Control #1 was 4,060.

Control #2

The 741 control births with missing latitude and longitude of birth on their birth certificate who were not interviewed controls, included a zip code for the mother's residence at birth. ArcGIS 10.2.1 was used to calculate the geographic centroid of each zip code (using the 2010 ZCTA shapefile downloaded from the US Census). Approximately 98% of these births (725 of 741) were assigned the latitude and longitude of this centroid as their location of birth. The remaining 16 zip codes were not included in the 2010 or 2000 ZCTA shapefile. This method allowed for a larger, more inclusive control group (n=4,785).

Control #3

This control group consists of singleton births whose mothers responded to a mailed request from the research group at the University of Pittsburgh. Mothers who were eligible and consented to be included in the study (n=226) were asked questions from the same questionnaire as the case mothers. For this analysis, the population was restricted to singleton births, so Control #3 had 217 participants.

4.3.2 Birth certificate variables

All adjustment variables were extracted from the PA birth certificate for both cases and controls. Mother's age and father's age were examined as continuous variables. Low birth weight (< 2500 g), preterm birth (< 37 weeks of gestation), cesarean delivery, breech presentation, gestational diabetes, gestational hypertension, and first birth were included as dichotomous variables.

Smoking was examined in three categories: no smoking in the three months prior to pregnancy through the entire pregnancy, smoking in the three months prior to pregnancy and/or the first trimester only, and smoking at least into the second trimester of pregnancy. Race was defined from the mother's self-selected race as white, black, or other. Mother's education was categorized as less than a high school education, a high school diploma with or without some years of college, and an undergraduate degree or more.

4.3.3 Exposure assessment

TRI reports for the years 2004 through 2009 were downloaded from the US EPA website.[19] To reduce the occurrence and severity of edge effects, TRI sites in the 6 county study area, as well as TRI sites in adjacent counties were considered. Industries within this geographical range with any air emissions of "Chromium" or "Chromium Compounds" were included in the analysis. The TRI does not differentiate between Cr(III) and Cr(VI).

An inverse distance (1/distance) weighted (IDW) average of the total air emissions of chromium for the 10-15 closest TRI sites was calculated for each year between 2004 and 2009. This surface was then used to predict chromium exposure for each year at each residence of birth (cases and all controls). However, each birth only used two of these yearly exposure predictions to calculate the assigned exposure value. The average of the TRI chromium exposure predictions for the year of birth and the year prior to birth was used as the assigned exposure value for each birth. ArcGIS 10.2.1 was used for all IDW and exposure assignment procedures.

4.3.4 Statistical analysis

Logistic regression was used to investigate the association between IDW estimates of chromium exposure and ASD status. The assigned exposure value for each birth (cases and all controls) were explored in a univariable model and then the models were adjusted for variables that were significantly different between cases and all control groups. Odds ratios for all control groups were based on an increase of 100 pounds of chromium emissions for ease of interpretation and comparison between groups. StataSE 12 statistical software was used for all analyses.

4.4 RESULTS

ASD cases did not significantly differ from any of the three control groups by gender of the child, year of birth, low birth weight, preterm birth, breech presentation, gestational diabetes, or being first born (Table 11). All control groups were significantly different from the ASD case by mother's race, education, age, and smoking status. However, the control groups were not consistent in their differences. Mothers of cases were more likely to be white than Control #1 and Control #2 mothers, but less likely to be white than Control #3 mothers. Mothers of cases were more likely to have at least a college degree than Control #1 and Control #2 mothers, but less likely to have at least a college degree than Control #3 mothers. Mothers of ASD cases were significantly older than Control #1 and Control #2 mothers, but significantly younger than Control #3 mothers. Finally, mothers of cases were more likely to be non-smokers than Control #1 and Control #2 mothers, but less likely to be non-smokers that Control #3 mothers. Mother's county of residence was significantly different between cases and Control #1, but not between

cases and the other two control groups. Father's age, the likelihood of being born via cesarean, and likelihood of the mother having gestational hypertension are significantly different between cases and Control #1 and Control #2, but not between cases and Control #3.

Table 11. Demographic and risk factor characteristics of autism cases and control groups

Characteristic	Cases (n=198)	Control #1 (n=4,060)	Control #2 (n=4,785)	Control #3 (n=217)
Gender: Male child – n(%)	154 (77.8)	3,238 (79.8)	3,840 (80.3)	170 (78.3)
County at birth – n(%)		**		
Allegheny	120 (60.6)	2,480 (61.1)	2,778 (58.1)	128 (59.0)
Armstrong	7 (3.5)	68 (1.7)	145 (3.0)	2 (0.9)
Beaver	7 (3.5)	335 (8.3)	378 (7.9)	18 (8.3)
Butler	14 (7.1)	337 (8.3)	388 (8.1)	17 (7.8)
Washington	19 (9.6)	364 (9.0)	433 (9.0)	22 (10.1)
Westmoreland	31 (15.7)	476 (11.7)	663 (13.9)	30 (13.8)
Year of birth – n(%)				
2005	40 (20.2)	913 (22.5)	1,118 (23.4)	53 (24.4)
2006	52 (26.3)	957 (23.6)	1,106 (23.1)	41 (18.9)
2007	45 (22.7)	872 (21.5)	1,030 (21.5)	45 (20.7)
2008	33 (16.7)	730 (18.0)	848 (17.7)	37 (17.1)
2009	28 (14.1)	588 (14.5)	683 (14.3)	41 (18.9)
Mother's race – n(%)		**	**	**
White	178 (89.9)	3,299 (81.5)	3,897 (81.7)	212 (97.7)
Black	16 (8.1)	578 (14.3)	667 (14.0)	4 (1.8)
Other	4 (2.0)	169 (4.2)	203 (4.3)	1 (0.5)
		14 missing	18 missing	
Mother's education – n(%)		**	**	**
< High school graduate	6 (3.0)	386 (9.6)	461 (9.7)	3 (1.4)
High school graduate and some college	84 (42.4)	2,032 (50.3)	2,448 (51.4)	43 (19.8)
≥ College graduate	108 (54.5)	1,623 (40.2)	1,854 (38.9)	171 (78.8)
		19 missing	22 missing	
Mean (SD) maternal age	30.4 (5.3)	28.5 (6.0)**	28.4 (6.0)**	31.7 (4.7)**
			1 missing	
Mean (SD) paternal age	32.7 (5.9)	31.5 (6.6)**	31.4 (6.6)**	33.8 (6.0)
	13 missing	519 missing	614 missing	7 missing
Low birth weight – n(%) (< 2500g)	12 (6.1)	209 (5.2)	250 (5.2)	7 (3.2)
		11 missing	12 missing	
Preterm birth – n(%) (< 37 weeks)	20 (10.3)	351 (8.9)	413 (8.8)	15 (7.0)
	4 missing	95 missing	115 missing	4 missing
Cesarean Delivery – n(%)	75 (38.1)	1,120 (27.6)**	1,324 (32.9)**	64 (29.5)
	1 missing	2 missing	3 missing	
Smoking – n(%)		**	**	**
None	170 (86.3)	3,048 (76.5)	3,567 (76.0)	202 (93.1)
Pre and 1st trimester only	10 (5.1)	275 (6.9)	322 (6.9)	8 (3.7)
At least into 2nd trimester)	17 (8.6)	661 (16.6)	803 (17.1)	7 (3.2)
	1 missing	76 missing	93 missing	
Breech Presentation – n(%)	9 (4.5)	104 (2.6)	119 (2.5)	8 (3.7)
Gestational Diabetes – n(%)	11 (5.6)	133 (3.3)	166 (3.5)	5 (2.3)
Gestational Hypertension – n(%)	17 (8.6)	172 (4.2)**	203 (4.2)**	12 (5.5)
First Birth – n(%)	96 (48.5)	1,748 (43.2)	2,051 (43.0)	89 (41.2)
		13 missing	14 missing	1 missing

** significantly different from the case group at p≤0.05

Table 12 contains descriptions and amounts of the industrial sites that emitted chromium or chromium compounds into the air between the years of 2004 and 2009. Fossil fuel electric power generation, iron and steel mills, and steel foundries were the major sources of chromium emissions into the air in this geographical area. Together, these industrial sources accounted for more than half of the yearly total pounds emitted. Other sources of industrial emissions of chromium included steel and nonferrous metal finishing plants, dye, pigment, paint, and coating manufacturing, and glass manufacturing. Total number of pounds of emissions were highest in 2006 (73,937 pounds) and lowest in 2009 (41,690 pounds). Additionally, 2009 had the fewest total number of industrial sites that emitted chromium or chromium compounds into the air (n=87).

Table 12. Primary industrial sources in the TRI with air emissions of chromium

Year	Total number of sources	Total pounds of emissions	Fossil Fuel Electric Power Generation (NAICS code: 221112)		Iron and Steel Mills (NAICS code: 331111)		Steel Foundries (NAICS code: 331513)	
			Number of sources (% of total)	Pounds of emissions (% of total)	Number of sources (% of total)	Pounds of emissions (% of total)	Number of sources (% of total)	Pounds of emissions (% of total)
2004	96	50,418	18 (18.8)	12,829 (25.4)	13 (13.5)	21,875 (43.4)	9 (9.4)	5,651 (11.2)
2005	98	58,766	17 (17.3)	11,349 (19.3)	13 (13.3)	25,044 (42.6)	8 (8.2)	7,917 (13.5)
2006	93	73,937	17 (18.3)	12,393 (16.8)	13 (14.0)	39,397 (53.3)	8 (8.6)	7,981 (10.8)
2007	97	59,391	17 (17.5)	12,401 (20.9)	14 (14.4)	23,282 (39.2)	8 (8.2)	6,827 (11.5)
2008	88	60,851	13 (14.8)	10,718 (17.6)	15 (17.1)	22,115 (36.3)	7 (8.0)	7,253 (11.9)
2009	87	41,690	13 (14.9)	8,599 (20.6)	16 (18.4)	12,964 (31.1)	7 (8.1)	4,656 (11.2)

Figure 1 illustrates the IDW surfaces for 2004 through 2009. These surfaces were used to estimate exposure to chromium at each case and control birth residence.

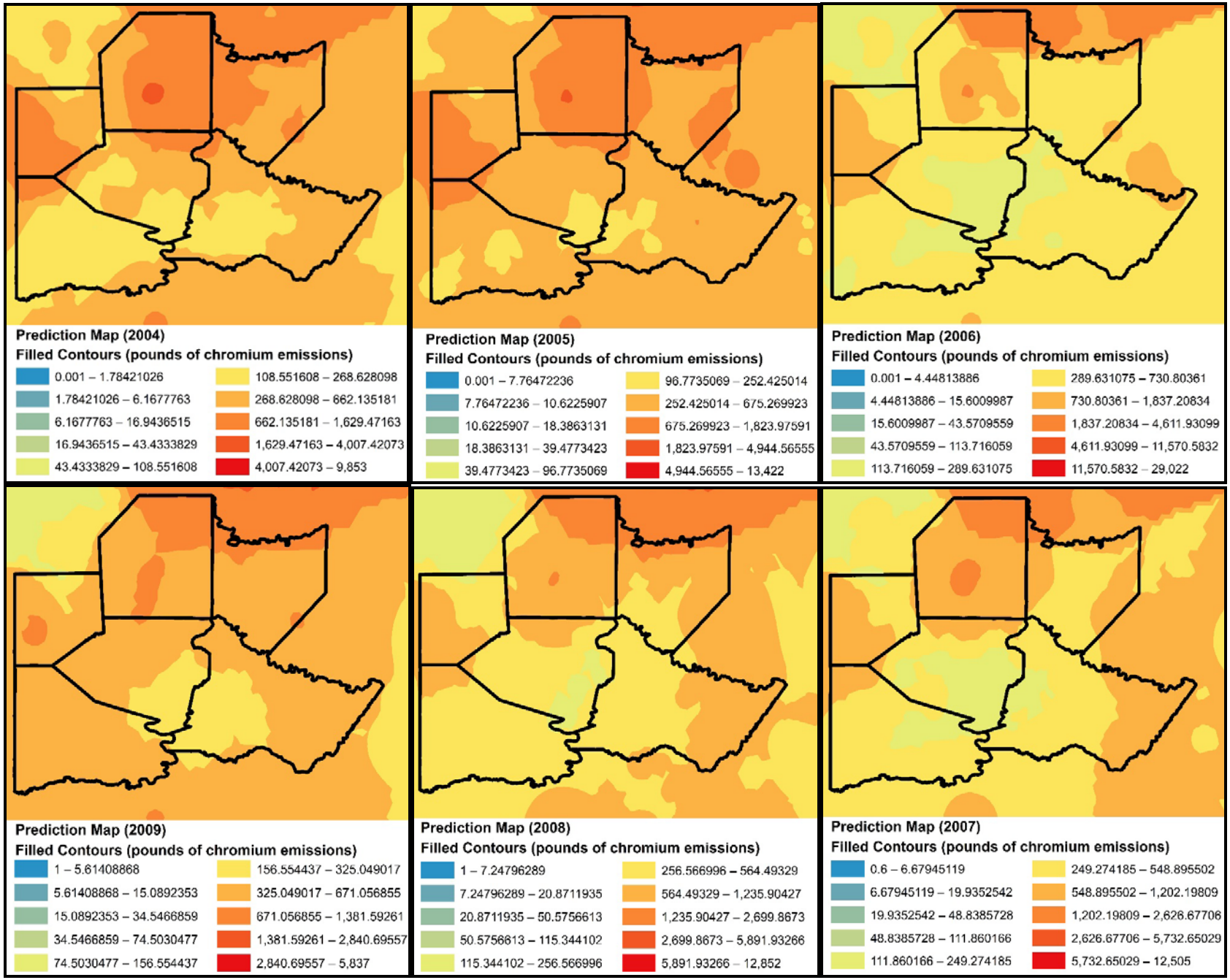

Figure 1. IDW prediction maps for exposure estimates of chromium (2004-2009)

Table 13 contains descriptive statistics of the assigned exposure variable. The comparison of exposure between the ASD cases and each of the three control groups illustrates that the cases have the lowest mean exposure of the four groups. However, Control #1 has the lowest median exposure of the four groups.

Table 13. Chromium exposure comparison between cases and controls

Group	Mean chromium exposure (pounds of air emissions)	Median chromium exposure (pounds of air emissions)	Standard deviation
Cases (n=198)	381.81	313.41	235.06
Control #1 (n=4,060)	394.12	312.07	253.27
Control #2 (n=4,785)	400.47	316.14	257.76
Control #3 (n=217)	396.05	321.76	224.04

Table 14 contains the odds ratios for an increase of 100 pounds of chromium emissions for cases versus each control group. None of the logistic regression results were elevated or statistically significant.

Table 14. Odds ratios for ASD status for an increase of 100 pounds of chromium emissions

Cases versus	Odds Ratio (95% CI)*	p-value*	Odds Ratio (95% CI)**	p-value**
Control #1	0.979 (0.922, 1.041)	0.503	0.968 (0.907, 1.032)	0.318
Control #2	0.969 (0.911, 1.031)	0.317	0.957 (0.896, 1.022)	0.187
Control #3	0.973 (0.894, 1.059)	0.528	0.983 (0.899, 1.075)	0.707

* univariable models
** models adjusted for mother's age, race, education, and smoking status

4.5 DISCUSSION

This analysis of the association between TRI measured chromium and ASD status had null results. Each of the three control groups had strengths and limitations. Control #1 used only

those birth residences that could be ascertained from the birth certificate, or were interviewed as a part of the Case-Control Study of Risk Factors for Childhood Autism in Southwest Pennsylvania. Although actual latitude and longitude of mother's residence at birth were used, this control group had a statistically different county of birth composition than the controls. As geographical location in relation to industrial plants was central to this analysis, this difference was most likely very important. Control #2 used all of the potential control births sent by the PA DOH, the geographical placement was more representative of all births in the area for the years 2005 to 2009. However, 725 of these control births were assigned the latitude and longitude of the centroid of their zip code. Again, as location is imperative to this analysis, this is not ideal. Control #3 consisted of a smaller group of interviewed participants. These women were distinct from the case group in different ways than the first two control groups were. The women in this control group were more likely to be older, more educated, and white than the women in the case group. These demographics suggest that these women are also less likely to live near industrial sites.[25]

There were TRI sites during the study years that emitted minimal chromium to the air, but were included in our exposure assessment. We performed an analysis omitting TRI sites that emitted less than 50 pounds in a particular year to investigate the validity of including these sites. Predictably, the mean and median IDW estimated chromium exposures for each study group (cases and the three control groups) were higher. Although this analysis resulted in slightly different odds ratios for each control group, the results were not elevated or significant.

Additional analyses were performed to determine if birth certificates without latitude and longitude of mother's address at birth were different than those containing that information. Women who lived in Armstrong county were the most likely to have missing locational

information, while women living in Allegheny county were the least likely. Over half (55.8%) of the women living in Armstrong county at the time of their delivery did not have latitude and longitude on their birth certificates, compared to 11.3% of the women in Allegheny county missing this same information during this time period.

A preliminary investigation using NATA estimates of exposure with this same study population found that women living in areas of the highest quartile of chromium exposure at birth had 1.78 times the odds (95% CI, 1.17-2.72) of having an ASD diagnosed child than women living in areas of the lowest quartile of chromium at birth after adjusting for age, race, education, and smoking status of mother, cesarean delivery, and gestational hypertension.[18] The NATA uses point, non-point, on road, non-road, and background estimates of air pollutants to model their total concentration estimates by census tract. The TRI listing includes what would be considered point sources only. Although chromium is estimated to originate primarily from point sources in the study area, important non-point and background sources and levels were not included in this analysis.

Although chromium has not been considered in a proximity analysis in the past, Volk et al.[15] studied ASD diagnosis and proximity to roadways. Chromium is one constituent in the complex mixture of vehicle exhaust.[3] Volk et al. published a manuscript from the Childhood Autism Risks from Genetics and the Environment (CHARGE) study on the relationship between traffic-related air pollution and autism in California.[20] The 2011 study consisted of 304 autism cases and 259 typically developing controls. Pre-school aged cases and controls were recruited from Southern California between 2003 and 2009. Controls were matched on age, sex, and general geographical area. Volk et al. calculated the distance from each residence to the nearest freeway or major road. After adjusting for sex, ethnicity, education of parent, maternal age, and

maternal smoking during pregnancy, children who lived within 309 meters of a freeway had 1.86 the odds (95% CI, 1.04-3.45) of having autism as children who lived further from a freeway.

Palmer et al. published two studies investigating the association between autism and another heavy metal using the same industrial release database as the current study.[23, 24] In their 2006 study,[23] counts of autism cases in Texas by school district were obtained for the school years 2000-2001. Although autism diagnosis was not standardized, the cases were identified by special education professionals. Pounds of industrial mercury releases at the county level, as reported in the EPAs TRI, were used in a multilevel Poisson regression to estimate counts of autism cases by school district. After adjusting for percent European American, percent poverty, district wealth, and measures of urbanicity, Palmer et al. found that for every 1000 pounds of mercury released from industry, autism rates increase by 61% (95% CI, 48.7%-75.2%).

In 2009, Palmer et al. conducted another study on the association of industrial mercury releases and autism counts in Texas school districts.[24] They identified autism cases for the 2002 school year in each school district through educational records. Palmer et al. mapped 95 industrial facilities, including 39 coal-fired power plants, which reported mercury emissions to the EPA for the 1998 TRI. Within each school district, the investigators calculated the distance from the centroid to each mercury emitting industrial facility to determine a weighted distance. Using this method, each child in a given school district was assigned the same proximity to sources/pounds of emission exposure level. Palmer et al. found that pounds of mercury released were no longer significant in this adjusted model. However, the Poisson model adjusted for 1997 autism rates, district wealth, measures of urbanicity, percent white, and pounds of mercury released, resulted in a 2% decrease in the rate of autism for every 10 miles farther away from the

source ($p<0.05$). This study suggests that distance from a mercury source is more indicative of autism risk than the amount of mercury emitted from the source.

Two studies that investigated the association of metals with ASD status using NATA estimates of exposure found associations with a combined metals score, but not with chromium individually. Windham et al. published one of the first studies to look at an association between air pollutants and autism.[26] The investigators used a case-control design with 284 autism cases and 657 randomly chosen matched controls from the same birth cohort of infants born in the San Francisco Bay Area in 1994. Autism cases were identified through active surveillance of existing medical records. A 2 to 1 ratio of controls to cases was used, and controls were matched on sex and month of birth. Cases and controls were linked to their birth certificate to ascertain the residence of birth. Using modeled concentrations of air toxics by census tract as their exposure measure (NATA, 1996), Windham et al. found that women who were exposed to the highest interquartile level of exposure to metals (OR=1.50; 95% CI, 1.05-2.12) had higher odds of having an autistic child when compared to women who gave birth in census tracts with the lowest interquartile levels of these substances. Arsenic, cadmium, chromium, lead, manganese, mercury, and nickel were included in the metals index. When the researchers further investigated these groups of pollutants, they found that cadmium (OR=1.54; 95% CI, 1.08-2.20), mercury (OR=1.92; 95% CI, 1.36-2.71), and nickel (OR=1.48; 95% CI, 1.04-2.06) in the metals group were significantly associated with autism diagnosis in adjusted logistic regression models.

Roberts et al. investigated the association of air pollutant exposures in a cohort of women from the Nurses' Health Study II.[27] Mothers reported ASD diagnosis status for selection of the 325 autism cases and 22,098 controls to comprise the first study on the association between air pollution and ASD to have a national scope in the US. The study population was selected from

children born to mothers in the Nurses' Health Study II between the years of 1987 and 2002. Depending on birth year, Roberts et al. assigned quintile levels of air pollution concentration by census tract from the 1990, 1996, 1999, or 2002 NATA assessments. Comparing the highest quintile of exposure to the lowest for both sexes, the investigators found a significant association between ASD and exposure to overall metals (OR=1.5; 95% CI, 1.0-2.3). Their metals index included antimony, arsenic, cadmium, chromium, lead, manganese, mercury, and nickel. The investigators also found that lead (OR=1.6; 95% CI, 1.1-2.3), manganese (OR=1.5; 95% CI, 1.1-2.2), mercury (OR=2.0; 95% CI, 1.2-3.3), and nickel (OR=1.7; 95% CI, 1.1-2.5) were individually associated with ASD status. These models were adjusted for individual and census tract level covariates as well as an indicator for NATA year model used.

Exposure assessment limitations were present in the current study, as well as in all of the studies mentioned above. The US EPA only monitors 6 air pollutants regularly (carbon monoxide, nitrogen oxides, sulfur dioxide, particulate matter, lead, and ozone), while other hazardous air pollutants are measured sporadically, if at all. This leaves the environmental health researcher with two options: specialized monitoring for the purposes of the health study or applying useful, but often inadequate national modes or emissions data. Chromium exposure comes from various sources, including vehicle emissions, and this study only considered industrial sources of chromium. Additionally, all exposures were assessed at residence of birth. This method does not account for any variation in exposure throughout the pregnancy period based on change of residence or significant periods of time spent away from home, which may result in misclassification of exposures.[28]

4.6 CONCLUSIONS

This analysis did not find an elevated or statistically significant association between chromium emitted to the air from TRI sites between 2004 and 2009 and ASD diagnosis. However, future studies into the association of ASD and heavy metals such as chromium are warranted. Although monitored levels of air toxics for exposure assessment are limited, they would add to the accuracy of future studies on association with ASD.

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5.0 CONCLUSIONS

This research is one of the first to investigate ASD in the southwestern PA region, and it adds to the literature on non-genetic risk factors for the disease. Additionally, it adds to the growing body of work on the investigation into the association of ASD with air pollutants. Independent associations were found with maternal, prenatal, and obstetric characteristics as well as with extrinsic exposures. Three different methods of exposure assessment were used in this study: parental and pregnancy factors extracted from the birth certificate, NATA estimated concentrations, and proximity to TRI industrial sites.

Maternal age, education, gestational hypertension, cesarean delivery, maternal infections, and being first born were associated with ASD status in the investigation using birth certificate risk factors only. Arsenic, chromium, and lead were found to have a significant association with ASD using NATA estimates of exposure. While this is the first study to find a significant association between ASD and both arsenic and chromium, it is the third study to find a significant association between ASD and lead exposure. However, an examination concentrating on TRI sources that emit chromium did not find an elevated or statistically significant association with ASD status.

Two of the non-genetic risk factors associated with ASD status in this study are also associated with inflammation, or more importantly, with the inflammatory response. Mothers of ASD cases had 2.17 the odds (95% CI, 1.11-4.27) of having at least one of six STD infections

during pregnancy than mothers of controls. This finding was independent of mother's age, race, education, and smoking status, cesarean delivery, and gestational hypertension. Additionally chromium is a known immune system toxicant. Women living in areas of the highest quartile of chromium exposure at the birth of their child had 1.78 times the odds (95% CI, 1.17-2.72) of having an ASD diagnosed child than women living in areas of the lowest quartile of chromium at the birth of their child after adjusting for age, race, education, and smoking status of mother, cesarean delivery, and gestational hypertension. These two risk factors were among the strongest associations found in this study. Emerging research suggests that the immune system and the neurological system are highly inter-related, especially during the prenatal period. This relationship means that a dysfunction in the immune system during pregnancy can lead to abnormalities in neurologic development.

Additional studies in varying geographical areas and with more precise exposure methods, must be conducted to verify and expand upon all previous findings. It would be advantageous to conduct a prospective study to evaluate the association of environmental factors and ASD. This would allow for more robust and unbiased exposure assessment. Although personal and monitored levels of air toxics are limited and expensive, they would add to the accuracy of future studies on association with ASD.

6.0 PUBLIC HEALTH SIGNIFICANCE

ASD is a neurodevelopmental disorder which is characterized by abnormalities in two categories - social interactions, including verbal and non-verbal communication and relationships, and repetitive and stereotypical actions or behaviors. People with ASD often have one or more comorbid conditions including epileptic seizures, severely impaired speech, increased brain size, and intellectual disability. However, the spectrum includes highly functioning cases as well as those that require life-long care.

ASD is a significant public health concern because it is increasing in prevalence and because its etiology is largely unknown. Surveillance data from the CDC funded Autism and Developmental Disabilities Monitoring (ADDM) Network indicates that autism prevalence increased from 6.7 per 10,000 (1 in 150) children born in 1992 to 14.7 per 10,000 (1 in 68) children born in 2002. This more than two-fold increase can partially be attributed to factors such as changes in diagnostic procedures and increased awareness. However, research also suggests an actual increase in ASD incidence over the past few decades.

Although ASD is recognized as having strong genetic links, current research also suggests non-genetic contributions to the disease. Research into etiology is not only imperative to elucidate causal mechanisms, but also to determine mutable risk factors. Ascertaining non-genetic risk factors for ASD could go a long way in preventing the continued growth in prevalence of ASD in the US and worldwide. This investigation adds to the small but growing

number of studies linking certain classes of air toxics (e.g. metals) and perinatal risk factors to the risk of ASD.

APPENDIX A: EXPLORATORY ANALYSES FOR ARTICLE ONE

A.1 RESULTS OF ANALYSIS OF MATERNAL SMOKING STRATIFIED BY RACE

Table 15 illustrates that white mothers and black mothers had a similar smoking frequency in this study.

Table 15. Frequency of smoking status by mother's race

Smoking status	White mothers n (%)	Black mothers n (%)
Non-smokers	3,048 (75.6)	499 (76.0)
Smoking: pre and 1st trimester only	304 (7.5)	27 (4.1)
Smoking: At least into 2nd trimester	682 (16.9)	131 (19.9)

Table 16 shows the association of maternal smoking stratified by race. Although there was a significant association between smoking status and ASD in the unadjusted logistic models for white mothers only, it was no longer significant after adjusting for mother's age and education, cesarean delivery, and gestational hypertension. There was some indication of elevated odds for ASD in black mothers who smoked, but this association was not statistically significant in either the unadjusted or adjusted models. As there were few black mothers in our case group (n=16), this is most likely due to small sample size.

Table 16. Association of maternal smoking and ASD, stratified by race

Mother's race and smoking status	Odds Ratio (95% CI)*	p-value*	Odds Ratio (95% CI)**	p-value**
White mothers				
Pre and 1st trimester only vs none	0.57 (0.29, 1.13)	< 0.001	0.69 (0.35, 1.39)	0.0645
At least into 2nd trimester vs none	0.36 (0.20, 0.64)		0.51 (0.28, 0.93)	
Black mothers				
Pre and 1st trimester only vs none	1.56 (0.20, 12.5)	0.908	1.97 (0.24, 16.36)	0.821
At least into 2nd trimester vs none	0.95 (0.26, 3.42)		1.07 (0.29, 4.00)	

* = univariable analysis

** = adjusted for age and education of mother, cesarean delivery, and gestational hypertension

A.2 EXPLORATORY ANALYSIS OF BIRTH WEIGHT, GESTATIONAL AGE, AND SMOKING STATUS USING ALTERNATE CUT-POINTS

Birth weight, gestational age, and smoking status of mother were not associated with ASD status in the primary analysis. These variables were analyzed further as this finding was unexpected.

A.2.1 Methods

Low birth weight (LBW) is defined as when an infant is less than 2500 grams at birth. As birth weight is an indicator of both infant morbidity and mortality, alternate cut-points have been investigated in the birth outcome literature. Very low birth weight (VLBW) and extremely low birth weight (ELBW) are defined as when an infant is less than 1500 grams and 1000 grams respectively. Logistic regression modelling was used to investigate the association between these more severe low birth weight categories and ASD status. Additionally, birth weight was explored as a continuous variable.

Preterm birth is defined as when an infant is born before 37 weeks of gestation. A shortened gestational period is also an indicator of both infant morbidity and mortality. Therefore, very preterm births (< 32 weeks of gestation) and extremely preterm births (≤ 25 weeks of gestation) were investigated for association with ASD status. Additionally, gestational age was explored as a continuous variable in logistic regression models.

In the primary analysis for Article One, maternal smoking was divided into three categories: no maternal smoking three months prior to pregnancy or during the entire pregnancy,

maternal smoking in the three months prior to pregnancy and/or during the first trimester only, and maternal smoking at least into the second trimester of pregnancy. This analysis also investigates maternal smoking as a dichotomous variable (any maternal smoking vs no maternal smoking) in logistic regression models.

A.2.2 Results

Table 17 contains frequencies and statistics for alternate categories for birth weight, gestational age, and smoking status. None of the birth weight or gestational age categories were statistically different between cases and controls. However, smoking status was significantly different between case mothers and control mothers. This difference can be seen whether smoking is investigated categorically or dichotomously.

Table 17. Alternate descriptive statistics of neonatal characteristics and smoking status

Variables	Case (n=198) n (%)	Control (n=4,801) n (%)	p-value	Number missing (cases/controls)
Birth Weight Categories				
Low birth weight (< 2500g)	12 (6.1)	250 (5.2)	0.604	0/12
Very low birth weight (< 1500g)	4 (2.0)	38 (0.8)	0.064	
Extremely low birth weight (< 1000g)	1 (0.5)	7 (0.1)	0.216	
Mean (SD) birth weight	3399.1 (629.7)	3362.5 (551.2)	0.363	
Gestational Age Categories				
Preterm birth (< 37 weeks)	20 (10.3)	413 (8.8)	0.473	4/115
Very preterm (< 32 weeks)	4 (2.1)	47 (1.0)	0.155	
Extremely preterm (≤ 25 weeks)	0 (0)	3 (0.06)	0.724	
Mean (SD) gestational age	38.7 (2.2)	38.6 (1.8)	0.685	
Smoking Categories				
Smoking (categorical) None Pre and 1st trimester only At least into 2nd trimester	 170 (86.3) 10 (5.1) 17 (8.6)	 3,574 (75.8) 324 (6.9) 814 (17.3)	0.003	1/89
Smoking (dichotomous) None Any	 170 (86.3) 27 (13.7)	 3,574 (75.8) 1,138 (24.2)	0.001	

Table 18 contains the odds ratios for select ASD risk factor alternate categories. The odds ratios are progressively larger for more severe categories of low birth weight. This can be seen in both the unadjusted models and in models adjusted for maternal age, race, education, and smoking status, and cesarean delivery and gestational hypertension. However, none of these associations reached statistical significance. In this population, smoking mothers are less likely to have children who are later diagnosed with ASD. Whether smoking status is investigated categorically or dichotomously, this risk factor is significant in unadjusted models, but not statistically significant in models adjusted for maternal age, race, education, and smoking status, and cesarean delivery and gestational hypertension.

Table 18. Odds ratios for risk factors by alternate categories (cases vs controls)

Risk Factor	Odds Ratio (95% CI)*	p-value*	Odds Ratio (95% CI)**	p-value**
Birth Weight Categories				
Low birth weight (< 2500g)	1.17 (0.64, 2.12)	0.604	1.21 (0.65, 2.24)	0.545
Very low birth weight (< 1500g)	2.58 (0.91, 7.29)	0.074	1.96 (0.66, 5.80)	0.224
Extremely low birth weight (< 1000g)	3.47 (0.42, 28.3)	0.246	2.42 (0.28, 21.0)	0.423
Birth weight (continuous)	1.00 (1.00, 1.00)	0.363	1.00 (1.00, 1.00)	0.931
Gestational Age Categories				
Preterm birth (< 37 weeks)	1.19 (0.74, 1.91)	0.473	1.10 (0.67, 1.81)	0.699
Very preterm (< 32 weeks)	2.08 (0.74, 5.83)	0.164	1.95 (0.68, 5.59)	0.216
Extremely preterm (≤ 25 weeks)	NA		NA	
Gestational age (continuous)	1.02 (0.94, 1.10)	0.684	1.04 (0.96, 1.13)	0.363
Smoking Categories				
Pre and 1st trimester only vs none	0.65 (0.34, 1.24)	0.003	0.77 (0.40, 1.48)	0.171
At least into 2nd trimester vs none	0.44 (0.27, 0.73)		0.61 (0.36, 1.05)	
Any vs none	0.50 (0.33, 0.75)	0.001	0.66 (0.43, 1.03)	0.069

* = univariable analysis

** = adjusted for age, race, smoking status, and education of mother, cesarean delivery, and gestational hypertension

APPENDIX B: CORRELATION ANALYSIS BETWEEN NATA AND TRI EXPOSURE ESTIMATES FOR CHROMIUM

This study found an association between NATA estimates of chromium exposure (by census tract of birth residence) and ASD status. However, it did not find an association between TRI estimates of chromium exposure (latitude and longitude of birth residence). In an effort to understand this dichotomy, both Pearson and Spearman correlations between the two exposure variables were calculated.

B.1 COMPARISON OF VARIABLES

The US EPA uses emissions estimates of point sources, non-point sources, on-road sources, and off-road sources, as well as background levels to model the concentration, exposure, and health risks of toxic air pollutants for its NATA database. Specific Aim #2 of this current study used the total concentration of seven individual metals (arsenic, cadmium, chromium, lead, manganese, mercury, and nickel) as the independent exposure variable. Specific Aim #3 of this current study created a spatial interpolation (IDW) of chromium emissions from the US TRI database as the independent exposure variable. The US EPA reports the total yearly emissions (in pounds) of toxic air pollutants from larger industrial sources for its TRI database. These larger industrial sources are analogous to the point sources in the NATA database. Therefore, it is hypothesized

that the TRI estimates of chromium exposure used in Specific Aim #3 and the NATA estimates of chromium exposure used in Specific Aim #3 are correlated. Additionally, the correlation between the TRI estimates of chromium exposure and the point source concentrations of chromium in the NATA database should be stronger.

B.2 RESULTS

The Pearson correlation tests indicate significant correlations for TRI, total chromium and chromium point concentrations (at $p<0.01$); although these correlation coefficients are not above 0.2. Somewhat perplexing is the negative correlation with the Spearman, which is a nonparametric test of correlation (Table 19).

Table 19. Correlation between yearly TRI and 2005 NATA estimates of chromium exposure

	TRI/IDW estimate	NATA total concentration	NATA point concentration
TRI/IDW estimate			
Pearson coefficient	1		
Spearman coefficient	1		
NATA total concentration			
Pearson coefficient	0.097*	1	
Spearman coefficient	-0.196*	1	
NATA point concentration			
Pearson coefficient	0.132*	0.996*	1
Spearman coefficient	0.005	0.834*	1

* = significant at the $p<0.01$ level

B.3 DISCUSSION

The correlation between NATA and TRI estimates of chromium was not strong, although they were significant. However, the temporal and spatial differences in the databases most likely explain this variation. NATA estimates are three year averages, where TRI emissions are reported yearly. This study used 2005 NATA estimates for all births, but used a spatially interpolated average of TRI emissions from the year of birth and the year prior to birth for each birth in the population. NATA estimates are also spatially averaged; the estimates are the same for an entire census tract. Our TRI estimates were point estimates determined from a spatial interpolation of chromium emissions. Perhaps most importantly, if these two exposure estimates were highly correlated, we would have seen an association between ASD status and TRI estimates of chromium exposure.

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