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Prenatal exposure to beta-2 adrenergic receptor agonists in relation to autism/autism spectrum disorder: a case-control study

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Abstract

Prenatal exposure to beta-2 adrenergic receptor agonists in relation to autism/autism spectrum disorder: a case-control study

By Konny Haywon Huh

Background

Beta-2 adrenergic receptor (B2AR) agonists are a class of drugs that are administered to mothers during pregnancy to treat various indications including preterm labor, asthma, allergy and respiratory infection. B2AR agonists are able to cross the placenta and blood-brain barrier of the fetus. Based on studies linking structural brain abnormalities to autism/autism spectrum disorder (AU/ASD), the in utero period has been hypothesized as an etiologically relevant risk period, and animal studies have shown an association between exposure to B2AR agonist drugs in the prenatal period and subsequent neurodevelopmental damage.

Methods

The **CH**ildhood **A**utism **R**isks from **G**enetics and the **E**nvironment (CHARGE) Study is a case-control study that enrolled 879 children and their caretakers from families with an index child with AU/ASD, with a developmental delay but not AU/ASD, or from the general population. AU/ASD diagnoses for the children were evaluated using the Autism Diagnostic Observation Schedules (ADOS) and the Autism Diagnostic Interview-revised (ADI-R). Maternal B2AR agonist drug use was based on a structured telephone interview with the mother. All analyses used conditional logistic regression controlling for maternal birth place and the matching factors: regional catchment center, child's sex and child's age at enrollment. Sampling fractions were used to weight the analyses to represent the general California population.

Results

Prenatal exposure to B2AR agonists is associated with a decreased odds of having a child diagnosed with AU/ASD when taken: *(1)* at any time during the pregnancy period (adjusted Odds Ratio (OR_{adj}) 0.53; 95% Confidence Interval (CI) 0.46 – 0.61) or *(2)* in the third trimester (OR_{adj} 0.49; 95% CI 0.40 – 0.61).

Conclusions

Although prior studies have reported B2AR agonist use during pregnancy increases the risk of AU/ASD, the results of this large study did not confirm these earlier findings.

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Chapter One

1. Introduction and Background

Autism/Autism Spectrum Disorder (AU/ASD)

Autism and autism spectrum disorder (AU/ASD) are neurodevelopmental disorders that are major global public health issues. Autism spectrum disorder is manifested by 3 years of age and entails a range of developmental disabilities characterized by atypical development in socialization, communication, and behavior (1).

Descriptive Epidemiology

AU/ASD affects approximately 1 in every 88 children in the United States and an estimated 1 in every 150 children living in developed countries worldwide (2, 3). Moreover, research has shown that the incidence of AU/ASD is increasing (4, 5).

Possible Risk Factors

Although the etiology of AU/ASD is unknown, several potential risk factors have been identified. Possible risk factors for AU/ASD include biologic factors such as male gender (4, 6, 7), advanced maternal and paternal age (6, 8-12), earlier and later birth order (8, 10, 11), pregnancy complications (8, 11, 13) including premature labor (14), maternal illness and stress (8, 11, 13-15); environmental factors such as medication (8), month of conception (16), maternal birth abroad (8, 17), residential proximity to highways (18); and genetic factors including increased signaling prevalence and transmission of genetic polymorphisms of the beta-2 adrenergic receptor (19-21). Given the variety of risk factors, it is widely believed that the etiology of AU/ASD likely consists of a combination of environmental, biologic, and

genetic factors. Advanced maternal age and male gender are two of the strongest and most consistent predictors of AU/ASD.

Maternal Age

Advanced maternal age has been found to be strongly associated with AU/ASD. A retrospective cohort study of approximately 3.5 million children born to mothers who were California residents from 1989 to 1994 observed an increased risk of autism among children with increasing maternal age (6). In children born to mothers who were between 30-34 years old and greater than 35 years old, the risk of autism was respectively 3.4 (95% CI 2.3 – 3.1) and 2.7 (95% CI 2.9 – 4.0) times that of mothers who were less than 20 years old after adjusting for child sex, birth weight, plurality, birth order, maternal race/ethnicity, maternal birth place, and maternal education.

In a more recent retrospective cohort study, the risk of autism increased 18% for every 5-year increase in maternal age, thus showing an incremental increase in odds of autism per 5-year age interval (adjusted OR 1.18, 95% CI 1.04 – 1.33)(12). A categorical analysis of the same data found the odds of giving birth to a child with AU/ASD among mothers who were older than 40 at the time of birth was 1.77 (95% CI 1.56 – 2.00) times that of mothers who were less than 25 years old at delivery after adjusting for parent's age, both parents race/ethnicity, parity, year of birth, insurance type, and sum of parental education. An Australian study that included children born between 1980 and 1995 also observed that advanced maternal age was associated with an elevated risk of child AU/ASD and that the risk increased incrementally across maternal age categories (11). When children diagnosed with AU/ASD (n = 465) were compared with control children (n = 1,794), children who

were born to mothers more than 34 years old at delivery had a 54% increased odds of having an autistic child compared with mothers who delivered their children at 25-29 years old (OR 1.54, 95% CI 1.04 – 2.30). Several large studies have reported strong and precise estimates of effect that support advanced maternal age as a risk factor for AU/ASD.

Sex

Male gender has also been shown to be strongly associated with AU/ASD. When a study of 3.5 million children born to California-resident mothers compared the risk of AU/ASD among male children to female children, boys had a risk of AU/ASD that was about 4 times that of girls after adjusting for birth weight, plurality, birth order, maternal age, maternal race/ethnicity, maternal birth place, and maternal education (Risk Ratio 4.3, 95% CI 3.9 – 4.6)(6). In an analysis of the National Health Interview Surveys that focused on trends in the prevalence of developmental disability in US children in a 12-year period from 1997-2008, the prevalence of developmental disabilities generally was about two times higher overall in males compared to females (18.0% vs. 9.5%), and about four times higher in males than females for specifically autism (0.7% vs. 0.2%)(4). Moreover, a Taiwanese study that assessed AU/ASD prevalence stratified by gender among children ages 3-17 using registry data found that from 2004 to 2010, the rate of autism in boys was about 6 times higher than girls each year (2004: Rate Ratio 5.75, 95% CI 5.26 – 6.29; 2010: Rate Ratio 6.06, 95% CI 5.69 – 6.47)(7).

Etiologically Relevant Window of Time

Maternal factors may expose the developing fetus to potentially neurodevelopmentally toxic substances *in utero*. The in utero period has been hypothesized as an etiologically relevant risk

period based on studies linking structural brain abnormalities to autism. These studies suggest that neurodevelopmental damage manifested physiologically by brain abnormalities may begin prenatally when the fetal brain is developing (22, 23).

2. Maternal Medications

Based on the hypothesis that the in utero period is the etiological risk period, maternal medications are important factors to consider as a variety of different medications with unknown or unclear levels of neurodevelopmental toxicity are being prescribed to mothers while their children are developing in utero. A recent systematic review and meta-analysis of epidemiological studies on prenatal exposures and AU/ASD reported a 46% increased risk of AU/ASD associated with exposure to medication taken during pregnancy (8). This finding is limited, however, due to its broad reference to all maternal medications. Beta-2 adrenergic receptor (B2AR) agonists are one class of drugs that may be prescribed during pregnancy that are of particular interest due to their widespread use to treat a variety of indications (e.g., preterm labor, respiratory infection, allergy, asthma).

B2AR agonists

B2AR agonists are used clinically to treat a range of indications including asthma and preterm labor. Asthma medications are prescribed during pregnancy as uncontrolled asthma can lead to poor birth outcomes (24). B2AR agonists are also used off-label to treat preterm labor by delaying or inhibiting labor contractions during the birth process. B2AR agonists perform smooth muscle relaxation via the sympathetic nerve pathway resulting in widening of blood vessels and relaxation of the bronchial tube, uterus, bladder, and gut (25). The sympathetic nerve pathway is part of the sympathetic nervous system that affects heart rate,

blood vessel constriction, and blood pressure. The effect of B2AR agonists on AU/ASD based on current research is debated (26-30). Terbutaline is a B2AR agonist drug commonly found in asthma medications that is most frequently administered off-label to treat preterm labor. In recent times, terbutaline has been studied more than other B2AR agonist drugs in relation to AU/ASD.

Biologic Plausibility

Terbutaline is able to cross the placenta and blood-brain barrier (31-33). Moreover, animal studies have shown that exposure to terbutaline during the prenatal or early life period is associated with considerable neurodevelopmental damage (34-36). Despite the biological plausibility and supporting evidence from animal studies, however, the prenatal neurotoxicity of B2AR agonists in humans remains unclear since very few studies have conducted research on this particular class of medication and its particular relation to a woman giving birth to offspring who are later diagnosed with a developmental delay.

Studies of Exposure to Terbutaline and AU/ASD

A case-control study investigating the association between in utero exposure to terbutaline (continuous exposure for 2 weeks or longer) and ASD among dizygotic twins found that continuous B2AR agonist exposure was associated with an elevated risk of ASD concordance (both twins diagnosed with ASD, 11 twins pairs) in comparison to ASD discordance (only one twin diagnosed with ASD, 24 twin pairs) (Risk Ratio 2.0; p-value 0.16)(19). Twin pairs without ASD diagnoses were excluded from the study. When pairs that included one female child or had additional siblings with ASD were excluded (16 twin pairs),

the relative risk was 4.4 comparing exposure to the B2AR agonist among concordant twin pairs and discordant twin pairs (p-value 0.04).

A case-control study reported that abnormal neurobehavioral test results were more frequent among 8 children with ASD who were exposed to terbutaline in utero compared to 163 children without ASD who were not exposed (37). The study did not include ASD children who were unexposed. All children received neurophysiological and neuropsychological tests. The ASD children who were exposed to terbutaline had an average of 6.8 abnormalities compared to an average of 0.9 abnormalities among non-ASD children who were unexposed. However, given that all the children exposed to terbutaline were diagnosed with ASD, it is expected that they would demonstrate more abnormal neurobehavioral characteristics than the control children without neurodevelopmental diagnoses. Further, the ASD children were not independent because they were birthed by only two mothers, and the number of ASD cases was small.

One of the largest studies to date on B2AR agonist exposure and ASD incidence is a case-control study conducted out of the Northern California region in which maternal B2AR agonist exposure during pregnancy (within the time frame of 30 days before conception through delivery) was obtained from the Kaiser Permanente Northern California pharmacy database (38). The study sample consisted of 575 women, 291 women who gave birth to at least one child diagnosed with ASD and 284 women who gave birth to children with normal development. Cases were frequency matched to controls on sex, birth year, and delivery hospital. While investigators found that there was no difference in having a child diagnosed with ASD among mothers who were administered any B2AR agonist during the pregnancy

period compared to mothers who were not (OR 1.2, 95% CI 0.7 – 2.0, adjusted for maternal education, maternal age, birth type, gestational age, parity, birth hospital, birth year, sex, asthma and preterm labor indications), investigators found an elevated but imprecise risk of ASD among mothers who were administered terbutaline during the third trimester for more than 2 days (OR 4.4, 95% CI 0.8 - 24.6, adjusted for the same variables). Similar results were found for exposure to terbutaline for 2 weeks or more during the third trimester (OR 4.3, 95% CI 0.5 - 38.5). The investigators concluded that exposure to terbutaline during the third trimester for more than 2 days may be associated with an increased risk of autism; however, the imprecise estimates preclude any definitive conclusion.

Studies with Other Developmental Disorder Outcomes

Other studies that have researched outcomes related to developmental delay but not specifically AU/ASD have shown elevated risk of psychopathology, poor school performance, decreased cognitive ability, and decreased motor function among children exposed to the B2AR agonists in utero compared to the unexposed children (39, 40). A longitudinal, prospective study out of Germany, which included 347 infants born between 1986 and 1988, measured the motor skills, cognitive ability and psychopathology of children at ages 2, 4.5 and 8 years old. Full term (not preterm) children who were exposed to B2AR agonist drugs in utero (n = 34) had a prevalence of psychiatric disorders that was about 2-3 times the prevalence among full term children who were not exposed (n = 159) across the three ages when the assessments were performed (% diagnosed with a psychiatric disorder among exposed vs. unexposed: at 2 years, 28% vs. 10%; 4.5 years, 45% vs. 22%; 8 years, 39% vs. 23%). When cognitive ability and motor skills were assessed, full term children who were exposed to B2AR agonist drugs in utero had decreased scores on psychometric tests of

cognitive development and motor skills tests that were significantly different from the scores of full term children who were unexposed (p-value 0.01 and p-value < 0.05, respectively). The same pattern of increased psychopathology and decreased cognitive ability and motor skills, however, was not exhibited among children who were born preterm (40).

In contrast, several studies did not find an increased risk of developmental delay among children who were exposed to B2AR agonist drugs in utero compared to children who were not (41-44). In a study conducted by The Canadian Preterm Labor Investigators Group, 708 women who were at risk of preterm delivery were assigned to receive ritodrine (n = 352), a B2AR agonist drug, or a placebo drug (n = 356)(41). A total of 771 children were born with 380 delivered by women who received ritodrine and 391 delivered by women who received the placebo. Among these children, 125 from the ritodrine group and 121 from the placebo group were randomly selected and evaluated at 18 months for their psychomotor and mental development using the Bayley Psychomotor Development Index and the Bayley Mental Development Index. The two groups observed similar results for both tests with the children from the ritodrine group demonstrating slightly higher scores (Average Psychomotor Development Index Score for Ritodrine Group 110.9±16.8 and Placebo 108.7±15.5; Average Mental Development Index Score for Ritodrine Group 100.3±18.3 and Placebo 95.1±18.8). Compared to the children who did not receive the B2AR agonist drug in utero, the children who were exposed to a B2AR agonist drug did not show an increased risk of developmental damage at 18 months old.

A French study also concluded that there was not an association between maternal exposure to ritodrine and developmental damage to the child (43). Exposed infants were born to

mothers who took ritodrine during pregnancy and unexposed infants were born to mothers who did not take ritodrine (n = 84). Exposed infants were matched to unexposed infants based on birth place, gestational age, sex, and birth weight. The mothers' mean duration of ritodrine use was 24 days (range 3 – 93 days). The study examined diseases of the central nervous system, psychiatric disorders as well as psychomotor development outcomes, and found that there was no difference between exposed and unexposed children at 1 – 2 years old. The psychomotor development assessment, which was evaluated according to the age a child achieved a particular milestone in psychomotor development, showed no meaningful difference between the two groups overall or for any individual milestone (Mean age in months, Able to stand unaided for one minute: Ritodrine 8.1±1.4, Control 7.8±1.6; Able to walk ten steps unaided: Ritodrine 14.3±3.5, Control 13.9±3.1; Able to use 3 or 4 isolated words: Ritodrine 18.3±4.4, Control 16.8±3.8; Able to say a phrase with 3 or 4 words: Ritodrine 26.2±4.2, Control 25.0±4.8).

Another study enrolled infants born to mothers taking ritodrine to treat preterm labor between 24 – 34 gestational weeks and infants born to mothers from the same hospital population matched on race, sex, gestational age, and birth weight (n = 40). This study reported no meaningful difference in development between the two groups of children who were evaluated at 7 – 9 years old (44). An assessment of visual-motor integration measured by the Koppitz visual-motor perceptual age score indicated similar results (Exposed VMI score 41.37±22.96; Unexposed VMI score 40.83±24.70). In addition to this test, neurologic evaluations of cranial nerves and of motor, sensory, and cerebellar function as well as psychometric evaluations of intellectual function, academic achievement, and personality were conducted and showed similar results for both groups.

Finally, a Swedish study of children born preterm to mothers treated with terbutaline intravenously until 4 hours before delivery reported a low incidence of abnormal neurodevelopmental conditions (n = 3 or 5.5%) among 53 children at 2 years old (42). The study did not have a comparison group.

3. Contribution

Existing research regarding the association between in utero exposure to B2AR agonist drugs and AU/ASD is inconsistent and imprecise. The **CH**ildhood **A**utism **R**isks from **G**enetics and the **E**nvironment (CHARGE) Study at the UC Davis M.I.N.D. Institute (Medical Investigation of Neurodevelopmental Disorders) is a large study of children and their caretakers. The CHARGE Study was established to assess which genes and environmental exposures are contributing factors to atypical patterns of development such as AU/ASD. The CHARGE data have several advantages over prior studies including being a large study (n = 879) and having data on a wide range of potential confounding variables. We will use the CHARGE Study to evaluate the effect of in utero exposure to B2AR agonist drugs on AU/ASD diagnosis. This research will contribute to the understanding of the potential neurodevelopmental toxicity of maternal medications on the developing fetus. Further, the findings will enable future mothers and their physicians to make more informed decisions about taking or prescribing drugs of the B2AR agonist class during the pregnancy period.

Chapter Two

AU/ASD is manifested by 3 years of age and entails a range of developmental disabilities characterized by atypical development in socialization, communication, and behavior (1). Currently, AU/ASD affects approximately 1 in every 88 children in the United States and an estimated 1 in every 150 children living in developed countries worldwide (2, 3). Further, research has shown that the incidence of AU/ASD is increasing (4, 5). While the cause of AU/ASD is unknown, it is widely believed that the etiology of AU/ASD likely consists of a combination of environmental, biologic, and genetic factors. Advanced maternal age (6, 8, 11, 12) and male gender (4, 6, 7) are two of the strongest and most consistent risk factors for AU/ASD.

The developing fetus may be affected by environmental factors to which the mother is exposed in a manner that is neurodevelopmentally toxic. The in utero period has been hypothesized as an etiologically relevant risk period for AU/ASD based on studies linking structural brain abnormalities to autism. These studies suggest that neurodevelopmental damage manifested physiologically by brain abnormalities may begin prenatally when the fetal brain is developing (22, 23). Maternal exposure to medication during pregnancy was associated with a 46% increased risk of AU/ASD according to a 2009 systematic review and meta-analysis of epidemiological studies on prenatal exposures (8). Due to its broad reference to all maternal medications, however, the findings are limited in applicability.

Beta-2 adrenergic receptor (B2AR) agonists are one class of drugs prescribed to mothers during pregnancy that are of particular interest due to their use to treat a variety of

indications (e.g., preterm labor, respiratory infection, allergy, asthma). B2AR agonists perform smooth muscle relaxation via the sympathetic nerve pathway resulting in widening of blood vessels and relaxation of the bronchial tube, uterus, bladder, and gut (25). Terbutaline is a B2AR agonist drug commonly found in asthma medications and is most frequently administered off-label to treat preterm labor. Terbutaline is able to cross the placenta and blood-brain barrier (31-33). Moreover, animal studies have shown that exposure to terbutaline during the prenatal or early life period is associated with considerable neurodevelopmental damage (34-36).

In recent times, terbutaline has been studied more than other B2AR agonist drugs in relation to AU/ASD. Three case-control studies have suggested an increased risk of AU/ASD or neurodevelopmental abnormalities among children who were exposed prenatally to terbutaline compared to children who were not exposed albeit all results were imprecise (19, 37, 38). Studies with outcomes related to developmental delay but not specifically AU/ASD have also shown elevated risk of psychopathology, poor school performance, decreased cognitive ability, and decreased motor function among children exposed to the B2AR agonists in utero compared to the unexposed children (39, 40). Several other studies, however, did not find an increased risk of developmental delay (41-44).

Despite the biological plausibility and supporting evidence from animal studies, the prenatal neurotoxicity of B2AR agonists in humans remains unclear. In addition to the small number of studies on this class of medication and AU/ASD, the results from these studies are inconsistent, imprecise, and difficult to interpret due to the nature of the study methodology. As a result, the effect of B2AR agonists on AU/ASD is debated and additional studies are

needed (26-30).

We will use data from the **CH**ildhood **A**utism **R**isks from **G**enetics and the **E**nvironment (CHARGE) Study at the UC Davis M.I.N.D. Institute (Medical Investigation of Neurodevelopmental Disorders) to evaluate the effect of in utero exposure to B2AR agonist drugs on AU/ASD diagnosis.

1. Methods

Study Design

The CHARGE Study was launched in 2002 to identify the causes and contributing factors for autism including a wide spectrum of biologic and chemical exposures, endogenous susceptibility factors, and their interactions.

The study uses a case-control design, enrolling families with an index child from one of three groups: children with autism, children with developmental delay but not autism, and children from the general population. All index children must fulfill the following requirements: (1) be between two and five years of age, (2) reside in the study catchment area, (3) be born in California, and (4) live with a biological parent who speaks either English or Spanish. Children from the general population are used as the control group and are frequency matched to children with autism or developmental delay based on age at enrollment, gender, and residential area.

Recruitment and Data Collection

Families with children in the autism/autism spectrum disorder (AU/ASD) and developmental delay (DD) groups are recruited primarily through the California Department

of Developmental Services (DDS) Regional Center System, other studies at the UC Davis M.I.N.D. (Medical Investigation of Neurodevelopmental Disorders) Institute, and general publicity. The families from the population-based control group are selected from State birth files by stratified random sampling, with probabilities aimed at representing the overall distribution of age, gender, and general geographic area of residence among the study's autistic children.

Informed consent is administered at the M.I.N.D. Institute by CHARGE Study staff. A complete psychometric and medical evaluation of the index children are performed at the M.I.N.D. Institute by trained CHARGE clinical assessment personnel who have attained research reliability on the diagnostic instruments. Children with AU/ASD and their primary caretakers are evaluated using the Autism Diagnostic Observation Schedules (ADOS) and the Autism Diagnostic Interview-revised (ADI-R), respectively, to confirm their diagnosis. The primary caretaker of each index child completed an interview including questions about medication use during pregnancy. B2AR agonist exposure was determined based on this information.

Potential sociodemographic and environmental confounders were assessed from State birth files and from an extensive telephone interview with the primary caretaker. For the telephone interview, the interviewers were kept blind to the case status of the family as much as possible. Further details about recruitment and data collection in the CHARGE Study have been previously published (45). All data were collected only after informed consent was obtained and all protocols were approved by the UC Davis School of Medicine and State of California Institutional Review Boards.

Data Analysis

Candidate confounders included maternal age, maternal birth place, maternal race and ethnicity, maternal education, maternal smoking and alcohol use, breast feeding duration, prenatal vitamin intake, parity, asthma during index pregnancy, maternal history of asthma, paternal age, paternal race and ethnicity, and paternal education. Those covariates showing associations with both B2AR agonist drugs and case status based on bivariate and Directed Acyclic Graph (DAG) analyses were considered for inclusion in the regression models.

In the final analyses, conditional logistic regression models were used to predict the odds of giving birth to a child diagnosed with AU/ASD relative to giving birth to a child with typical development as a function of prenatal exposure to B2AR agonist drugs controlling for maternal birth place and the matching factors: child's sex, child's age at enrollment, and area of residence. Based on survey research methods that aim for results that represent the study population, each participant was assigned a weight equal to the inverse of the estimated response probability within strata of case group and demographic factors (46).

2. Results

Population Characteristics

The descriptive characteristics of the study population are portrayed in Table 1 (n = 879). Children with AU/ASD were different from typically developing children on four primary factors: mother's birth place, prenatal vitamin intake, length of breast feeding, and maternal smoking.

The mothers of children with AU/ASD were more likely to be born outside of the United States compared to the mothers of typically developing children (p-value 0.03). Although there was no significant difference in terms of whether mothers breast fed their children, mothers of children with AU/ASD tended to breast feed for a shorter duration of time compared to mothers of typically developing children. Moreover, mothers of children with AU/ASD were more likely to smoke during pregnancy and less likely to take prenatal vitamins. Fathers of children with AU/ASD were less likely to be younger than 25 years at the time of birth (p-value 0.20).

The two most common indications for which women used B2AR agonists were asthma and preterm labor. Among the 61 women who were exposed to B2AR agonists, 72.1% used B2AR agonists to treat asthma indications (n = 44) and 23.0% to treat preterm labor indications (n = 14). Women used B2AR agonists to treat respiratory infections and allergies as well.

Women who were exposed to B2AR agonists at any time during the pregnancy period had a decreased odds of having a child diagnosed with AU/ASD (Table 2; Odds Ratio (OR) 0.53; 95% CI 0.46 – 0.61 adjusted for regional catchment area, child's sex, child's age at enrollment, and maternal birth place).

When analyses were conducted for B2AR exposure during each trimester, the odds of having a child diagnosed with AU/ASD was similar for women who were exposed to B2AR agonists in the first trimester or the second trimester compared to women who were never exposed. However, the odds of having a child diagnosed with AU/ASD for mothers who

were exposed to B2AR agonists in the third trimester was half the odds of unexposed mothers.

As an indirect assessment of the true duration of B2AR agonist exposure, analyses were also performed based on the number of different pregnancy months of prenatal B2AR agonist exposure (none, 1-2, or 3-9). Mothers who were exposed to B2AR agonist(s) in 1 or 2 different pregnancy months had a decreased odds of having a child diagnosed with AU/ASD (OR 0.25; 95% CI 0.22 – 0.29, adjusted for regional catchment area, child's sex, child's age at enrollment, and maternal birth place). On the other hand, mothers who were exposed in 3 to 9 different pregnancy months had a similar risk of having a child diagnosed with AU/ASD compared to unexposed mothers.

3. Discussion

This is the largest study to date evaluating the effect of prenatal exposure to B2AR agonist drugs on AU/ASD case status after birth. In this study, we found that prenatal exposure to B2AR agonists decreases the odds of having a child diagnosed with AU/ASD when taken: *(1)* at any time during the pregnancy period, *(2)* in the third trimester or *(3)* in only 1 or 2 different months during the pregnancy period. Prenatal exposure to B2AR agonists in each of these circumstances resulted in precise effect estimates. However, these results have not been seen in previous studies of B2AR agonists and AU/ASD.

Due to disparate study design and methods of analysis, a direct comparison of our results to the majority of previous studies is difficult. The Kaiser Permanente Northern California study is the most similar to ours because it evaluated the effect of all B2AR agonists in each

trimester as well as for the overall pregnancy period using a case-control study design (38). The Kaiser Permanente study is also the largest prior study (n = 575). In the Kaiser Permanente study, the odds of having a child diagnosed with ASD was slightly elevated among mothers who were exposed to B2AR agonists in the first trimester (OR 1.6, 95% CI 0.6 – 4.0, adjusted for maternal education, maternal age, birth type, gestational age, parity, birth hospital, birth year, sex, asthma and preterm labor indications). The estimate was imprecise, however, and they reported no difference between mothers who were exposed to B2AR agonists in the second trimester, third trimester, or when the exposure was dichotomized to ever during the pregnancy period versus never.

We also observed no difference in having a child diagnosed with AU/ASD between mothers who were exposed to B2AR agonists in the second trimester and those mothers who were not exposed during that time. Unlike the Kaiser Permanente study, however, we also found that there was no difference between mothers exposed or not exposed to B2AR agonists during the first trimester. Moreover, in contrast to the Kaiser Permanente study, we found a decreased odds of having a child diagnosed with AU/ASD among mothers who were administered a B2AR agonist any time during the pregnancy period. This association appeared to be driven by exposure during the third trimester. In contrast to the Kaiser study, our results suggest that prenatal exposure to B2AR agonists may not have damaging effects on the neurological development of a child.

We also found that prenatal exposure to B2AR agonists in 1 or 2 different pregnancy months was associated with a strongly reduced odds of having a child diagnosed with AU/ASD whereas exposure in 3 to 9 different pregnancy months only showed a slight

decrease. The intent of categorizing B2AR agonist exposure in this manner was to estimate the duration of B2AR agonist use by the mother. Therefore, based on these estimations, our results indicate that shorter duration of B2AR agonist use was associated with a reduced odds of having a child diagnosed with AU/ASD to a greater degree than longer duration of B2AR agonist use. However, we do not have a true measure of cumulative dose of exposure as individuals who took B2AR agonists twice during two different months are not distinguishable from individuals who took B2AR agonists daily for two months.

Analyses of specific types of B2AR agonists (e.g., terbutaline, albuterol, salmeterol, combination medications, etc.) were not performed due to small sample sizes that would have resulted in imprecise results. Focused analyses restricted to specific B2AR agonists are strongly encouraged for future studies that have sufficient sample sizes for different B2AR agonists. Although all B2AR agonists react with the same beta-2 adrenergic receptor on the human cell resulting in the same general physiologic response of smooth muscle relaxation, the particular substance(s) of each B2AR agonist may have unique neurodevelopmental effects on the developing fetus that would be obscured if B2AR agonists are only evaluated collectively.

Despite evidence from previous AU/ASD studies and animal studies showing neurodevelopmental damage after prenatal exposure to B2AR agonists, the results from our study show that prenatal exposure to B2AR agonists may not have an effect on the fetus in a way that leads to developmental conditions such as AU/ASD. As AU/ASD incidence continues to rise, exploring the role of prenatal exposure to B2AR agonists in relation to AU/ASD is important for several reasons. First, the in utero period may be the etiologically

relevant risk period for the child based on studies that suggest neurodevelopmental damage may start prenatally when the fetal brain is developing. Second, little is known about exposure to medications in general during pregnancy let alone any specific class of maternal medication. Lastly, B2AR agonists are prescribed to mothers during pregnancy to treat a variety of indications.

This study and the Kaiser study suggested that exposure to any B2AR agonist in the second trimester, third trimester, or during the pregnancy period overall is not harmful. However, the Kaiser study found that B2AR agonist exposure was harmful when administered during the first trimester while we found no effect in our study. A substantive conclusion about the relationship between prenatal B2AR agonist exposure and AU/ASD cannot be drawn from existing research because results are imprecise and inconsistent. Therefore, when recommending B2AR agonist medication use during pregnancy, it is important to weigh the indicators for treatment with B2AR agonists against the inconsistent results from studies on B2AR agonists and AU/ASD and other neurodevelopmental conditions.

References

1. *American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders*. 4th, Text Revision ed. Washington, DC: American Psychiatric Association; 2000.
2. Baio J. Surveillance Summaries: Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators. *Morbidity and Mortality Weekly Report (MMWR)*. Atlanta, GA: CDC National Center on Birth Defects and Developmental Disabilities, 2012:1-19.
3. Fombonne E. Epidemiology of pervasive developmental disorders. *Pediatr Res* 2009;65(6):591-8.
4. Boyle C, Boulet, S., et al. Trends in the prevalence of developmental disabilities in US children, 1997-2008. *Pediatrics* 2011;127(6):1034-42. Epub 2011 May 23.
5. Hertz-Picciotto I, Delwiche, L. The rise in autism and the role of age at diagnosis. *Epidemiology* 2009;20(1):84-90.
6. Croen LA, Grether, J.K et al. Descriptive epidemiology of autism in a California population: who is at risk? *J Autism Dev Disord* 2002;32(3):217-24.
7. Lai DC, Tseng YC, Hou YM, et al. Gender and geographic differences in the prevalence of autism spectrum disorders in children: Analysis of data from the national disability registry of Taiwan. *Res Dev Disabil* 2012;33(3):909-15.
8. Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. *Br J Psychiatry*. England, 2009:7-14.
9. Hultman CM, Sandin S, Levine SZ, et al. Advancing paternal age and risk of autism: new evidence from a population-based study and a meta-analysis of epidemiological studies. *Mol Psychiatry*, 2010.

10. Zhang X, Lv CC, Tian J, et al. Prenatal and perinatal risk factors for autism in China. *J Autism Dev Disord* 2010;40(11):1311-21.

11. Glasson EJ, Bower, C. et al. Perinatal factors and the development of autism: a population study. *Arch Gen Psychiatry* 2004;61(6):618-27.

12. Shelton JF, Tancredi DJ, Hertz-Picciotto I. Independent and dependent contributions of advanced maternal and paternal ages to autism risk. *Autism Res* 2010;3(1):30-9.

13. Lyall K, Pauls DL, Spiegelman D, et al. Pregnancy complications and obstetric suboptimality in association with autism spectrum disorders in children of the Nurses' Health Study II. *Autism Res* 2011.

14. Buchmayer S, Johansson S, Johansson A, et al. Can association between preterm birth and autism be explained by maternal or neonatal morbidity? *Pediatrics*. United States, 2009:e817-25.

15. Croen LA, Grether JK, Yoshida CK, et al. Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. *Arch Pediatr Adolesc Med*. United States, 2005:151-7.

16. Zerbo O, Iosif AM, Delwiche L, et al. Month of conception and risk of autism. *Epidemiology* 2011;22(4):469-75.

17. Lauritsen MB, Pedersen CB, Mortensen PB. Effects of familial risk factors and place of birth on the risk of autism: a nationwide register-based study. *J Child Psychol Psychiatry*. England, 2005:963-71.

18. Volk HE, Hertz-Picciotto I, Delwiche L, et al. Residential proximity to freeways and autism in the CHARGE study. *Environ Health Perspect* 2011;119(6):873-7.

19. Connors SL, Crowell DE, Eberhart CG, et al. beta2-adrenergic receptor activation and genetic polymorphisms in autism: data from dizygotic twins. *J Child Neurol* 2005;20(11):876-84.

20. Cheslack-Postava K, Fallin MD, Avramopoulos D, et al. beta2-Adrenergic receptor gene variants and risk for autism in the AGRE cohort. *Mol Psychiatry*. England, 2007:283-91.

21. Tassone F, Qi L, Zhang W, et al. MAOA, DBH, and SLC6A4 variants in CHARGE: a case-control study of autism spectrum disorders. *Autism Res* 2011;4(4):250-61.

22. Nickl-Jockschat T, Habel U, Maria Michel T, et al. Brain structure anomalies in autism spectrum disorder-a meta-analysis of VBM studies using anatomic likelihood estimation. *Hum Brain Mapp* 2011.

23. Courchesne E, Mouton PR, Calhoun ME, et al. Neuron number and size in prefrontal cortex of children with autism. *JAMA*. United States, 2011:2001-10.

24. Dombrowski MP, Schatz M. Asthma in pregnancy. *Clin Obstet Gynecol*. United States, 2010:301-10.

25. Morgan JG, MS M, MJ M. Chapter 12. Adrenergic Agonists & Antagonists. In: Morgan JG, MS M, MJ M, eds. *Clinical Anesthesiology*. New York: McGraw-Hill, 2006.

26. Witter FR, Zimmerman AW, Reichmann JP, et al. In utero beta 2 adrenergic agonist exposure and adverse neurophysiologic and behavioral outcomes. *Am J Obstet Gynecol*. United States, 2009:553-9.

27. Henry A, Kennedy D, Lowe S. In utero beta 2 adrenergic agonist exposure and adverse neurophysiologic and behavioral outcomes. *Am J Obstet Gynecol*. United States, 2010:e14; author reply e-5.

28. Rodier P, Miller RK, Brent RL. Does treatment of premature labor with terbutaline increase the risk of autism spectrum disorders? *Am J Obstet Gynecol*. United States, 2011:91-4.

29. Witter FR. Does treatment of premature labor with terbutaline increase the risk of autism spectrum disorders? *Am J Obstet Gynecol*, 2011.

30. Rodier PM, Miller RK, Brent RL. Reply. *American Journal of Obstetrics and Gynecology* 2011;205(2):e12-e3.

31. Bergman B, Bokstrom H, Borga O, et al. Transfer of terbutaline across the human placenta in late pregnancy. *Eur J Respir Dis Suppl* 1984;134:81-6.

32. Hsu CH, Robinson CP, Basmadjian GP. Tissue distribution of 3H-terbutaline in rabbits. *Life Sci* 1994;54(20):1465-9.

33. Slotkin TA, Tate CA, Cousins MM, et al. Beta-adrenoceptor signaling in the developing brain: sensitization or desensitization in response to terbutaline. *Brain Res Dev Brain Res*. Netherlands, 2001:113-25.

34. Rhodes MC, Seidler FJ, Abdel-Rahman A, et al. Terbutaline is a developmental neurotoxicant: effects on neuroproteins and morphology in cerebellum, hippocampus, and somatosensory cortex. *J Pharmacol Exp Ther*. United States, 2004:529-37.

35. Slotkin TA, Seidler FJ. Anomalous regulation of beta-adrenoceptor signaling in brain regions of the newborn rat. *Brain Res*. Netherlands, 2006:54-8.

36. Zerrate MC, Pletnikov M, Connors SL, et al. Neuroinflammation and behavioral abnormalities after neonatal terbutaline treatment in rats: implications for autism. *J Pharmacol Exp Ther*. United States, 2007:16-22.

37. Kilburn KH, Thrasher JD, Immers NB. Do terbutaline- and mold-associated impairments of the brain and lung relate to autism? *Toxicol Ind Health*. England, 2009:703-10.

38. Croen LA, Connors SL, Matevia M, et al. Prenatal exposure to beta2-adrenergic receptor agonists and risk of autism spectrum disorders. *J Neurodev Disord* 2011.

39. Hadders-Algra M, Touwen BC, Huisjes HJ. Long-term follow-up of children prenatally exposed to ritodrine. *Br J Obstet Gynaecol* 1986;93(2):156-61.

40. Pitzer M, Schmidt MH, Esser G, et al. Child development after maternal tocolysis with beta-sympathomimetic drugs. *Child Psychiatry Hum Dev* 2001;31(3):165-82.

41. Moutquin J. Treatment of preterm labor with the beta-adrenergic agonist ritodrine. The Canadian Preterm Labor Investigators Group. *N Engl J Med* 1992;327(5):308-12.

42. Svenningsen NW. Follow-up studies on preterm infants after maternal beta-receptor agonist treatment. *Acta Obstet Gynecol Scand Suppl* 1982;108:67-70.

43. Freysz H, Willard D, Lehr A, et al. A long term evaluation of infants who received a beta-mimetic drug while in utero. *J Perinat Med* 1977;5(2):94-9.

44. Polowczyk D, Tejani N, Lauersen N, et al. Evaluation of seven- to nine-year-old children exposed to ritodrine in utero. *Obstet Gynecol* 1984;64(4):485-8.

45. Hertz-Picciotto I, Croen LA, Hansen R, et al. The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism. *Environ Health Perspect* 2006;114(7):1119-25.

46. Kalton G, Piesse A. Survey research methods in evaluation and case-control studies. *Stat Med* 2007;26(8):1675-87.

Table 1 Characteristics of study sample (n = 879) by diagnostic status in the CHARGE Study, California, years 2003 - 2011

Characteristic Variables	AU/ASD n (%)	TD n (%)	p-value
B2AR agonist exposure			0.01
No	486 (94.9)	332 (90.5)	
Yes	26 (5.1)	35 (9.5)	
Child's gender			0.43
Female	75 (14.7)	61 (16.6)	
Male	437 (85.4)	306 (83.4)	
Regional Center catchment area			<.0001
Alta, Far Northern, Redwood Coast	182 (35.6)	162 (44.1)	
North Bay	71 (13.9)	61 (16.6)	
East Bay, San Andreas, Golden Gate	84 (16.4)	68 (18.5)	
Valley Mountain, Central Valley, Kern	90 (17.6)	56 (15.3)	
All LA RCs*, Orange, San Diego, Tri-counties, Inland, San Gabriel/Pomona	85 (16.6)	20 (5.5)	
Maternal age			0.27
15 - 24	75 (14.7)	65 (17.7)	
25 -34	295 (57.6)	215 (58.6)	
35+	142 (27.7)	87 (23.7)	
Maternal birth place			0.03
USA	388 (75.8)	300 (81.7)	
Non-USA	124 (24.2)	67 (18.3)	
Maternal race			0.80
White	432 (84.4)	312 (85.0)	
Other	80 (15.6)	55 (15.0)	
Maternal ethnicity			0.26
Mother non-Hispanic	385 (75.2)	288 (78.5)	
Mother Hispanic	127 (24.8)	79 (21.5)	
Maternal education			0.07
≤ High school	72 (14.1)	55 (15.0)	
Some College	206 (40.2)	118 (32.2)	
College degree	154 (30.1)	136 (37.1)	
Higher degree	80 (15.6)	58 (15.8)	
Maternal smoking			0.03
No	458 (90.3)	341 (94.5)	
Yes	49 (9.7)	20 (5.5)	
Missing	11		
Maternal alcohol intake			0.45
No	366 (72.3)	268 (74.7)	
Yes	140 (27.7)	91 (25.4)	
Missing	14		
Maternal breastfeeding			0.17
No	72 (15.3)	42 (11.9)	
Yes	400 (84.8)	311 (88.1)	
Missing	54		

Characteristic Variables	AU/ASD n (%)	TD n (%)	p-value
Prenatal vitamins			0.03
No	239 (47.8)	138 (40.0)	
Yes	261 (52.2)	207 (60.0)	
Missing	34		
Parity			0.14
1	237 (46.3)	156 (42.5)	
2	190 (37.1)	131 (35.7)	
3+	85 (16.6)	80 (21.8)	
Gestational age			0.95
Very preterm or preterm (25-36 weeks)	51 (10.1)	37 (10.3)	
Term or post-term (37+ weeks)	453 (89.9)	324 (89.8)	
Missing	14		
Preterm Labor			0.78
No	469 (92.3)	336 (91.8)	
Yes	39 (7.7)	30 (8.2)	
Missing	5		
Asthma during index pregnancy			0.71
No	231 (87.2)	254 (86.1)	
Yes	34 (12.8)	41 (13.9)	
Missing	319		
Maternal history of asthma			0.19
No	338 (81.3)	216 (77.1)	
Yes	78 (18.8)	64 (22.9)	
Missing	183		
Paternal age			0.20
15-24	44 (8.8)	182 (50.1)	
25-34	246 (49.3)	44 (12.1)	
35+	209 (41.9)	137 (37.7)	
Missing	17		
Paternal race			0.80
White	432 (84.4)	312 (85.0)	
Other	80 (15.6)	55 (15.0)	
Paternal ethnicity			0.83
Non-Hispanic	400 (78.1)	289 (78.8)	
Hispanic	112 (21.9)	78 (21.3)	
Paternal education			0.47
≤ High school	116 (22.8)	90 (24.5)	
Some College	158 (31.0)	109 (29.7)	
College degree	145 (28.4)	115 (31.3)	
Higher degree	91 (17.8)	53 (14.4)	
Missing	2		
Child's age, months, mean(SD)	44.12 (9.7)	42.83 (9.5)	0.05
Breastfeeding duration, months, mean(SD)	7.73 (7.5)	9.61 (8.2)	0.00

AU/ASD autism/autism spectrum disorder; *TD* typical development

*Los Angeles Regional Centers include Lanterman, Harbor, Westside, and Eastern, South Central, and North Los Angeles

Table 2 Odds ratios (ORs) and 95% confidence intervals (CIs) for prenatal exposure to B2AR agonists and AU/ASD in the CHARGE Study (n = 879), California, years 2003 - 2011

Exposure	No. (%)		Adjusted OR (95% CI)[b]	Adjusted OR (95% CI)[c]
	AU/ASD (n=512)	TD (n=367)		
Never[a]			1.00	1.00
Pregnancy period	26 (5.1)	35 (9.5)	0.51 (0.44 - 0.59)	0.53 (0.46 - 0.61)
Never[a]			1.00	1.00
First trimester[d]	14 (2.7)	15 (4.1)	1.10 (0.87 - 1.39)	1.17 (0.94 - 1.47)
Second trimester	21 (4.1)	23 (6.3)	1.21 (1.02 - 1.43)	1.19 (1.01 - 1.40)
Third trimester	20 (3.9)	22 (6.0)	0.48 (0.38 - 0.60)	0.49 (0.40 - 0.61)
None[a]			1.00	1.00
1-2 months[e]	9 (1.8)	21 (5.7)	0.24 (0.21 - 0.29)	0.25 (0.22 - 0.29)
3-9 months	17 (3.3)	14 (3.8)	0.91 (0.69 - 1.20)	0.96 (0.73 - 1.26)

OR odds ratio; *CI* confidence interval; *AU/ASD* autism/autism spectrum disorder; *TD* typical development

[a] Reference group had no exposure to any B2AR agonists during the pregnancy period

[b] Observations were weighted to account for key sociodemographic factors and adjusted for matching variables regional catchment area, child's sex and child's age at enrollment

[c] Observations were weighted and adjusted for the same variables above in addition to maternal birth place

[d] Model simultaneously adjusted for all 3 trimesters. 12 AU/ASD mothers and 9 TD mothers had B2AR agonist exposure during each trimester; 1 AU/ASD mother and 2 TD mothers had B2AR agonist exposure during trimester 1 and 2; 1 TD mother had B2AR agonist exposure during trimester 1 and 3; 4 AU/ASD mothers and 4 TD mothers had B2AR exposure during trimester 2 and 3

[e] Number of different month(s) of B2AR agonist exposure during the pregnancy period

Appendix A

EMORY UNIVERSITY

Institutional Review Board

TO: Konny Huh
Principal Investigator
Global Health

DATE: October 24, 2011

RE: **Expedited Approval**
IRB00054047
Prenatal exposure to β2-adrenergic agonists in relation to autism, autism spectrum disorder and developmental delay: a case-control study

Thank you for submitting a new application for this protocol. This research is eligible for expedited review under 45 CFR 46.110 and/or 21 CFR 56.110 because it poses minimal risk and fits the regulatory category F5 as set forth in the Federal Register. The Emory IRB reviewed it by expedited process on 10/24/2011 and granted approval effective from **10/24/2011** through **10/23/2012**. Thereafter, continuation of human subjects research activities requires the submission of a renewal application, which must be reviewed and approved by the IRB prior to the expiration date noted above. Please note carefully the following items with respect to this approval:

- A complete HIPAA/Consent waiver was granted

Any reportable events (e.g., unanticipated problems involving risk to subjects or others, noncompliance, breaches of confidentiality, HIPAA violations, protocol deviations) must be reported to the IRB according to our Policies & Procedures at www.irb.emory.edu, immediately, promptly, or periodically. Be sure to check the reporting guidance and contact us if you have questions. Terms and conditions of sponsors, if any, also apply to reporting.

Before implementing any change to this protocol (including but not limited to sample size, informed consent, study design, you must submit an amendment request and secure IRB approval.

In future correspondence about this matter, please refer to the IRB file ID, name of the Principal Investigator, and study title. Thank you

Andrea Goosen, MPH, CIP
Research Protocol Analyst
This letter has been digitally signed