The Effect of In Utero Exposure to Beta-2 Adrenergic Receptor Agonists and Selective Serotonin Reuptake Inhibitors on the Risk for Autism Spectrum Disorders

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Dedication

For Mom and Dad.

Thank you for instilling the importance of hard work.
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I would like to offer my gratitude to a number of individuals for their guidance and encouragement throughout my graduate career. My advisor, Dr. Craig Newschaffer, has been a mentor and an inspiration. A true scholar and exceptional teacher who made this experience a thoughtful and rewarding journey. Dr. Michael Yudell, for the patient guidance and mentorship he provided to me from when I was first a master student to the completion of this degree. Dr. Brian Lee, for the friendly guidance, thought provoking suggestions and encouragement, which were invaluable. I have learned so much from our conversations. Thank you Dr. Igor Burstyn for your insightful comments and constructive criticisms at different stages of my research. Without the support of Dr. Erik Mortensen in Denmark, this thesis would never have been possible. Thank you for your encouragement and making this idea a reality. I would also like to thank members of my dissertation committee -Drs. Yvonne Michael and Dani Fallin, for their help over the years as my ideas evolved to into a completed study. Every baby step I took was larger than life because I was standing on the shoulders of these giants.

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ABSTRACT
The Effect of In Utero Exposure to Beta-2 Adrenergic Receptor Agonists and Selective Serotonin Reuptake Inhibitors on the Risk for Autism Spectrum Disorders
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Objective: This dissertation explored associations between two specific classes of drug exposures, beta-2 adrenergic receptor (B2AR) agonists and selective serotonin reuptake inhibitors (SSRIs) and the risk for ASD.

Study Design: This project had two components: 1) a case-control study focused on estimation of exposure main effects from Denmark’s population-registers and 2) a candidate gene-environment interaction analysis using both exposure and genotype data from a large ongoing US autism case-control study.

Methods: From the Danish registers, one case per ten controls was individually matched on birth month and year. Conditional logistic regression was used to estimate unadjusted and adjusted drug exposure odds ratios (OR) and 95% confidence intervals (CI). Estimates were calculated in each drug class for any exposure, any exposure by preconception or trimester, dose, and duration. Special attention was paid to confounding by indication, as well as a sensitivity analysis for exposure and indicating condition misclassification. The gene-environment interaction analysis was based on unmatched case-control data and explored whether effects of in utero B2AR agonist drugs used during pregnancy are modified by polymorphisms in the ADRB2 gene (Gly16 and Glu27 polymorphisms). Logistic regression was used to estimate adjusted ORs and 95% CI.
Results: In parental age and child sex adjusted models we observed an increased risk for ASD associated with any in utero B2AR agonist drugs exposure (OR 1.3, 95% CI: 1.1-1.5). Similarly, any exposure to SSRIs during pregnancy was associated with ASD (OR 2.0 [95% CI, 2.0 [1.6-2.6]) compared to the unexposed reference group. Lastly, for the gene-environment interaction analysis we found some suggestion that the ASD risk associated with prenatal B2AR exposure may be modified by ADRB2 genotype; although estimates were imprecise.

Conclusion: The combination of low exposure prevalence during pregnancy and modest odds ratios we had observed for these two classes of drugs implies that the population attributable risks associated with both exposures will be fairly small. The possible increase in ASD risk from exposure to these medications must be weighed against effects of uncontrolled indications during pregnancy and the growing concern over the potential risk for the developing fetus and the mother as well as benefits of medication to mother and fetus despite possible ASD risk.
CHAPTER 1: OVERVIEW
OVERVIEW

Introduction

Autism spectrum disorders (ASD) are a group of developmental disabilities characterized by core deficits in three domains: social interaction, communication, and repetitive or stereotypic behavior [1]. Prenatal pharmacologic exposures are one of the few known environmental risks factors for ASD [2]. Specifically, three prescription medications have been identified as potential prenatal environmental risk factors for this neurodevelopmental disorder—thalidomide [3], antiepileptic drugs [4], and misoprostol [5]. Although these drugs are now rarely prescribed during pregnancy they provide proof of principal for associations between prenatal pharmacologic exposures and ASD. The fact that the teratogenic risks, especially with respect to neurodevelopmental outcomes, of many other drugs is still undetermined, it is important to consider further prenatal maternal prescription drug use as ASD risk factors. For women with certain chronic medical conditions such as epilepsy, diabetes, psoriasis, inflammatory bowel disease and asthma, the use of drugs is an important part of standard care [6]. However, since clinical trials during drug development commonly exclude pregnant women due to ethical reasons, there are often many open questions regarding the effects of drugs commonly used for chronic conditions on the developing human fetus even as these medications come into use in populations of pregnant women [7]. For example, a number of observational studies have found associations between medications that became commonly prescribed during pregnancy and birth defects [8-10].

Further, the potential for exposure to pregnant women is substantial. Data from the Baltimore-Washington Infant Group Study suggest that 68% of women used at least
one prescription or non-prescription drug during pregnancy [11]. The mean number of
drugs reported is 1.2; however evidence suggest that this might be underestimated due to
exclusion of some drug categories [11]. Two studies conducted in European populations
raise concerns regarding the potential harm in some prescription drugs given to pregnant
women. Olesen et al found from 1991 to 1996, 18% of pregnant women in Denmark
reported receiving at least one drug during pregnancy with “proven or anticipated
harmful fetal effects” [12]. A study in southwest France reported that 59% of the
women had a prescription for a drug that demonstrated fetal risk[13].

In this dissertation, two classes of drugs that have been commonly used over the
last two decades, beta-2-adrenergic receptor (B2AR) agonist drugs and selective
serotonin reuptake inhibitors (SSRIs), were investigated to explore a potential association
with ASD. Long-acting B2AR agonist drugs such as salmeterol and formoterol are used
to reduce asthma exacerbations and provide asthma control in adults [14]. Estimates of
the prevalence of B2AR agonist drug exposure during pregnancy range from 4%-20%
[15, 16] in the U.S. and 1.9-7.5% in Denmark [17, 18]. B2AR agonist drugs are thought
to have the potential to impact the fetal brain because they cross the placenta [19] and
have been shown in animal and in vitro studies to disrupt either replication or
differentiation of the developing neurons [20]. Selective serotonin reuptake inhibitors are
among the most commonly used medications, with the prevalence of prenatal prescription
use ranging from 3.8-5% in the US and Denmark [21-23]. SSRIs also can cross the
placenta [24]. Serotonin is known to play an important role in human brain development
by regulating both, serotoninergic outgrowth and maturation of target regions [25, 26].
Data from animal models suggest early exposure to SSRIs can disrupt the normal
maturation of the serotonin system however it is not known whether this effect of SSRIs is paralleled in humans [27].

**AIMS OF THIS DISSERTATION**

The aims of this dissertation are to evaluate the associations between two drugs commonly prescribed during pregnancy and ASD risk. In addition, for one of the drugs, an interaction between a known susceptibility genotype and the drug exposure-ASD risk relationship will be explored.

**Aim 1.** Estimate the main effects of two medications commonly used during the prenatal period on autism risk in a large population-based cohort

-Aim 1a. Estimate the effects of prenatal B2AR agonist exposure on ASD risk for any exposure and for exposure by trimester, dose and duration

-Aim 1b. Estimate the effects of prenatal SSRI exposure on ASD risk for any exposure and for exposure by trimester, dose and duration

**Aim 2.** Explore whether effect of exposure to B2AR is modified by maternal susceptibility genotypes in the ADRB2 gene.

**ORGANIZATION OF THE DISSERTATION**

This dissertation is comprised of a total of six chapters. Following the brief overview provided in this chapter is a more comprehensive review of the relevant literature in Chapter 2. Chapter 3 describes a large population based case-control study capitalizing on environmental data in a large epidemiological sample from Denmark’s health registers to provide detailed information regarding prescription drugs used during
pregnancy and autism diagnosis, as well as health and socioeconomic status by linking individuals’ unique civil-register number. The chapter investigates the association between maternal B2AR agonist drug use and ASD risk. This is then followed by Chapter 4, which uses the same study sample described in Chapter 3, to estimate the effect of prenatal SSRI use and risk associated with ASD. Chapter 5 investigates the possibility of gene-environment interaction between maternal B2AR agonist drug use during pregnancy and single nucleotide polymorphisms in the ADRB2 gene using a different sample from the US. The Study to Explore Early Development (SEED), a multisite investigation case-control study aimed at addressing knowledge gaps in autism phenotype and etiology. The sixth Chapter is a summary and discussion of the findings.

The dissertation was designed to investigate associations between two common classes of drug exposures still prescribed during pregnancy and the risk for ASD. It took advantage of the existence of both population based genetic and environmental data from two different epidemiological samples to investigate their effect on this developmental disorder. Results from this dissertation add to the limited amount of epidemiologic evidence on pharmacologic ASD risk factors, particularly exposures during pregnancy. It will help target further research on maternal pharmacological exposures as well as susceptibility genotypes.
References


CHAPTER 2: A REVIEW OF THE LITERATURE
Autism Spectrum Disorders

Autism spectrum disorders (ASD) are a group of developmental disabilities characterized by core deficits in three domains: social interaction, communication, and repetitive or stereotypic behavior [1]. There is variability in level of disability among individuals with ASD, and it is a much more common disorder than previously believed. The disorder is largely of unknown cause, and there is variability of impairment among individuals with ASD [2]. Currently, ASD has a US prevalence of 11.3 cases per 1,000, and a prevalence of seven cases per 1,000 in Denmark [3-5]. There has been strong evidence of a heritable component in ASD etiology however no definitive gene has been identified [6-10]. Molecular genetics of ASD are complicated by a combination of phenotypic heterogeneity [11], the possibility of multiple interacting loci [12] and gene-environment interaction [13]. Initial early linkage studies found only a few genes associated with ASD, but with technical advancements such as the development of DNA microarrays to measure the expression levels of large numbers of genes simultaneously and cytogenetics, more recent studies have focused on genome wide association (GWA) and copy number variation (CNV). In addition, studies using micro-array-based comparative genomic hybridization (array-CGH) have showed that the genomes of unrelated healthy individuals vary significantly with respect to the number of copies an individual has of each DNA segment [14]. Small cytogenic abnormalities and overall rate of copy number variants (deletion, insertion, duplication or a complex multisite variant that can be inherited or may arise de novo on a paternally or maternally inherited chromosome) are increased in individuals with ASD compared to controls [15].
In addition to the complexities in identifying the genetic influence of ASD, identifying environmental risk factors are also largely unknown. Prenatal pharmacologic exposures are one of the few known environmental risks factors for ASD [16]. Specifically, three prescription medications have been identified as potential prenatal environmental risk factors for ASD - thalidomide, antiepileptic drugs, and misoprostol. Synthesized in the 1950s, thalidomide was used as a potent sedative which was withdrawn from the market in 1961 after it was learned that children were being born with serious malformations associated with prenatal exposure [17]. A study of 100 Swedish thalidomide embryopathy cases, found at least four cases that met full criteria for DSMIII-R autistic disorder and ICD-10 childhood autism, indicating that there was a possible association of thalidomide embryopathy with ASD [17]. Antiepileptic drugs such as carbamazepine monotherapy, valproate monotherapy, phenytoin monotherapy, and polytherapy used during pregnancy have also been suggested as ASD risk factors [18-21]. Mothers taking antiepileptic drugs are also more likely to have a child with developmental delays than in children of epileptic mothers who were unexposed [22]. Lastly, misoprostol has been used in the treatment of gastric ulcers and also used in abortions since it causes uterine contractions. In a case series study of 23 patients with Möbius sequence, five children met criteria for autism, three of which had a positive history of misoprostol exposure during the first trimester of pregnancy [23].

Although the above mentioned drugs are now considered a rare prenatal exposure, given the suggested association between prenatal pharmacologic exposures with ASD and the fact that the teratogenic risks of most other drugs is still undetermined, especially with respect to neurodevelopmental outcomes [24], it is important to consider further
prescription drug use as ASD risk factors. For women with certain chronic medical conditions such as epilepsy, diabetes, inflammatory bowel disease and asthma, the use of drugs is essential, and it is commonly believed that the benefits for mother and child will outweigh risks, and exposure to a range of medications in pregnancy continues today. Since clinical trials during drug development commonly exclude pregnant women due to ethical reasons, there have been many questions regarding the effects of the drug on the developing human fetus. Several observational studies have found associations between many commonly used prescription drugs and birth defects [25-27]. Two classes of drug exposures that have been commonly used over the last two decades, B2AR agonists and SSRIs, were investigated in this dissertation to explore a potential association with ASD. The table below described the three epidemiological findings thus far.
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**Beta-2-adrenergic receptor agonist**

The prevalence of asthma among pregnant and all childbearing-aged women from the US national health surveys indicate that it is currently between 3.7% - 8.4% [28]. In Denmark the prevalence of asthma increased from 5.3% in 1986 to 11.7% in 2001 [29]. Long-acting B2AR agonist drugs such as terbutaline have been used to reduce asthma...
exacerbations and provide asthma control in adults [30]. In addition, these drugs have been also commonly used off-label as a tocolytic agent since they appear to have an immediate and comparable profound effect on uterine activity in term labor to stop early contractions [31]. The prevalence of terbutaline exposure during pregnancy in the US is estimated to be between 4%-20% [32, 33]. In Denmark, 1.9-7.5% of women received prescriptions for asthma medication during pregnancy [34, 35].

Beta-2 adrenergic receptors play an important role in the pathophysiology of asthma. In regards to the treatment of asthma, beta-2 adrenergic receptor agonist drugs relax the muscles surrounding the airways to relieve symptoms. In mature adults, these receptors are important for pulmonary and cardiovascular physiology and are activated by hormones such as adrenaline and noradrenaline [36]. The binding of these hormones to the receptor plays a critical role in the sympathetic nervous system, resulting in the fight or flight response [36]. Once the hormones bind to the receptor, a downstream signaling molecular response leads to the various stress responses such as accelerated heart rate, inhibition of stomach and upper-intestinal action, constriction of blood vessels in many parts of the body, dilation of blood vessels for muscles, and dilation of pupil (mydriasis) to name a few [37]. In the developing fetus, these receptors have a much different role, since they are involved in tissue differentiation and axonal growth [38]. Transcription, which is the first step leading to gene expression, is down regulated for beta 2 adrenergic receptors, preventing cells from being over stimulated and maintaining the balance between sympathetic and parasympathetic nervous system [38].

Since B2AR agonist drugs, such as tocolytics like terbutaline and asthma medications mentioned previously can cross the placenta, they have the potential to
impact the fetal brain by reaching and directly binding to fetal beta 2 adrenergic receptors. This can result in the disruption of either replication or differentiation of the developing neurons [39]. Therefore, aberrations within this system could result in an imbalance of natural cell proliferation and differentiation during gestation when the brain is developing [40]. Exposures during pregnancy that increase beta 2 adrenergic receptor signaling or over stimulate the receptor could have wide spread effects in light of the function of these receptors during pre- and postnatal life [41]. However, the observed main effects are subtle in pregnancies exposed to B2AR agonist drugs, which indicate the possibility for gene-environment interaction effects.

Animal studies

Animal models have been developed to understand the neurodevelopmental effects of B2AR agonist drugs and results from this work appear relevant to ASD pathogenesis [42, 43]. It has been shown that during gestation, overstimulation of the beta 2- adrenergic receptors from an exogenous source during the development of the nervous system may have harmful effects on the development of the brain and the peripheral nervous system [42, 44]. The mechanisms within the mature beta 2-adrenergic receptors protect against over-stimulation; however, fetal receptors do not have the ability to regulate any imbalance and may in fact become sensitized [45]. Early research has shown that B2AR agonist drugs administered to pregnant rats during the second trimester of gestation alters neural cell replication and differentiation, synaptogenesis, and expression of synaptic proteins involved in neurotransmission [45]. These animal model experiments also found abnormalities within the central and
peripheral nervous system and a decrease in the cell numbers in the fetal brain and liver of rats that were exposed prenatally [42]. The effect on the rats is gender selective with males experiencing more abnormalities, which is analogous to the male predominance of ASD in humans [42].

**Human studies of B2AR agonist drugs and autism risk**

Human studies of B2AR agonist drugs and ASD or other neurodevelopmental disorders are limited. In a study investigating the effects of prenatal overestimation of the beta 2 adrenergic receptors in dizygotic twins who were exposed to terbutaline to treat premature labor, there was an increased concordance for ASD in dizygotic twins than among similar unexposed twins [43]. In addition, this study found a further increase in the risk for male twins with no other affected siblings [43]. A recent, study looking at the association between maternal exposure to B2AR agonist drugs during pregnancy and ASD risk using 291 cases and 284 controls found that exposure for more than two days during the third trimester was associated with a six fold increased risk for ASD independent of the indication (adjusted odds ratio (OR) = 6.01, 95% confidence interval (CI) 1.05-34.28), although the confidence intervals were wide [46]. Along with considering B2AR agonist drugs as a tocolytic agent, this study also considered B2AR agonist drugs prescribed for other purposes (e.g., asthma control) and B2AR- mimics which also impact cellular cAMP levels (e.g., inhaled steroids, steroids administered through other routes, theophylline and the tocolytic magnesium sulfate). The analysis including all agonists and mimics showed exposure in the 30 days before conception to be associated with three times higher ASD risk (adjusted OR 2.98, 95% CI 1.01-8.83)
B2AR mimics were defined as drugs that impact cellular cAMP levels, including inhaled steroids, steroids administered through other routes, theophylline and the tocolytic magnesium sulfate [46]. As mentioned, the effect estimates for specific exposure windows and doses in this investigation were statistically imprecise and the mechanism behind the pre-conception exposure has not been described.

Effect of indicating condition (asthma) on autism risk

Among mothers who are severe asthmatics, avoidance of asthma treatment during pregnancy has been linked to birth complications including preterm birth, low birth weight, preeclampsia, and neonatal death [28, 48]. Since uncontrolled asthma has been associated with poor birth outcomes, mothers are often still recommended to continue asthma medication [32, 49]. However, there is a high frequency of preterm labor and intrauterine growth restriction in corticosteroid-dependent asthmatic patients [49-51]. Infants with a gestational age at birth that is less than 35 weeks have shown to have more than two-fold increase risk for ASD [52]. Only one study has reported an association between maternal asthma and autism risk showing a more than a two-fold elevated risk of ASD associated with maternal asthma during the first (OR=2.8; 95% CI, 1.3-6.10) and second (OR=2.2; 95% CI, 1.1-4.2) trimester of pregnancy [53]. However, this study was not able to explore the independent role of indicating condition and medication exposure.

Genetic susceptibility

Polymorphisms in the beta 2-adrenergic receptor gene (ADBR2) have been shown to influence beta 2-adrenergic receptor sensitivity and consequently may enhance
susceptibility to the neurodevelopment effects of B2AR agonist drug exposure [54]. The mechanism of the genetic susceptibility that over stimulate the beta 2-adrenergic receptor may increase the risk for neurodevelopment impairment [42]. Located on chromosome 5q31-32, there are three polymorphisms, Arg16Gly, Gln27Glu, and Thr164Ile, which are common in the general population and influence receptor function [54]. In vivo studies to determine the responsiveness of the receptor have been investigated through forearm blood flow and hand vein dilation [55]. Muscle vasodilatation during mental stress and exercise appear to be higher in women who were homozygous Gly(16)/Glu(27) of the beta 2-adrenergic receptors [56]. Therefore, presence of these alleles indicates that there may be an increase in responsiveness and sensitivity of the beta 2-adrenergic receptor. A study using of 331 autism case parent trios from the Autism Genetic Resource Exchange (AGRE) found a Glu27 homozygous genotype to be associate with increased autism risk, while Gly16 did not reach statistical significance [57]. Exogenous B2AR agonist drug exposure in conjunction with the presence of the genetic polymorphisms may increase beta 2-adrenergic receptor activity and affect neurodevelopment.

**Selective serotonin reuptake inhibitors**

Selective serotonin reuptake inhibitors (SSRIs), such as many antidepressants, are among the most commonly used medications, with a prescription frequency of 5-13% in pregnant women in the US [58, 59]. In Denmark 10-12.3% of pregnant women received at least one prescription for an SSRI a year before delivery [60]. The extent of use of SSRIs before and during pregnancy and its trend over the years in the Netherlands indicated that the prescription rate for SSRIs was highest in the trimester before
conception, decreased in the first trimester and further in the second trimester, which was comparable to the third trimester [60]. SSRIs can potentially influence the intrauterine environment because it is known to cross the placenta [61].

There have been inconsistencies in early reports of SSRI treatment during pregnancy [62], however in 2005 the Food and Drug Administration (FDA) issued an advisory indicating that early exposure to paroxetine may increase the risk for cardiac defects (http://www.fda.gov/CDER/Drug/advisory/paroxetine200512.htm). Moreover, several studies have looked at the possible association between exposures to SSRIs in late pregnancy, and have reported an increased risk of persistent pulmonary hypertension in the newborns [63]. These resulted in either inconsistent findings ranging from no association to a six-fold increased risk [26, 64]. In a recent prospective study to determine the exposure prevalence of SSRI treatment in pregnant women found that over 20% of the pregnant women with major depressive disorder who were using SSRIs delivered a preterm infant [65]. There were 5% of women who had depression during their pregnancy and did not receive an SSRI [65]. Preterm labor has been known as a potential risk factor for ASD [52]. In addition, a recent study reports an odds ratio of 2.0 (95% CI, 1.3-3.2) for preterm birth in the women exposed to SSRIs compared with women with no history of psychiatric illness [66]. This suggests that the preterm birth rate appears to be significantly higher in SSRIs exposed pregnancy.

There is also growing concern over the potential risk for relapsed depression after discontinuing antidepressant medication during pregnancy. A recent study found that 68% of the women who discontinued antidepressant treatment during pregnancy relapsed in depression, with 50% experiencing recurrence of depression in the first trimester of
pregnancy and 90% by the end of the second trimester [67]. Weighing the risk-benefit decision for women with major depression who are treated with antidepressants such as SSRIs is vital. Concerns about prenatal exposure to these medications range from risk of malformations [68], obstetrical and peri-natal complications [69]. Mothers must also consider a relapse in depression during pregnancy [67], and the effect of untreated depression on the fetus.

SSRIs and autism risk

Abnormalities in serotonin metabolism are one of the few consistent biological findings that are thought to be a potential biomarker for ASD [70]. Elevated levels of serotonin in the blood (5-hydroxytryptamine, 5-HT) have been reported in patients with ASD [71]. Other studies have shown that more than 99% of whole blood serotonin is contained in platelets, which accounts for the elevated serotonin levels in found in autism [72]. Several lines of evidence suggest that alterations in the serotonergic neurotransmitter system might represent one of the biological pathways of ASD. Serotonin has been shown to play an important role in brain development by regulating both, serotonergic outgrowth and maturation of target regions [71, 73]. However, the mechanisms behind differences in circulating serotonin levels seen in individuals with ASD are not fully understood and whether this in anyway reflects neurodevelopmentally relevant pathology remains unclear.
Animal Studies

Animal models have shown that higher prenatal levels of serotonin produce adverse neurodevelopment such as a reduction in the number of β-adrenergic and serotonin receptors as well as cause abnormal brain serotonin receptor binding in the cerebral cortex [74]. Chronic neonatal exposure to SSRIs in male offspring of timed-pregnant rats resulted in reduced serotonin expression that persists into adulthood, which produces changes in the normal maturation of the serotonin system [75]. Rats exposed to SSRIs during the prenatal period have resulted in neuroautonomic alterations in somatosensory structures [76], as well as serotonin content and receptor binding sites [77]. Interestingly, following in utero exposure to serotonergic drugs significantly impacted the number of serotonin receptors expressed in the brains of rats [78]. Data from animal models indicate that early exposure to SSRIs can disrupt development of the serotonin system and alter serotonin-dependent neural processes, however it however it is not known whether this effect of SSRIs is paralleled in humans.

Human studies of health effects of prenatal SSRI exposure

Data on the relationship of birth defects and SSRI exposure are inconsistent; however, Louki et al found a significant association between the use of SSRIs and the occurrence of omphaloceles and septal defects [79]. In a more recent study of 298 cases and 1507 controls the investigators reported a two-fold increase of ASD associated with maternal SSRI use a year before delivery (adjusted OR 2.2 [95% CI, 1.2-4.3]), with the largest effect estimate found in the first trimester (adjusted OR 3.8 [95% CI, 1.8-7.8]) [80]. However, the small sample size and low exposure prevalence in the study
population should be considered when interpreting results. There have been several studies looking at neonatal outcomes following exposure to SSRIs, particularly affecting the respiratory, gastrointestinal and neurological systems, however the findings were also inconsistent [81]. A small retrospective case study has linked maternal SSRI exposure in the latter half of pregnancy with risk for persistent pulmonary hypertension, but these findings have yet to be replicated in a prospective study [82]. Other studies report a greater association of ‘withdrawal symptoms’ with SSRIs such as paroxetine and fluoxetine in children who were exposed to these drugs late in pregnancy; however one of the issues of making the interpretation of these studies is the lack of definition of the term ‘withdrawal symptoms’ [83]. The inconsistency in the literature, as well as some fundamental methodological limitations across many studies, including lack of control for independent effect of maternal depression, has made it difficult to interpret findings.

Effect of indicating condition on autism risk

Diagnosis for psychiatric disorders before the birth of the child have been more commonly reported among parents of children with ASD than parents of children without a diagnosis of ASD, with the condition-specific ORs for depression stronger for mothers than fathers [84]. In addition, elevated rates of clinical psychiatric disorders, distinct from autism, including schizophrenia, anxiety, depression, and social phobias, have been reported among the relatives of individuals with autism, [85]. A case-control study explored the association between peri-natal factors and risk of autism reported an OR of 3.44 (95% CI, 2.21-5.58) for parental psychiatric history of affective disorder [85]. Associations between maternal depression and developmental psychopathology in early
children have been widely reported; however, it is difficult to determine whether these effects are due to a genetic predisposition, intrauterine environment, maternal prescription drug use, or the effects of parenting. Inconsistencies in the literature are perhaps mainly due to reporting bias since one would need to examine parents’ psychiatric diagnoses not only after their child had received an ASD diagnosis, but also before the child’s birth [86]. Researchers must often struggle with whether parents who report depression display more difficulties compared to the general population. These difficulties are more pronounced when investigating parents of children with ASD, primarily due to determining whether or not the depression is a symptom of having a child on the spectrum. Although a number of studies have looked at the possible association between ASD and parental depression, the biological mechanism and causal pathway are still unknown.

Conclusion

Exposures during the pregnancy period have been a focus of several epidemiological studies [16, 41, 87-90]. As autism research moves closer to identifying risk factors and risk biomarkers during the pre-, peri- and neonatal periods, large population-based cohort designs can be used understand the etiology of this disorder. Emphasis on the pregnancy period is supported by a recently reported higher rate of concordance in dizygotic twins [13], which was substantially larger than the non-twin sibling recurrence risk (18.7%) [91]. This implicates a shared environment, such as in utero, as a critical time period for development and suggests the need to explore potential environmental triggers or causes during pregnancy, which could lead to autism.
A current review of the literature, which is summarized in Table 1 above, reveal that there are only two epidemiological studies that have investigated prenatal exposure to B2AR agonist and risk for ASD, and one observational study on SSRIs and risk for ASD. Although these studies were all population based, they had a relatively small sample size and may not have enough statistical power to adequately untangle the effects of the indicating condition and risk for ASD. Further research is needed to replicate the findings thus far and utilize various statistical techniques to address confounding by indication such as restriction methods along with various correction techniques for exposure and outcome misclassification. Given the evidence supporting the associations between the indications for both these medications and risk for ASD, as mentioned above, statistical methods to parse out the effects are needed to fully understand the possible causal pathways.

These medications are known to cross the placenta and have deleterious effects from studies using animal models, and the biological mechanism and causal pathways remain unclear. As described previously, asthma exacerbations, maternal stress, and relapse of depression have the potential to be dangerous for the fetus and mother, therefore to recommend avoidance of all medications during pregnancy is unrealistic. About 8% of pregnant women need permanent drug treatment due to their chronic diseases [92]. Thus, there is increasing need to understand the etiology of neurodevelopmental disorders such as autism, identify critical time periods of development for the fetus, and adequately balance the risks and benefits of medication use during pregnancy.
References


CHAPTER 3: IN UTERO EXPOSURE TO BETA-2-ADRENERGIC RECEPTOR AGONIST DRUGS AND RISK FOR AUTISM SPECTRUM DISORDERS
Abstract

Objectives: The purpose of this study was to investigate associations between use of beta-2-adrenergic receptor (B2AR) agonist drugs during pregnancy and risk for autism spectrum disorders (ASD).

Methods: The matched case-control study sample consisted of children born in Denmark between 1997 and 2006. Health register data provided detailed information regarding maternal indications and prescription drug use, ASD diagnoses, and socioeconomic status. Conditional logistic regression models were used to estimate odds ratios (OR) and confidence intervals (CI) for any B2AR agonist drug exposure during pregnancy, preconception and by trimester.

Results: In models adjusted for parental age, sex of the child, maternal asthma, and family socioeconomic status, increased risk for ASD was associated with any exposure to B2AR agonist drugs during pregnancy (OR: 1.3, 95% CI: 1.1-1.5) with similar effect size across all other exposure periods of interest. These estimates remained robust to various sensitivity analyses.

Conclusion: Our results indicate that B2AR agonist drug exposure during pregnancy may be associated with an increased risk for ASD. While this association is biologically plausible, epidemiologic associations should be replicated in additional populations. Further research is needed to determine underlying biological mechanisms, and if this relationship is real, pregnant women must balance their treatment decisions against benefits of indicated medication use.
Introduction

Autism spectrum disorders (ASD) are a group of developmental disabilities characterized by core deficits in three domains: social interaction, communication, and repetitive or stereotypic behavior [1] and currently has a US prevalence of 11.3 per 1,000 [2]. Although ASD has moderate to high heritability [3] several reviews of autism genetics have suggested the possibility for an interaction between genetic mechanisms and environmental exposures in autism etiology [4, 5]. A large population-based twin study recently conducted by Hallmayer et al, found a dizygotic twin concordance higher than previously reported [6], which was substantially larger than the non-twin sibling recurrence risk (18.7%) [7]. This implicates a shared experience between twin individuals, such as the prenatal environment, as a critical period of life with respect to environmental influences.

In utero exposures such as obstetric complications, maternal illness, and prenatal stress may be etiologically relevant [8-10]. Prenatal pharmacologic exposures have also been implicated as potential risk factors for ASD [11-13]. In particular, concerns have been expressed that exposure to terbutaline, a beta-2 adrenergic receptor (B2AR) agonist drug, used as indicated throughout pregnancy to reduce asthma exacerbations and provide asthma control [19] and used off-label later in pregnancy as a tocolytic agent [14], may increase the risk for neurodevelopmental disorders in offspring [15-17]. A study of in utero B2AR agonist drug exposure in a small series of dizygotic twin pairs suggested an increased risk associated with ASD[18]. A more recent case-control study of 291 cases and 284 controls found that exposure for more than two days during the third trimester was associated with increased risk for ASD, although estimates were imprecise (adjusted
odds ratio (OR) 6.01, 95% confidence interval (CI) 1.05-34.28) [19]. B2AR agonist drug utilization will be strongly associated with indicating conditions. Croen et al 2005 observed autism was associated with women with a diagnoses of asthma or allergies during the second trimester, which suggest that disease severity may be more strongly correlated with fetal neuropathologic conditions or that a critical period for dysregulation in neurodevelopment occurs in mid-pregnancy [8]. However, only one study has thus far reported an association between maternal asthma and autism, reporting a more than a two-fold increased risk of ASD associated with maternal asthma during the first (OR, 2.8; 95% CI, 1.3-6.10) and second (OR, 2.2; 95% CI, 1.1-4.2) trimesters of pregnancy [8]. However, that study was not able to explore the independent role of indicating condition and medication exposure.

Beta-2 adrenergic receptors within the catecholamine system are present in normal nervous system development and for the function of both neural and non-neural tissues in adults [20]. If used during pregnancy, these drugs can impact the fetal brain by crossing the placenta, resulting in the disruption of either replication or differentiation of the developing neurons [21]. However, because uncontrolled asthma has been associated with poor birth outcomes, mothers are often still recommended to continue asthma medication [22, 23]. Given the biological plausibility and limited knowledge regarding risks of prenatal pharmacological exposures, which need to be balanced against the benefits of indicated medication use by pregnant mothers, we conducted a large population-based case-control study to determine the associations of maternal exposure to outpatient B2AR agonist drugs used during preconception and pregnancy and risk associated with delivering a child who goes on to develop ASD.
Methods

Participants

All children (n = 749,755) born in Denmark in the period from January 1, 1997 to December 31, 2006 were identified through the Danish Civil Register System (DCRS). The study population was then drawn from all biological singletons and one child randomly selected from multiple births. Children were also excluded if they could not be linked to their biological mother (n = 1,139), if their mother was not living in Denmark a year before delivery (n = 10,806), or if they were born extremely preterm or post-term (gestational ages less than 23 weeks or greater than 43 weeks, n = 1,774). There were a total of 628,408 live births in the study population.

Cases were identified from the Danish Psychiatric Central Register (DPCR) with an International Classification of Diseases code (ICD-10) for ASD diagnoses identified using records up to March 31, 2011. ICD-10 codes for childhood autism, atypical autism, Asperger’s syndrome and pervasive developmental disorder-unspecified using ICD-10 codes F840, F841, F845, F848, and F849, respectively. Controls were defined as never receiving an ASD diagnosis. Ten controls per case were individually matched on birth month and year to assure that controls have the same length of follow-up time as a case, and thus the same opportunity to acquire an ASD diagnosis and be entered in the registers. Matching on birth month also controlled for birth seasonality. The Institutional Review Board of Drexel University and the University of Copenhagen, Danish Data Protection Agency (Record No. 2010-41-4861) approved this study.
Outcome

As mentioned above, outcome was based on the ICD-10 codes from both the Danish National Hospital Register (DNHR) and DCPR. Data from both the DNHR and DCPR includes information on all inpatient and outpatient care from psychiatric hospitals and psychiatric wards in general hospitals in Denmark. General practitioners or school psychologists refer children who are suspected of having ASD to a child psychiatric ward where they can receive a diagnosis and both inpatient and outpatient treatment. A recent study was able to measure the validity of the reported ASD diagnosis in the register. This validation study evaluated the concordance of reported diagnosis of childhood autism in the DPCR with a record review method developed at the Centers for Disease Control and Prevention (CDC) for population-based surveillance of ASDs in the United States and adopted by CDC’s Autism and Developmental Disabilities Monitoring (ADDM) Network. The investigators concluded that the diagnosis of childhood autism was confirmed in 94% of cases with childhood autism in the DPCR. As many as 486 cases (97%) fell within the autism spectrum and only five cases were found not to have an ASD diagnosis [24].

Exposure

Pharmacologic information was drawn from the Danish Drug Prescription Register (DDPR) which contains information regarding unique personal identification number, dispense date, drug code using the WHO Anatomical Therapeutic Chemical classification system (ATC), name of drug, number of units in the container, number of packets dispensed, strength of the medication, form, and defined daily dosage (DDD) on
all dispensed medication from any pharmacy, except hospital dispensaries, in Denmark (see Table A in the Appendix for medication codes). Therefore, we did not have information on in-hospital use of B2AR agonist drugs, which limits our ability to study maternal B2AR agonist drugs used as a tocolytic, since inpatient prescription of this is common. The drug codes included in the analysis are listed in (see Table A in the Appendix for medication codes under β2 adrenergic receptor agonist). The DDPR includes ATC and the DDD for the expressed purpose of facilitating drug utilization research [25] and these coding schemes have been employed in a variety of studies [26-29].

The Danish Medical Birth Register (DMBR) contains information estimated date of conception (EDC) which is calculated from mother’s self-reported last menstrual period, and corrected with an early ultrasound estimate if the woman reported contraceptive use in the four months prior to conception, had irregular periods or an abnormal last menstrual period. The exposure windows were defined as preconception (90 days prior to the EDC), first trimester (within 90 days after the EDC), second trimester (within 90-180 days after EDC), and third trimester (180 days after the EDC to delivery date). A child was considered exposed if the dispense date fell within the specified exposure period or the days supplied overlapped any portion of the exposure period or interest. Children born to women who did not fill a prescription for B2AR agonist drugs for the entire period from 90 days before EDC through the date of delivery were considered unexposed.

To calculate the cumulative dose dispensed during a particular exposure window, we first calculated the prescribed dose by multiplying the strength of the medication by
the number of units in the container to give the total dose in the package. The prescribed
dose was then calculated by dividing the total package dose by the number of DDD in the
package. Cumulative dose over each exposure period of interest was divided into
quartiles (low, medium, medium-high, and high) based on the distribution of the exposed
controls.

To calculate duration of use for each prescription, the number of packets
dispensed was multiplied by the DDD in the package. Duration was dichotomized into
two categories representing use for more or less than half (0-45 days and \( \geq 45 \) days) in
each exposure window. The reference group for determining the risk of dose and
duration of use was no exposure during any period of interest.

**Covariates**

Candidate covariates include parental age, sex of the child, gestational age, birth
weight, history of parental autoimmune disease, history of parental psychiatric disorder,
maternal infection during pregnancy, number of live births, obstetric complications, and
family socioeconomic status (a combination of parental income and highest level of
education). These covariates were identified from available data in the Danish Fertility
Database, DMBR, DCPR and DNHR. Covariates were selected based on prior literature
suggesting them as potential risk factors for ASD [10, 30-36]. Parental psychiatric
history was categorized as either absent or falling into one of four severity categories
following the algorithm of a prior study [34] developed by Larrson et al in 2005. A
parent was defined as having a psychiatric history if a psychiatric diagnosis had been
recorded before the date of birth of the child. Diagnosis considered in the severity score
included schizophrenia-like psychosis followed by affective disorder in the second most severe category. Diagnoses were ranked by Larsson et al 2005 according to severity and each parent with a psychiatric history received a score equal to the highest ranked condition. The score of the parent with the highest-ranking diagnosis determined the specific category assigned.

Candidate covariates such as maternal illness and obstetric complications were identified from available data in the Danish Fertility Database, DMBR, and DNHR. These covariates were selected based on prior literature suggesting these as potential risk factors for ASD and the exposure[37]. Maternal illness such as autoimmune disease was identified using ICD-8 and ICD-10 codes present in the register prior to the delivery of the child. Maternal infection during pregnancy and other obstetric complications were identified also using ICD-8 and ICD-10 codes if they were present a year before delivery and by trimester.

History of maternal asthma before the birth of the child was included as a covariate because of concerns over potential confounding by indication - when confounding is introduced because a known or perceived indication (or contraindication) for the treatment under study and is also a risk factor for an outcome [38]. Inpatient and outpatient information on maternal asthma was obtained from the DNHR using ICD-8 and ICD-10 codes. Data on maternal asthma were extracted as far back as 1973 for all mothers. Presence of both primary and secondary diagnoses of asthma anytime before delivery was used. From 1973-1993 ICD-8 codes were used in Denmark’s national registers, and were replaced after 1993 by ICD-10 code.


**Analytic method**

Conditional logistic regression analysis was conducted to estimate crude and adjusted associations with no exposure during any period of interest as the reference group. Parental age and gender were forced in all adjusted models as covariates. Other covariates were included only if they resulted in a 10% or greater change in unadjusted log odds ratios when added individually. To guard against joint confounding, any excluded covariate was added back to the adjusted model and retained if the log odds ratios changed by 10%. Additional adjustment for maternal asthma was conducted to control for confounding by indication. To further consider confounding by indication we also examined the effect of the exposure in a sample restricted to mothers with an asthma diagnosis history.

**Sensitivity analyses**

A number of sensitivity analyses were also conducted. Since outpatient data were added to the DCPR in 1993, we wanted to ensure that our estimates were not influenced by this change. Consequently, we re-analyzed data limited to the birth cohort born between 1998 and 2002 whose outcome status would be based on both inpatient and outpatient data. Because some time was likely needed for any impact of the inclusion of outpatient data to be felt, we chose to restrict the birth cohort at 1998 rather than 1993. We also compared effect estimates based on different criteria for considering an individual exposed during a specific window. For example, exposure within a window (e.g., first trimester) was first defined as any maternal B2AR agonist drug use during that
window regardless of whether B2AR agonist drugs were also used in other windows. The alternate definition considered only individuals exclusively exposed during that window.

We also explored the potential effect of maternal B2AR agonist drug exposure misclassification. In our data the observed maternal B2AR agonist drug use prevalence was 3%, while data from national health registers in other Nordic countries such as Finland report exposure prevalence to 4% [39]. Although the exposure prevalence that we observed only differs slightly from what is reported in the literature, any exposure misclassification could lead to bias effect estimates [40]. A Monte Carlo simulation of maternal B2AR agonist drug use and ASD effect estimates was conducted after re-classifying exposure status according to published prevalence estimates [41]. To stimulate increased the prevalence of maternal B2AR agonist drug exposure, we used 1,000 simulated datasets and randomly assigned an unexposed mother to being exposed from a normal distribution. From each of the 1,000 simulated datasets we obtained the OR and then took 1,000 samples from each OR normal distribution. We then took the median effect estimate from these 1,000,000 samples as the OR and the 2.5% and 97.5% percentile to estimate the 95% confidence interval. We assumed under-reporting was non-differential with respect to outcome by keeping sensitivity and specificity equal in case and control groups. Specificity was assumed to be 100%, because it is unlikely a prescription for a B2AR agonist drug would be documented in the register when none was given, and sensitivity was assumed to be 73% because that corresponds to a true B2AR agonist drug use prevalence of 4%.

In addition, we also examined the impact of misclassification of asthma, which would lead to incomplete control for confounding by indication using the same Monte
Carlo simulation approach described above. Our simulation adjusted asthma prevalence from the observed prevalence in the Denmark register data of 1.3% to a prevalence of 2%, which is the prevalence indicated from another national health register [41] assuming that this misclassification was also non-differential with respect to outcome. Specificity was assumed to be 100%; in other words, all mothers truly without a history of maternal asthma where assumed to be correctly classified as without asthma in the register, and sensitivity was set to 66% to reflect the under-reporting needed where the true asthma prevalence of 2%.

In both Monte Carlo simulations (correcting for misclassification of maternal B2AR agonist drug use and for misclassification of asthma) we also considered the maternal B2AR agonist drug use effect in the overall dataset and in the data stratified by maternal asthma status.

Results

Description of study sample

Over the entire pregnancy period, children with ASD (n = 5,200) were more likely than controls (n=52,000) to be male, have a higher parental age and have a mother with an asthma diagnosis prior to the birth of the child (Table 1). For each of the exposure periods examined, similar patterns were observed (as compared with over the entire pregnancy period). In the source population, which consisted of all live births from January 1996 to December 2006 the frequency of any ASD was 0.86%.

The frequency of exposure to maternal B2AR agonist drugs use throughout each exposure period was higher in children with ASD compared to controls. In the
preconception period, 2.0% (n = 102) of cases and 1.6% (n = 816) of controls were exposed. For the pregnancy exposure period 3.7% (n = 190) of cases and 2.9% (n = 1,489) of controls were exposed to B2AR agonist drugs in utero.

**Associations of any B2AR agonist use with ASD**

Table 2 shows unadjusted and adjusted estimates of the association of maternal B2AR agonist use with ASD. Since no additional covariates resulted in a 10% change in the log odds ratios, we show estimates from one adjusted model including parental age and sex of the child and a second adjusted model adding the maternal asthma history variable. In conditional regression models controlling for child birth year and month, sex of the child, and parental age we found an increased risk for any ASD diagnosis associated with exposure to B2AR agonist drugs during pregnancy (OR, 1.3 [95% CI, 1.1-1.5]). The largest effect estimate was found for exposure in the second trimester (adjusted OR, 1.4 [95% CI, 1.1-1.7]) compared to those who are unexposed, although confidence limits of this estimate overlap with point estimates for the maternal B2AR agonist drug use effect in other trimesters. For exposure during the preconception period the adjusted OR was 1.3 (95% CI, 1.0-1.5) for ASD compared to those who were unexposed.

**Analyses of B2AR agonist dose and duration of use**

The mean cumulative dose over the whole pregnancy period for cases was slightly higher than controls (205.3 mg versus 198.0 mg) for any ASD (Table 3). Median cumulative dose was the same (50 mg) for any ASD and controls throughout pregnancy. Across each trimester, the median cumulative dose did not vary substantively, however
the highest dosage among cases and controls occurred during in the second trimester. Results as displayed in Table 4 do not consistently suggest dose response.

Overall, the mean and median duration of use was similar between cases and controls within each period of interest (Table 5). For example the mean duration for the pregnancy period was 62.2 days vs 63.9 days and median duration of maternal B2AR agonist drug use during the pregnancy period was 50 days for both cases and controls. In conditional logistic regression results (Table 6), the point estimates for the longer duration exposure category (>45 days) tended to be slightly larger than that for the shorter duration exposure category (1-45 days) during the preconception and first and second trimesters periods; however the confidence limits for longer duration exposure effects overlapped substantively with those for shorter duration exposure.

Additional statistical adjustment for maternal asthma did not substantively change effect estimates as seen by comparing Adjusted Model 2 and Adjusted Model 1 findings in Tables 2, 4, and 6. Statistical adjustment for maternal asthma resulted in consistently elevated estimates (adjusted OR, 1.3 [95% CI, 1.1-1.5]) for any exposure during pregnancy, adjusting for parental age, sex of the child and risk for any ASD diagnosis compared to the unexposed group. Analyses restricting to only mothers with asthma (Table 7; n = 863) resulted in estimates suggesting elevated effect estimates, although the 95% confidence interval around the odds ratio included the null (adjusted OR, 1.3 [95% CI, 0.8-2.1]).
Sensitivity Analyses

Effect estimates in analyses restricting the birth cohort to those born between 1998-2002, a cohort where all members classified with ASD in the Psychiatric Register would have been entered after outpatient data were included, were comparable to estimates we had observed using our entire sample population (Table 8). In the second sensitivity analysis where we contrasted trimester-specific effects under alternative approaches for defining exposure status our estimates were reasonably close, with overlapping confidence intervals (data not shown). In these analyses we were comparing the estimates obtained when we defined exposure during that period but not limited to other exposure time periods verses exposure in a single trimester only. For example, exposure during the first trimester only had a crude OR of 1.6 (95% CI 0.6-3.7) compared to exposure during the first trimester, but not limited to other exposure periods (crude OR 2.3 [95% CI 1.8-3.0]).

Table 9 and 10 present the analysis results that reflect the impact of potential misclassification of any maternal B2AR agonist drug exposure in pregnancy and maternal asthma, respectively. In the full sample analysis, maternal B2AR agonist drug exposure misclassification (Table 9) did not appear to lead to any change in point or confidence bound estimates. The sensitivity analysis of misclassification of the indication condition (asthma) (Table 10) also suggested minimal impact of this potential misclassification on the effect estimate. Table 9 and 10 also show observed and simulation analysis effect estimates stratified by maternal asthma status and suggest that the finding mentioned above, where B2AR effects persists when looking only at mothers with the indicating condition, persists after considering misclassification of covariates.
Discussion

The present study examined maternal preconception and prenatal use of B2AR agonist drugs and subsequent ASD risk in an offspring in a large population-based register. To our knowledge this is the largest study to-date to look at this drug class and risk associated with ASD, as well as the first study to look at both dose and duration. Results suggest that exposure during the prenatal period was associated with modest association for ASD compared to those who were unexposed in utero. Adjustment for covariates considered did not appreciable change results. Specific exposure window effect estimates were higher in the second and third trimesters. However, trimester-specific effect estimates had confidence intervals with substantial overlap, indicating that results do not provide strong evidence suggesting a particular critical exposure window. Furthermore, we observed an association between exposure during the preconception period and ASD.

These findings are consistent with other epidemiological evidence regarding in utero exposure to B2AR agonist drugs and risk for ASD in that a modest increase effect estimates for ASD were also reported [18, 19]. Connors et al 2005 reported an increased concordance for ASD in dizygotic twins exposed to terbutaline [18]. In a more recent study, the findings from Croen et al 2010 also suggest a modest association for ASD, (adjusted OR 1.39 and 95% CI 0.87-2.22) for exposure to maternal B2AR agonist drug use or mimics and time during pregnancy. These estimates were similar to what we report, especially with regards to the second trimester effect estimates. Exposure to maternal B2AR agonist drugs or mimics during the second trimester and risk for any
ASD, Croen et al 2010 report an adjusted OR of 1.50 95% CI (0.69-3.25). However this same study found that exposure to maternal B2AR agonist drugs or mimics in the 30 days before conception to be associated with three times higher ASD risk (adjust OR 2.98, 95% CI 1.01-8.83) [43]. The effect estimates for specific exposure windows and doses in the study conducted by Croen et al 2010 as well as this present study were statistically imprecise and the mechanism behind the pre-conception exposure has not been described. The preconception effect sizes that were observed may be the result of induced persistent changes in the intracellular signals, although there has been no evidence of bioaccumulation of B2AR agonist drugs in the body, or can be due to uncontrolled confounding by indication.

This is the first study to look at both dose and duration of prenatal use of B2AR agonist drugs and risk for ASD. Epidemiological studies thus far have only looked at duration of use and report the highest risk with prolonged use (>2 days) [19], which is also consistent with our findings. In a study investigating the effects of prenatal overestimation of the B2AR receptors in dizygotic twins who were exposed to terbutaline to treat premature labor, the investigators were able to assess duration. However, they defined duration in their exposed group only as two weeks of continuous treatment with terbutaline in utero. In this present study, we defined duration in preconception and in each trimester into two categories, 1-45 days and more than 45 days of use, and included all B2AR agonist drugs. Although we had defined duration differently from Connor et al 2005, both results agree that increased length of duration is associated with ASD.

In regards to dose for any ASD diagnosis, the second trimester was the only exposure window where the largest dose quartile had the highest risk associated with
ASD, which was higher than the lowest dose quartile, suggesting a possible dose response mechanism for the second trimester. All confidence intervals overlapped each effect estimates, which remained elevated throughout each dose category. However, this evidence does not suggest any particular cumulative dose range or trimester specific exposure for dose.

Although we cannot definitively implicate any specific exposure window, our results do raise concerns around the second trimester exposure period. Croen et al 2010 found associations between B2AR agonist drugs and mimics and increased ASD risk associated with exposure around the time of preconception and in the third trimester, and did not find an associate for the second trimester use. However, our analyses were able to detect the highest effect estimate in the second trimester, which is also supported by animal models [42, 43]. It has been shown that during gestation, overstimulation of the beta-2- adrenergic receptors from an exogenous source during the development of the nervous system may have harmful effects on the development of the brain and the peripheral nervous system [44, 45]. The mechanisms within the mature beta-2-adrenergic receptors protect against over-stimulation; however, fetal receptors do not have the ability to regulate any imbalance and may in fact become sensitized [43]. Early research has shown that B2AR agonist drugs administered to pregnant rats during the second trimester of gestation alters neural cell replication and differentiation, synaptogenesis, and expression of synaptic proteins involved in neurotransmission [43]. This was evidence for chemical and structural damage to the cerebellum, hippocampus, and somatosensory cortex [44, 45], as well as deficiencies in the cerebellar Purkinje cell numbers [45]. These animal models also found abnormalities within the central and
peripheral nervous system and a decrease in the cell numbers in the fetal brain and liver of rats that were exposed prenatally [44]. The effect on the rats is gender selective with males experiencing more abnormalities, which is analogous to the male predominance of autism in humans [44].

Although animal models shed some insight on the possible biological mechanisms that could lead to ASD, there has been evidence linking the indication for B2AR agonist drugs, maternal asthma, to ASD. Only one study has reported an association between maternal asthma and autism risk showing a more than a two-fold elevated risk of ASD associated with maternal asthma during the first (OR, 2.8; 95% CI, 1.3-6.10) and second (OR 2.2; 95% CI, 1.1-4.2) trimester of pregnancy [8]. However, that study was not able to explore the independent role of indicating condition and medication exposure.

Our analyses suggest that maternal B2AR agonist drug use, independent of indication, was associated with increased ASD. Both analytical techniques used to address confounding by indication, restriction and statistical adjustment, remained elevated. These methods have also been used by Croen et al 2010 in order to control for the indication, which also suggest an increased risk associated with ASD for exposure to B2AR agonist drugs during pregnancy. Among mothers who are severe asthmatics, avoidance of asthma treatment during pregnancy, has been linked to birth complications including preterm birth, low birth weight, preeclampsia, and neonatal death [46, 47]. Since uncontrolled asthma has been associated with poor birth outcomes, mothers are often still recommended to continue asthma medication [22, 23, 48]. A high frequency of preterm labor and intrauterine growth restriction in corticosteroid-dependent asthmatic
patients has been reported [22, 49, 50]. Infants with a gestational age at birth that is less than 35 weeks have shown to have more than two-fold increase risk for ASD [34].

Although this study had advantages compared to past work in terms of available sample size and ability to assess dose and duration, our study has several potential limitations. As a register-based study, data on outcome and exposure were limited to information available in the databases. Outcome misclassification is one potential concern; however, the quality of ASD classification in the DPCR has been previously investigated using expert review of a subset of children’s medical records [24]. Our additional sensitivity analysis restricting the birth cohort indicated that our findings were not due to the addition of outpatient data into the register after 1994.

There was a possibility for exposure misclassification. We were able to assess the impact of exposure misclassification by conducting a simulation-based sensitivity increasing the prevalence of exposure that to a rate similar to what is observed in the literature, specifically that of other Nordic countries [39]. In these sensitivity analysis we increased the prevalence of B2AR agonist use in register from 3% to 4% non-differential with respect to exposure status by reclassifying 1.38% unexposed cases and 1.07% unexposed controls as being exposed. In addition, since we believed that the possible misclassification was non-differential, we fixed the sensitivity among cases and controls to be 0.73, and assumed perfect specificity. In this sensitivity analysis, we did not observe any changes in the effect estimates. However, we have assumed that the mothers consumed all of the medication. It was, however, also possible that portions of prescriptions during the study period where unused or that subjects used B2AR agonist drugs during the study period that were prescribed outside the study period.
Overestimation of the exposure could lead to an underestimate of effect estimate however we would expect this to be consistent throughout each exposure window. Any resulting exposure misclassification would be non-differential with respect to outcome, and any bias in exposure effects would tend to be toward the null.

Our exposure data was also restricted to outpatient prescriptions. Although most prescriptions for B2AR agonist drugs, including those used for asthma indications are obtained in an outpatient setting, they are also prescribed as a tocolytic for in-hospital use. B2AR agonist drug exposure due to tocolytic use would most likely occur in the third trimester. Therefore, third trimester exposure may have been underestimated since drugs prescribed during hospital admission are not included. Therefore, if terbutaline administered as a tocolytic does in fact increase ASD risk as previously reported [19] our estimates of third trimester associations may in fact underestimate the true association. Croen et al 2010 was able to isolate the effect of terbutaline, which was used to prevent preterm labor and accounted for the majority of third trimester exposures for both cases and controls [19]. Our findings were consistent with Croen et al 2010 in that we also observed an association for maternal B2AR agonist drug use and ASD in the third trimester [19]. Both Croen et al 2010 and our results provide evidence to further investigate the indication for using B2AR agonist drugs, as a tocolytic.

Finally, as with most observational epidemiology, the unmeasured covariates were also a limitation. However, we were able to consider a number of potential covariates available in the Danish register. Unmeasured covariates that were not represented in the register include in-home environmental exposures and pollutants, which may potentially be associated with ASD [51]. One covariate of potential interest
that was unavailable in the register was maternal smoking which has been suggested as an ASD risk factor and, since it is linked to maternal asthma exacerbation [52], may be associated with maternal B2AR agonist drug use. However, the relationship between maternal smoking and ASD is not proven [53].

This case-control analysis benefits from a large study population and prospective documentation of pharmacologic utilization through the DNPR. As registers are population-based, selection bias is not anticipated to be a large problem in these analyses because all information from Danish citizens are intended to be included in the register. Because of the large sample size, even with relatively small exposure prevalence, the study was powered to detect modest effects. The combination of low exposure prevalence and modest effect size implies that population attributable risks associated with this exposure will be small. Prevalence of asthma drugs used during pregnancy in our study was 2.9% and we observed a 1.3 odds ratio associated with exposure and delivering a child with ASD, the population attributable risk was 0.9%. If the effect is in fact real, this still suggests that a small proportion of autism cases in the population could be prevented if they were not exposed to B2AR agonist drugs during pregnancy.

Given the clinical and suspected etiologic heterogeneity of the ASDs, it is likely that attributable risks associated with implicated exposures will be quite small. Consideration of the biological mechanisms underlying exposure effects as they emerge might lead to an understanding of common etiologic pathways in ASD. As mentioned, animal models have been developed to understand the neurodevelopmental effects of maternal B2AR agonist drug use in conjunction with the results from this work appear relevant to ASD pathogenesis [18, 44]. Exposures during pregnancy that modify beta-2
adrenergic receptor signaling could have widespread effects during critical periods of development [15]. It is also possible that downstream signaling abnormalities including the dysregulated cAMP generation could be a pathway in the development of this disorder[15, 18].

Results from this study add to the limited knowledge on prenatal pharmacological exposures as potential ASD risk factors. Specifically, three prescription medications have been already been identified as potential prenatal environmental risk factors for ASD [12, 13, 54]. Although the effect sizes that we have observed for maternal B2AR agonist drug use during the prenatal period are modest and exposure carries a small population attributable risk, given the fact that the neurodevelopmental consequences of many medications used in pregnancy is still undetermined [55, 56] there is a need to continue to carefully explore prenatal prescription drug use as ASD risk factors. At the same time, while it is important to detect any such associations and consider their etiologic implications, the public health implications may not be straightforward. With respect to maternal B2AR agonist drug use, uncontrolled asthma in pregnancy has been associated with poor birth outcomes [23, 50]. During an asthma exacerbation in pregnancy, prenatal maternal stress response may be elevated, especially before 32 weeks of gestation, when the fetal limbic system is considered to be the most vulnerable to such a stress response [9]. Consequently, any ASD risk associated with maternal B2AR agonist drug use needs to be balanced against the benefits of indicated medication use by pregnant mothers. Additional studies need to replicate the present study before the implications of prenatal B2AR agonist drug exposure through maternal use of these
agents for asthma control on ASD risk be considered when making individual decisions about asthma control in pregnancy.
References


Table 1. Study demographics for B2AR analysis

<table>
<thead>
<tr>
<th>Maternal age</th>
<th>Any ASD</th>
<th>Controls</th>
<th>Any ASD</th>
<th>Controls</th>
<th>Any ASD</th>
<th>Controls</th>
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<th>Controls</th>
<th>Any ASD</th>
<th>Controls</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pregnancy</td>
<td>Preconception</td>
<td>1st Trimester</td>
<td>2nd Trimester</td>
<td>3rd Trimester</td>
<td>1st Trimester</td>
<td>2nd Trimester</td>
<td>3rd Trimester</td>
<td>1st Trimester</td>
<td>2nd Trimester</td>
</tr>
<tr>
<td>No. (%)</td>
<td>5200 (9.1)</td>
<td>52000 (90.9)</td>
<td>5112 (9.1)</td>
<td>51120 (90.9)</td>
<td>5096 (9.1)</td>
<td>50960 (90.9)</td>
<td>5116 (9.1)</td>
<td>51160 (90.9)</td>
<td>5117 (9.1)</td>
<td>51170 (90.9)</td>
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<tr>
<td>Maternal age</td>
<td>≤25 years</td>
<td>814 (15.7)</td>
<td>7461 (14.4)</td>
<td>805 (15.8)</td>
<td>7302 (14.3)</td>
<td>798 (15.7)</td>
<td>7169 (14.1)</td>
<td>799 (15.6)</td>
<td>7189 (14.0)</td>
<td>805 (15.7)</td>
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<tr>
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<td>26-30</td>
<td>1747 (33.6)</td>
<td>18763 (36.1)</td>
<td>1715 (33.6)</td>
<td>18398 (36.0)</td>
<td>1709 (33.5)</td>
<td>18256 (36.0)</td>
<td>1718 (33.6)</td>
<td>18670 (36.3)</td>
<td>1716 (33.5)</td>
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<tr>
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<td>≥36</td>
<td>901 (17.3)</td>
<td>7790 (15.2)</td>
<td>879 (17.2)</td>
<td>7656 (15.0)</td>
<td>881 (17.3)</td>
<td>7744 (15.2)</td>
<td>882 (17.2)</td>
<td>7700 (15.1)</td>
<td>882 (17.1)</td>
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<tr>
<td>Paternal age</td>
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<td>13089 (25.2)</td>
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<td>12623 (24.7)</td>
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<td>12895 (25.2)</td>
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<td>29-39</td>
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<td>27643 (54.1)</td>
<td>2610 (51.2)</td>
<td>27587 (54.2)</td>
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<td>27598 (54.0)</td>
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<td>40-49</td>
<td>532 (10.2)</td>
<td>4276 (8.2)</td>
<td>525 (10.3)</td>
<td>4294 (8.4)</td>
<td>527 (10.3)</td>
<td>4251 (8.3)</td>
<td>524 (10.3)</td>
<td>4147 (8.1)</td>
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<td>50-54</td>
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<td>284 (0.6)</td>
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<td>247 (0.5)</td>
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<td>243 (0.5)</td>
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<td>258 (0.5)</td>
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<td>≥55</td>
<td>20 (0.4)</td>
<td>118 (0.2)</td>
<td>20 (0.4)</td>
<td>126 (0.3)</td>
<td>20 (0.4)</td>
<td>112 (0.2)</td>
<td>20 (0.4)</td>
<td>119 (0.2)</td>
<td>20 (0.4)</td>
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<tr>
<td>Child sex</td>
<td>Boys</td>
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<td>4198 (82.0)</td>
<td>26274 (51.4)</td>
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Table 1 (continued). Study demographics for B2AR analysis (continued)

<table>
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<tr>
<th>Maternal Asthma</th>
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<th>Pregnancy Preconception 1st Trimester 2nd Trimester 3rd Trimester</th>
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<td>Yes</td>
<td>83 (1.6)</td>
<td>673 (1.3) 66 (1.3) 588 (1.2) 70 (1.4) 545 (1.1) 69 (1.4) 559 (1.1) 73 (1.4) 608 (1.2)</td>
</tr>
<tr>
<td>No</td>
<td>4986 (95.9) 50716 (97.6) 4901 (95.9) 49850 (97.5) 4887 (95.9) 49741 (97.6) 4907 (95.9) 49864 (97.5) 4904 (95.8) 49904 (97.5)</td>
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<tr>
<td>Paternal history of psychiatric disorder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rank 1</td>
<td>24 (0.5)</td>
<td>137 (0.3) 24 (0.5) 143 (0.3) 23 (0.5) 112 (0.2) 22 (0.4) 139 (0.3) 24 (0.5) 119 (0.2)</td>
</tr>
<tr>
<td>Rank 2</td>
<td>22 (0.4)</td>
<td>161 (0.3) 22 (0.4) 157 (0.3) 22 (0.4) 163 (0.3) 22 (0.4) 161 (0.3) 22 (0.4) 167 (0.3)</td>
</tr>
<tr>
<td>Rank 3</td>
<td>25 (0.5)</td>
<td>187 (0.4) 25 (0.5) 193 (0.4) 24 (0.5) 198 (0.4) 25 (0.5) 192 (0.4) 24 (0.5) 172 (0.4)</td>
</tr>
<tr>
<td>Rank 4</td>
<td>143 (2.8)</td>
<td>799 (1.5) 140 (2.7) 777 (1.5) 140 (2.8) 746 (1.5) 140 (2.7) 804 (1.6) 143 (2.8) 808 (1.6)</td>
</tr>
<tr>
<td>No disorder</td>
<td>4986 (95.9) 50716 (97.6) 4901 (95.9) 49850 (97.5) 4887 (95.9) 49741 (97.6) 4907 (95.9) 49864 (97.5) 4904 (95.8) 49904 (97.5)</td>
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</tr>
<tr>
<td>Maternal history of psychiatric disorder</td>
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<td></td>
</tr>
<tr>
<td>Rank 1</td>
<td>13 (0.3)</td>
<td>82 (0.2) 15 (0.3) 60 (0.1) 13 (0.3) 73 (0.1) 13 (0.3) 63 (0.1) 13 (0.3) 75 (0.1)</td>
</tr>
<tr>
<td>Rank 2</td>
<td>22 (0.4)</td>
<td>102 (0.2) 22 (0.4) 100 (0.2) 21 (0.3) 104 (0.2) 20 (0.4) 96 (0.2) 21 (0.4) 93 (0.2)</td>
</tr>
<tr>
<td>Rank 3</td>
<td>12 (0.2)</td>
<td>74 (0.1) 13 (0.3) 73 (0.1) 12 (0.2) 77 (0.2) 12 (0.2) 73 (0.1) 12 (0.2) 68 (0.1)</td>
</tr>
<tr>
<td>Rank 4</td>
<td>125 (2.4)</td>
<td>695 (1.3) 121 (2.4) 696 (1.3) 120 (2.4) 657 (1.3) 121 (2.4) 711 (1.4) 123 (2.4) 689 (1.4)</td>
</tr>
<tr>
<td>No disorder</td>
<td>5028 (96.7) 51047 (98.2) 4941 (96.7) 50191 (98.2) 4930 (96.7) 50049 (98.2) 4950 (96.8) 50217 (98.2) 4948 (96.7) 50245 (98.2)</td>
<td></td>
</tr>
<tr>
<td>Family SES</td>
<td></td>
<td></td>
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<tr>
<td>Low</td>
<td>831 (16.0)</td>
<td>6624 (12.7) 812 (15.9) 6512 (12.7) 808 (15.9) 6525 (12.8) 805 (15.8) 6494 (12.7) 814 (15.9) 6560 (12.8)</td>
</tr>
<tr>
<td>Medium</td>
<td>2712 (52.2)</td>
<td>26298 (51.0) 2676 (52.4) 25671 (50.2) 2668 (52.4) 25652 (50.3) 2672 (52.2) 26001 (50.9) 2668 (52.1) 25554 (49.9)</td>
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<tr>
<td>High</td>
<td>1657 (31.9)</td>
<td>19066 (36.3) 1624 (31.8) 18932 (37.0) 1620 (31.8) 18772 (36.8) 1639 (32.0) 18656 (36.5) 1635 (32.0) 19051 (37.0)</td>
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<tr>
<td>Missing</td>
<td>0 (0.0)</td>
<td>12 (0.0) 0 (0.0) 5 (0.0) 0 (0.0) 11 (0.0) 0 (0.0) 9 (0.0) 0 (0.0) 5 (0.0)</td>
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</table>
### Table 2. Effect estimates for prenatal B2AR agonist drug exposure and risk for ASD.

<table>
<thead>
<tr>
<th>Outcome</th>
<th># Cases</th>
<th>Exposure Period</th>
<th>Exposed No. (%)</th>
<th>Unadjusted OR(^a) (95% CI)</th>
<th>Adjusted Model 1 OR(^b) (95% CI)</th>
<th>Adjusted Model 2 OR(^c) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any ASD</td>
<td>5112</td>
<td>Preconception</td>
<td>102 (2.0)</td>
<td>1.3 (1.0-1.5)</td>
<td>1.3 (1.0-1.6)</td>
<td>1.3 (1.0-1.6)</td>
</tr>
<tr>
<td></td>
<td>5200</td>
<td>Pregnancy</td>
<td>190 (3.7)</td>
<td>1.3 (1.1-1.5)</td>
<td>1.3 (1.1-1.5)</td>
<td>1.3 (1.1-1.5)</td>
</tr>
<tr>
<td></td>
<td>5096</td>
<td>First Trimester</td>
<td>86 (1.7)</td>
<td>1.1 (0.9-1.4)</td>
<td>1.2 (0.9-1.5)</td>
<td>1.1 (0.9-1.4)</td>
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<tr>
<td></td>
<td>5116</td>
<td>Second Trimester</td>
<td>106 (2.1)</td>
<td>1.4 (1.1-1.7)</td>
<td>1.4 (1.1-1.7)</td>
<td>1.5 (1.1-1.7)</td>
</tr>
<tr>
<td></td>
<td>5117</td>
<td>Third Trimester</td>
<td>107 (2.1)</td>
<td>1.3 (1.1-1.6)</td>
<td>1.4 (1.2-1.8)</td>
<td>1.4 (1.1-1.7)</td>
</tr>
</tbody>
</table>

Abbreviations: Autism spectrum disorders (ASD); Odds ratio (OR); Confidence Intervals (CI)

Reference: No exposure during any exposure period

a. Controls for matching variables of child year and month of birth through conditioning

b. Odds ratios adjusted for parental age and sex of the child (and conditions on matching variables of child birth month and year)

c. Odds ratios adjusted for parental age, sex of the child, history of maternal asthma (and conditions on matching variables of child birth month and year)

*P-value < 0.05

### Table 3. Cumulative dose of prenatal exposure to B2AR agonist drugs.

<table>
<thead>
<tr>
<th>Cumulative Dose of Prenatal Exposure to B2AR Agonists (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any ASD</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td><strong>Pregnancy</strong></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Variance</td>
</tr>
<tr>
<td>Mode</td>
</tr>
<tr>
<td>Minimum</td>
</tr>
<tr>
<td>25th percentile</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>75th percentile</td>
</tr>
<tr>
<td>Maximum</td>
</tr>
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</table>
Table 4. Effect Estimates for Any ASD Associated with Cumulative Dose of Prenatal Exposure to B2AR Agonists

<table>
<thead>
<tr>
<th>Exposure Period</th>
<th>Dose(^a)</th>
<th>Crude OR(^b) (95% CI)</th>
<th>Models</th>
<th>Adjusted Model 1 OR(^c) (95% CI)</th>
<th>Adjusted Model 2 OR(^d) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preconception</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1 (≤25 mg)</td>
<td></td>
<td>*1.5 (1.1-2.2)</td>
<td>*1.5 (1.0-2.2)</td>
<td>*1.5 (1.0-2.2)</td>
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</tr>
<tr>
<td>Quartile 2 (26-60 mg)</td>
<td></td>
<td>1.3 (0.9-2.0)</td>
<td>*1.5 (1.0-2.2)</td>
<td>1.4 (0.9-2.1)</td>
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<tr>
<td>Quartile 3 (61-100 mg)</td>
<td></td>
<td>1.3 (0.8-2.0)</td>
<td>1.3 (0.9-2.1)</td>
<td>1.3 (0.9-2.1)</td>
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</tr>
<tr>
<td>Quartile 4 (&gt;100 mg)</td>
<td></td>
<td>0.9 (0.6-1.5)</td>
<td>0.8 (0.5-1.3)</td>
<td>0.8 (0.5-1.4)</td>
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<tr>
<td>Pregnancy</td>
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<td>Quartile 1 (≤36 mg)</td>
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<td>1.5 (1.1-2.0)</td>
<td>*1.5 (1.1-2.0)</td>
<td>*1.5 (1.1-2.0)</td>
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<tr>
<td>Quartile 2 (37-98 mg)</td>
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<td>1.0 (0.7-1.5)</td>
<td>0.9 (0.6-1.3)</td>
<td>0.9 (0.6-1.3)</td>
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<tr>
<td>Quartile 3 (99-120 mg)</td>
<td></td>
<td>1.3 (1.0-1.7)</td>
<td>0.9 (0.6-1.3)</td>
<td>*1.3 (1.0-1.8)</td>
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<tr>
<td>Quartile 4 (&gt;120 mg)</td>
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<td>1.3 (1.0-1.7)</td>
<td>1.3 (1.0-1.7)</td>
<td>1.3 (1.0-1.7)</td>
<td></td>
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<tr>
<td>First Trimester</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Quartile 1 (≤20 mg)</td>
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<td>*1.7 (1.2-2.6)</td>
<td>1.3 (1.0-1.7)</td>
<td>*1.6 (1.0-2.2)</td>
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<tr>
<td>Quartile 2 (21-52 mg)</td>
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<td>0.8 (0.4-1.3)</td>
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<tr>
<td>Quartile 3 (53-100 mg)</td>
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<td>1.2 (0.7-1.9)</td>
<td>1.1 (0.6-1.8)</td>
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<tr>
<td>Quartile 4 (&gt;100 mg)</td>
<td></td>
<td>1.2 (0.7-1.9)</td>
<td>1.2 (0.7-1.9)</td>
<td>1.1 (0.6-1.8)</td>
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<tr>
<td>Second Trimester</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Quartile 1 (≤34 mg)</td>
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<td>1.2 (0.8-1.8)</td>
<td>1.2 (0.8-1.8)</td>
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<td>Quartile 2 (35-80 mg)</td>
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<td>1.4 (0.9-2.1)</td>
<td>1.4 (0.9-2.1)</td>
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</tr>
<tr>
<td>Quartile 3 (81-174 mg)</td>
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<td>1.6 (1.1-2.3)</td>
<td>*1.7 (1.1-2.5)</td>
<td>*1.6 (1.1-2.5)</td>
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</tr>
<tr>
<td>Quartile 4 (&gt;174 mg)</td>
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<td>1.3 (0.8-2.0)</td>
<td>1.3 (0.8-2.0)</td>
<td>1.3 (0.8-2.0)</td>
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<tr>
<td>Third Trimester</td>
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<td></td>
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</tr>
<tr>
<td>Quartile 1 (≤25 mg)</td>
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<td>1.3 (0.9-2.0)</td>
<td>1.3 (0.9-2.0)</td>
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<td>Quartile 2 (26-70 mg)</td>
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<td>1.4 (1.0-2.1)</td>
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</tr>
<tr>
<td>Quartile 3 (71-102 mg)</td>
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<td>1.2 (0.8-1.9)</td>
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<tr>
<td>Quartile 4 (&gt;102 mg)</td>
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<td>1.5 (1.0-2.2)</td>
<td>1.5 (1.0-2.2)</td>
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</tr>
</tbody>
</table>

The reference group is unexposed

a. Dose categories were based on the distribution of cumulative dose of exposed controls in the exposure period of interest.
b. Controls for matching variables of child year and month of birth through conditioning
c. Odds ratios adjusted for parental age and sex of the child (and conditions on matching variables of child birth month and year)
d. Odds ratios adjusted for parental age, sex of the child, history of maternal asthma (and conditions on matching variables of child birth month and year)

*P-value < 0.05
Table 5. Duration of prenatal exposure to B2AR agonist drugs.

<table>
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<th></th>
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<th>Controls Preconception</th>
<th>Any ASD 1st Trimester</th>
<th>Controls 2nd Trimester</th>
<th>Any ASD 3rd Trimester</th>
<th>Controls Any ASD Controls</th>
<th>Any ASD Controls</th>
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<td>Mean</td>
<td>66.2</td>
<td>63.9</td>
<td>38.1</td>
<td>35.4</td>
<td>40.6</td>
<td>37.6</td>
<td>54.4</td>
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<tr>
<td>Variance</td>
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<td>74.3</td>
<td>28.9</td>
<td>29.3</td>
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<td>28.4</td>
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<tr>
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<td>50.0</td>
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<td>90.0</td>
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<tr>
<td>Minimum</td>
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<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
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<td>25&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>15.0</td>
<td>15.0</td>
<td>10.0</td>
<td>10.0</td>
<td>15.0</td>
<td>13.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Median</td>
<td>50.0</td>
<td>50.0</td>
<td>34.0</td>
<td>27.0</td>
<td>41.5</td>
<td>30.5</td>
<td>54.0</td>
</tr>
<tr>
<td>75&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>72.0</td>
<td>65.0</td>
<td>50.0</td>
<td>50.0</td>
<td>51.0</td>
<td>50.0</td>
<td>88.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>296.0</td>
<td>301.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
</tr>
</tbody>
</table>
Table 6. Effect Estimates for Any ASD Associated with Duration of use of Prenatal Exposure to B2AR Agonists

<table>
<thead>
<tr>
<th>Exposure Period</th>
<th>Unadjusted OR* (95% CI)</th>
<th>Adjusted Model 1 ORb (95% CI)</th>
<th>Adjusted Model 2 ORc (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preconception</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-45 days</td>
<td>1.2 (0.9-1.5)</td>
<td>1.2 (0.9-1.5)</td>
<td>1.2 (0.9-1.5)</td>
</tr>
<tr>
<td>≥ 45 days</td>
<td>1.4 (1.0-2.0)</td>
<td>1.5 (1.0-2.1)</td>
<td>1.5 (1.1-2.1)</td>
</tr>
<tr>
<td>First Trimester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-45 days</td>
<td>1.0 (0.7-1.4)</td>
<td>1.1 (0.8-1.5)</td>
<td>1.1 (0.8-1.5)</td>
</tr>
<tr>
<td>≥ 45 days</td>
<td>1.4 (1.0-1.1)</td>
<td>1.3 (0.9-1.8)</td>
<td>1.2 (0.8-1.7)</td>
</tr>
<tr>
<td>Second Trimester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-45 days</td>
<td>1.2 (0.9-1.7)</td>
<td>1.2 (0.9-1.7)</td>
<td>1.2 (0.9-1.6)</td>
</tr>
<tr>
<td>≥ 45 days</td>
<td>1.5 (1.2-2.0)</td>
<td>1.5 (1.2-2.0)</td>
<td>1.5 (1.1-2.0)</td>
</tr>
<tr>
<td>Third Trimester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-45 days</td>
<td>1.3 (1.0-1.7)</td>
<td>1.4 (1.1-1.8)</td>
<td>1.4 (1.1-1.8)</td>
</tr>
<tr>
<td>≥ 45 days</td>
<td>1.4 (1.0-1.9)</td>
<td>1.4 (1.1-2.0)</td>
<td>1.4 (1.0-2.0)</td>
</tr>
<tr>
<td>≥ 45 days</td>
<td>1.4 (1.0-1.9)</td>
<td>1.4 (1.1-2.0)</td>
<td>1.4 (1.0-2.0)</td>
</tr>
</tbody>
</table>

The reference group is unexposed

The cut point at 45 days was determined since it was median number of days in each 90-day exposure period for preconception and each trimester.

a. Controls for matching variables of child year and month of birth through conditioning
b. Odds ratios adjusted for parental age and sex of the child (and conditions on matching variables of child birth month and year)
c. Odds ratios adjusted for parental age, sex of the child, history of maternal asthma (and conditions on matching variables of child birth month and year)

*P-value < 0.05
Table 7. Effect estimates for any ASD associated with any prenatal exposure to B2AR restricted to mothers with asthma

<table>
<thead>
<tr>
<th>Exposure Period</th>
<th>ASD Cases</th>
<th>Controls</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted Model 1 OR&lt;sup&gt;a&lt;/sup&gt; (95% CI)</th>
<th>Adjusted Model 2 OR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preconception</td>
<td>25 (37.9)</td>
<td>234 (35.5)</td>
<td>1.1 (0.7-1.9)</td>
<td>1.2 (0.7-2.1)</td>
<td>1.2 (0.7-2.1)</td>
</tr>
<tr>
<td></td>
<td>n=66</td>
<td>n=660</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>42 (50.6)</td>
<td>363 (43.7)</td>
<td>1.3 (0.8-2.1)</td>
<td>1.4 (0.9-2.3)</td>
<td>1.4 (0.9-2.3)</td>
</tr>
<tr>
<td></td>
<td>n=83</td>
<td>n=830</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Trimester</td>
<td>29 (41.4)</td>
<td>241 (34.4)</td>
<td>1.4 (0.8-2.3)</td>
<td>1.3 (0.8-2.3)</td>
<td>1.3 (0.8-2.2)</td>
</tr>
<tr>
<td></td>
<td>n=70</td>
<td>n=700</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second Trimester</td>
<td>28 (40.6)</td>
<td>228 (33.0)</td>
<td>1.4 (0.8-2.3)</td>
<td>1.6 (0.9-2.7)</td>
<td>1.6 (0.9-2.7)</td>
</tr>
<tr>
<td></td>
<td>n=69</td>
<td>n=690</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third Trimester</td>
<td>32 (43.8)</td>
<td>254 (34.8)</td>
<td>1.5 (0.9-2.5)</td>
<td>1.6 (0.9-2.8)</td>
<td>1.7 (1.0-2.9)</td>
</tr>
<tr>
<td></td>
<td>n=73</td>
<td>n=730</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The reference group had no exposure during the exposure period of interest.

a. Odds ratios were adjusted for parental age and child sex (matching on child birth month and year)

b. Odds ratios were adjusted for parental age, child sex, maternal asthma (matching on child birth month and year)

Maternal Asthma: ICD – 10 codes: J45, J45.0, J45.1, J45.8, J45.9, J46, J46.9; ICD – 8 codes: 493.00, 493.01, 493.02, 493.08, 493.09

<table>
<thead>
<tr>
<th>Exposed No. (%)</th>
<th>ASD Cases (N = 3252)</th>
<th>Controls (N = 32520)</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted Model 1 OR\textsuperscript{a} (95% CI)</th>
<th>Adjusted Model 2 OR\textsuperscript{b} (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>125 (3.3)</td>
<td>872 (2.7)</td>
<td>*1.5 (1.2-1.8)</td>
<td>*1.5 (1.2-1.8)</td>
<td>*1.5 (1.2-1.8)</td>
</tr>
</tbody>
</table>

The reference group had no exposure during the exposure period of interest.

\textsuperscript{a} Odds ratios were adjusted for parental age and child sex (matching on child birth month and year)

\textsuperscript{b} Odds ratios were adjusted for parental age, child sex, maternal asthma (matching on child birth month and year)

*P-value < 0.05
Table 9. Effect estimates from the sensitivity analysis using Monte Carlo simulation to examine the impact of misclassification of any B2AR agonist exposure in pregnancy in the register

<table>
<thead>
<tr>
<th></th>
<th>Observed</th>
<th>Sensitivity Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted odds ratios(^b) (95% confidence intervals) for prenatal exposure to B2ARs and risk for ASD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Full Sample(^a) (n = 57,200)</td>
<td>Full Sample(^a) (n = 57,200)</td>
</tr>
<tr>
<td></td>
<td>Stratified(^b) Asthma (n=756)</td>
<td>Stratified(^b) Asthma ((\bar{n}=)1,176)</td>
</tr>
<tr>
<td></td>
<td>No Asthma (n=56,444)</td>
<td>No Asthma (n=56,444)</td>
</tr>
<tr>
<td>Observed</td>
<td>1.3 (1.1-1.5)</td>
<td>1.2 (0.8-1.8)</td>
</tr>
<tr>
<td>Stratified(^b)</td>
<td>1.3 (0.8-2.0)</td>
<td>1.3 (1.1-1.5)</td>
</tr>
<tr>
<td>Full Sample(^a)</td>
<td>1.3 (1.1-1.5)</td>
<td>1.2 (0.8-1.8)</td>
</tr>
<tr>
<td>Sensitivity(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analysis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Reference group is no exposure during pregnancy

\(^b\) Odds ratios adjusted for child birth year and month, parental age, and sex of the child. Models using observed data are conditional logistic regressions conditioning on the matching variables (child birth year and month) and adjusting for other covariates while models in simulation were unconditional logistic regressions models adjusting for all variables as covariates.

\(^c\) Simulations increase prevalence of B2AR agonist drug use in register from 3% to 4% non differential with respect to outcome (1.38% unexposed cases; 1.07% unexposed controls were reclassified being exposed). This assumes that overall B2AR agonist sensitivity was 73%, was non-differential across cases and controls, and that asthma specificity was 100%.
Table 10. Effect estimates from the sensitivity analysis using Monte Carlo simulation to examine the impact of misclassification of asthma in the register.

<table>
<thead>
<tr>
<th>Observed</th>
<th>Sensitivity Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Full Sample&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Full Sample&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(n = 57,200)</td>
</tr>
<tr>
<td></td>
<td>(n=756)</td>
</tr>
<tr>
<td>1.3 (1.1-1.5)</td>
<td>1.3 (0.8-2.0)</td>
</tr>
</tbody>
</table>

- Reference group is no B2AR agonist exposure during pregnancy.
- Odds ratios adjusted for child birth year and month, parental age, and sex of the child. Models using observed data are conditional logistic regressions conditioning on the matching variables (child birth year and month) and adjusting for other covariates while models in simulation were unconditional logistic regressions models adjusting for all variables as covariates.
- Simulations increase prevalence of maternal asthma in register from 1.3% to 2% non differential with respect to outcome (1.38% unexposed cases; 1.07% unexposed controls were reclassified being exposed). This assumes that overall asthma sensitivity was 66%, was non-differential across cases and controls, and that asthma specificity was 100%.
CHAPTER 4: IN UTERO EXPOSURE TO SELECTIVE SEROTONIN REUPTAKE INHIBITORS AND RISK FOR AUTISM SPECTRUM DISORDERS
Abstract

Objective: The objective of this study was to investigate associations between a commonly used antidepressant, selective serotonin reuptake inhibitors (SSRIs), that is prescribed during pregnancy and risk for autism spectrum disorders (ASD).

Method: We performed a case-control study of children born in Denmark between 1997 and 2006. Linkages using Denmark’s health and population registers were performed to obtain information regarding prescription drugs used, ASD diagnosis and health and socioeconomic status. Separate analyses were performed to take into account the independent effect of maternal depression and to assess the potential for under-reporting in the register.

Results: Estimates were calculated for any exposure during pregnancy, exposure by trimester, and preconception exposure. In conditional logistic regression models adjusted for parental age and sex of the child, any exposure to SSRIs during pregnancy was associated with ASD (odds ratio (OR) 2.0 [95% confidence interval CI, 1.6-2.6]) compared to the unexposed reference group. Similar estimates were found during other specific exposure periods of interest. Statistical adjustment for maternal depression to control for confounding by indication did not substantively change effect estimates (adjusted OR for any in pregnancy exposure was 1.9 [95% CI, 1.5-2.5]). Although analyses restricted to only mothers with a history of depression resulted in attenuated SSRI effect estimates, when Monte Carlo simulations were used to gauge the impact of under-reporting of maternal depression in the register, results suggested that the independent SSRI effect persists.
**Conclusion:** We found evidence supporting the hypothesis that *in utero* exposure to SSRIs is associated with a child’s risk for ASD. Confounding by indication remains a concern and, although we attempted to control for this as best as possible. Additional studies should explore this alternative hypothesis further as well as attempt to understand the persistence of the association across the preconception and prenatal periods. Results from this study add to the limited knowledge on prenatal pharmacological exposures as potential ASD risk factors; yet emerging evidence of this nature needs to be balanced against the benefits of indicated medication use by pregnant mothers.
Introduction

Autism spectrum disorders (ASDs) are a complex group of neurodevelopmental disorders defined by core deficits in three domains: social interaction, communication, and repetitive or stereotypic behavior [1]. This disorder represents a significant public health concern, with a current US prevalence of 11.3 per 1,000 [2]. Although ASD has moderate to high heritability [3] several reviews of autism genetics have suggested the possibility for an interaction between genetic mechanisms and environmental exposures in autism etiology [4, 5]. A large population-based twin study recently conducted by Hallmayer et al, found a dizygotic twin concordance rate higher than previously reported [6] and at a level substantially larger than the non-twin sibling recurrence risk (18.7%) [7]. This implicates the prenatal period, when twins’ environments are shared more so than those of non-twin siblings, as a critical period of life with respect to environmental influences.

Selective serotonin reuptake inhibitors (SSRIs), such as many antidepressants, are among the most commonly used medications in pregnancy, with prescription frequency estimates ranging from 5% to 13% in pregnant US women [8-10]. SSRIs cross the placenta, thus exposing the developing fetus [11]. There is accumulating evidence that mothers treated with SSRIs during pregnancy may experience increased rates of adverse reproductive outcomes including atrium septum defects [12, 13] preterm delivery, a low Apgar score, and more complications resulting in intensive care unit admissions [14].

A recent case-control study conducted within the Kaiser Permanente Medical Care Program in Northern California reported an ASD odds ratio (OR) associated with any prenatal SSRI use of 2.2 (95% confidence interval (CI) 1.4, 2.3) [15]. The largest
effect was due to use in the first trimester, though confidence intervals on trimester-specific effects were very wide, and an effect was also observed for exposure during the preconception period [15].

Associations between maternal depression, the chief indicating condition for SSRI use, and developmental psychopathology in children have been widely reported [16-20]. Diagnosis for psychiatric disorders before the birth of the child have been more commonly reported among parents of children with ASD than parents of children without a diagnosis of ASD, with the condition-specific ORs for depression stronger for mothers than fathers [21]. Consequently, it may be challenging to separate the effect of SSRI use from other effects of maternal depression (e.g., shared genetic predisposition, altered intrauterine environment, or downstream effects of parenting). To address the analytic concerns about treatment versus underlying condition for which the treatment was prescribed, Croen et al 2011 statistically adjusted for maternal depression and a broader range of maternal psychiatric disorders in their models assessing exposure to SSRIs during pregnancy and risk for ASD [15]. This resulted in SSRI exposure during the first trimester to remain significantly associated with risk of ASD, as was a history of SSRI exposure anytime during the year before delivery [15]. In addition, they still observed an association after conducting a restriction analysis to a subgroup of women with a history of mental health disorder in the year before delivery, although confidence intervals were wide (OR, 1.6 [95% CI, 0.6-4.0]) [15].

To follow-up on this report, we conducted a population register-based case-control study of maternal prenatal SSRI use and risk of ASD. The study sample was drawn from the Danish national health registers, which allowed for a sufficient numbers
of exposed mothers even though prenatal SSRI use may be lower in Nordic countries compared to the US [22]. We considered a variety of factors that might be associated with either SSRIs or ASD, including the sex of the child, perinatal risk factors for ASD, and family factors such as parental education and income, as potential confounders. In addition, several sensitivity analyses were used to address the issues of confounding by indication and explore the impact of covariate misclassification.

Methods

Eligible Participants

All children (n = 749,755) born in Denmark in the period from January 1, 1997 to December 31, 2006 were identified through the Danish Civil Register System (DCRS). The study population was then drawn from all biological singletons and one child randomly selected from multiple births. Children were also excluded if they could not be linked to their biological mother (n = 1,139), if their mother was not living in Denmark a year before delivery (n = 10,806), or if they were born extremely preterm or post-term (gestational ages less than 23 weeks or greater than 43 weeks, n = 1,774). There were a total of 628,408 in the study population.

Case and control definitions

ASD diagnoses are based on data from the Danish National Hospital Register (DNHR) supplemented by data from the Danish Psychiatric Central Register (DPCR). These sources include information on all inpatient and outpatient care from psychiatric hospitals and psychiatric wards in general hospitals in Denmark. General practitioners or
school psychologists refer children who are suspected of having ASD to a child psychiatric ward where they can receive a diagnosis and both inpatient and outpatient treatment. The quality of the diagnosis of childhood autism in the Denmark registers has recently been investigated through a validation study that conducted detailed abstraction of the children’s medical records that were reviewed by autism experts. Register diagnosis of childhood autism was confirmed 94% of the time with an additional 3% falling within the autism spectrum [23].

Subjects’ DNHR and DCPR record’s from January 1, 1999 to March 31, 2011 were searched for International Classification of Diseases codes (ICD-10) of: F840, F841, F845, F848, and F849 (childhood autism, atypical autism, Asperger’s syndrome and pervasive developmental disorder-unspecified, respectively). Subjects were considered a case if any of these codes were present.

Controls were defined as never having register evidence of any of these ASD diagnostic codes. Ten controls per case were individually matched on birth month and year. Matching on birth month and year assured that controls have the same length of follow-up time as a case, and thus the same opportunity to acquire an ASD diagnosis and be entered in the register. Matching on birth month also controlled for birth seasonality, though this was not anticipated to be a strong confounder.

*Exposure definition*

Pharmacologic exposure information was drawn from the Danish Drug Prescription Register (DDPR). The DDPR contains data on all dispensed medication from any pharmacy, except hospitals’ dispensaries, in Denmark. Information extracted
included number of packets dispensed, dispense date, drug code using the WHO Anatomical Therapeutic Chemical classification system (ATC), name of drug, number of units in the container, strength of the drug, and defined daily dosage (DDD). The drug codes included in the analyses are listed in (see Table A in the Appendix for medication codes). The DDPR includes ATC and DDD codes for the expressed purpose of facilitating drug utilization research [24] and these coding schemes have been employed in a variety of studies [25-28].

Data for each subject were assembled for the three months prior to estimated-date-of-conception (EDC) to birth. EDC was estimated from the Danish Medical Birth Register (DMBR), which calculates EDC based on mother’s self-reported last menstrual period but corrects with an early ultrasound estimate if the woman reported contraceptive use in the four months prior to conception, had irregular periods, or an abnormal last menstrual period. Exposure windows were defined as preconception (90 days prior to EDC), first trimester (within 90 days after EDC), second trimester (within 90-180 days after EDC), and third trimester (180 days after EDC to delivery date). A child was considered exposed during any window if the dispense date fell within the specified exposure period or the number of days supplied overlapped any portion of the time period.

To calculate the cumulative dose dispensed during a particular exposure window, we first calculated the prescribed dose by multiplying the strength of the medication by the number of units in the container to give the total dose in the package, which was then divided by the number of DDD in the package. The cumulative dose over each exposure
period of interest was divided into quartiles (low, medium, medium-high, and high) based on the distribution in the exposed controls.

To calculate duration of use for each prescription, the number of packets dispensed was multiplied by the DDD in the package. Duration was dichotomized into two categories representing use for more or less than half (0-45 days and ≥45 days) in each exposure window. The reference group for estimating associations of dose and duration of use was no exposure during any period of interest.

**Covariates**

Candidate covariates include parental age, parental psychiatric history, gestational age, birth weight, parental history of autoimmune disease, maternal infection during pregnancy, number of live births, obstetric complications, and family socioeconomic status (a combination of parental income and highest level of education). These were identified from available data in Danish Fertility Database, DMBR, and DNHR. These covariates were chosen *a priori* because they are potential risk factors for ASD [21, 29-35]. Family socioeconomic status was measured as a sum of father’s educational level, mother’s educational level, father income, and mother income. Education for each parent was categorized and scored from 1-3 and income from 1-4. Each parent’s socioeconomic status was a summation of income and education scores, and family socioeconomic status a sum of the mother and father’s scores (score ranges from 1-14).

Maternal psychiatric history prior to delivery was obtained by searching DCPR records (available back through 1973). Psychiatric conditions that are indicators for SSRI use were given special consideration. One variable was created for history of
maternal depression, the principal indication for SSRI use, and another for the presence of any other indication, including anxiety, obsessive-compulsive disorder, phobia, adjustment disorder and schizophrenia in the register. DCPR records from 1973-1993 used ICD-8 codes with ICD-10 codes used thereafter (appendix Table Y lists specific codes).

Analytic method

Conditional logistic regression analysis was conducted to estimate unadjusted and adjusted associations with no SSRI exposure during any period of interest as the reference group. Parental age and sex of the child were forced in all adjusted models as covariates. Other covariates were included only if they resulted in a 10% or greater change in unadjusted log odds ratios when added individually. To guard against joint confounding, any excluded covariate was added back to the adjusted model and retained if the log odds ratios changed by 10%. Additional adjustment for maternal depression and the term for other conditions associated with SSRI use was conducted to control for confounding by indication. To further consider confounding by indication, we also examined the SSRI effect in two restricted samples: mothers with a depression history and mothers with a history of either depression or other psychiatric conditions potentially indicating SSRI use.

Additional Sensitivity Analyses

A number of other sensitivity analyses were also conducted. First, we used simulation analyses to explore the effects of under-reporting of SSRI use and depression
in the register. For each simulation, a Monte Carlo approach was used simulating 1,000 datasets to randomly assign an unexposed mother to being exposed from a normal distribution. From each of the 1,000 simulated datasets we obtained the OR and then took 1,000 samples from each OR normal distribution. We then took the median effect estimate from these 1,000,000 samples as the OR and the 2.5% and 97.5% percentile to estimate the 95% confidence interval. Models used in the simulations were unconditional logistic regressions adjusting for the matching variables as covariates.

Because the observed SSRI exposure prevalence (0.7%) was below published estimates for other Nordic countries [22] we completed simulations increasing the observed prevalence of SSRI exposure from 0.7% to 3%. We assumed under-reporting was non-differential with respect to outcome by keeping sensitivity and specificity equal in case and control groups. Specificity was assumed to be 100%, because it is unlikely a prescription for an SSRI would be documented in the register when none was given, and sensitivity was assumed to be 26% because that corresponds to a true SSRI use prevalence of 3%.

Simulations also examined the impact of under-ascertainment of maternal depression (which would lead to incomplete control for confounding by indication) because published estimates of the prevalence of maternal depression during pregnancy in Europe range from 3-17% [14, 36-38] - higher than our observed prevalence of 0.6%. Simulations were done increasing maternal depression prevalence to 5% and then 15%. Maternal depression under-reporting was assumed to be non-differential with respect to ASD case status because depression is assessed prior to the birth of the child and, thus, could not be influenced by subsequent diagnosis of the offspring. Specificity was
assumed to be 100%; in other words, all mothers truly without a history of depression where assumed to be correctly classified as without depression in the register, and sensitivity was set to 4.7% to reflect the under-reporting needed where the true depression prevalence of 15%.

In addition, since outpatient data were added to the DCPR in 1993, we wanted to ensure that our estimates were not influenced by this change. Consequently, we reanalyzed data limited to the birth cohort born between 1998 and 2002 whose outcome definitions were based on both inpatient and outpatient data. Because some time was likely needed for any impact of the inclusion of outpatient data to be felt, we chose to restrict the birth cohort at 1998 rather than 1993. Then, finally, we also compared effect estimates based on different criteria for considering an individual as exposed during a specific window. In principal analyses, exposure within a window (e.g., first trimester) was defined as any SSRI use during that window regardless of whether SSRIs were also used in other windows. In our sensitivity analyses we considered an alternate definition where only individuals with medication use exclusively during that window were considered exposed.

Results

Description of Study Sample

There are 5215 children diagnosed with ASD and 52150 controls included in the study sample for analyses for exposure anytime during pregnancy. Children with ASD were more likely than controls to be male, have older parents, and have a mother with a
diagnosis for depression before to the birth of the child (Table 1). In the source population, which consisted of all live births from January 1996 to December 2006 the frequency of any ASD was 0.86%. For each of the exposure periods examined, similar patterns in descriptive statistics across case and control groups were observed (Table 1).

In each exposure period of interest, cases had a higher frequency of SSRI exposure compared to controls. For example, during the entire pregnancy period 1.5% (n = 76) of cases compared to 0.7% (n = 356) were exposed to SSRIs in utero. In addition, the highest exposure frequency occurred during the preconception period with 1.7% (n = 89) cases and 0.8% (n = 413) controls exposed.

**Associations of any SSRI use with ASD**

Table 2 presents conditional logistic regression results for dichotomous SSRI exposure in various exposure windows. Inclusion of no additional covariates changed the log odds ratios 10% or more in any model. Consequently, the four models presented for each exposure period of interest are a base model that includes only covariates for the matching variables (unadjusted Model), a model that adjusts additionally for parental age and sex of the child (Adjusted Model 1), a model that includes additional adjustment for indication of maternal depression (Adjusted Model 2), and a model that adjusts for parental age, sex of the child, and the presence of the broader range of SSRI indicating conditions (Adjusted Model 3).

Table 2 shows results for any SSRI exposure in the various exposure windows of interest for any ASD. Measures of association consistently suggest approximately doubling of risk for any ASD regardless of exposure window. The adjusted odds ratio of
any ASD for any SSRI exposure during pregnancy was 2.0 [95% CI, 1.6-2.6]). Effect estimate and confidence interval widths do not change dramatically with adjustment for other covariates. For example, the largest SSRI exposure effect estimates were seen for third trimester exposure was 3.1 (95% CI 2.2-4.5) and parental age and child gender-adjusted effect estimates were 3.1 (95% CI 2.1-4.5). Further adjustment for maternal depression or any SSRI indication reduced OR estimates to 2.7 (95% CI 1.8-4.0) and 2.5 (95% CI 1.7-3.7), respectively. Adjustment for the broader range of SSRI indications resulted in only slight additional attenuation of effect estimates.

**Analyses of SSRI duration and dose of use**

As shown in Table 3, case median duration of use during pregnancy was 119 days, which was higher than that for controls (75 days). Table 4 presents logistic regression results for exposure duration. The models presented adjust for the same covariates as described above for any exposure since the addition of covariates in these models, similarly, did not change duration effect estimates appreciably. As shown, SSRI use longer than 45 days, in each exposure window except for the third trimester, regardless of exposure period, resulted in slightly higher odds ratios than exposure of 45 days or less compared to the unexposed reference group. In the adjusted model 3 exposure to SSRIs during the first trimester for less than 45 days resulted in an odds ratio of 1.6 (95% CI 1.0-2.6), while exposure greater than or equal to 45 days had an odds ratio of 2.4 (95% CI 1.7-3.3) compared to children who were unexposed. No duration response was suggested for third trimester exposure. In these same models, exposure to SSRIs during the third trimester for less than 45 days had an odds ratio of 2.9 (95% CI
1.2-6.9), which was similar to exposure for greater than or equal to 45 days (OR 2.9; 95% CI 1.9-4.4), both compared to those in the unexposed reference group.

As shown in Table 5, the median cumulative dose for cases over the entire pregnancy period was 2710.0 mg, which was higher than controls (2000.0 mg). Overall, cases had a higher median cumulative dose than controls in each exposure period. Table 6 show logistic regression results for dose. Adjusted models are parameterized similarly to those for any exposure and exposure duration because the introduction of additional covariates did not change dose effect estimates. The highest dose categories tended to have slightly higher effect estimates in comparison to the unexposed reference than did lower dose categories; the exception being for first trimester exposure.

To further explore confounding by indication, logistic regression models were refit to datasets including, first, only mothers with a diagnosis of depression, and then only mothers with depression and other psychiatric conditions associated with SSRI use listed above in the methods before the birth of the child. In both these restricted samples, the SSRI effect estimates were close to the null, but had broad confidence intervals (Tables 8 and 9). In the sample of mothers with a history of depression, models adjusting for parental age and sex of the child resulted in an odds ratio of 0.9 (95% CI 0.4-1.8) for exposure during the pregnancy period compared to those who were unexposed. Odds ratio estimates for the mothers without SSRI indications where close to the estimates reported for the full data sets (data not shown).

*Additional sensitivity analysis*
Table 9 shows the results from simulation analyses exploring the influence of misclassification. The first row presents effect estimates and confidence bounds for any prenatal SSRI exposure from simulations assuming increased SSRI prevalence and non-differential misclassification as described above. Estimates for the full sample were very close to the comparable (Model 1) adjusted estimate in Tables 2, suggesting that exposure misclassification is unlikely to have strongly influenced results. The following rows show results from simulations investigating the potential effects of non-differential under-reporting of maternal depression. By increasing the assumed depression prevalence to 5%, the pooled effect estimate, 2.2 (95% CI 1.9-2.4), is extremely close to the comparable (Model 1) estimates shown in Table 2. At 15% assumed depression prevalence, the SSRI effect is only moderately attenuated. Under the assumed 15% depression prevalence scenario we also estimated depression-stratified effects. A positive association was observed in both strata, though the effect was smaller among mothers with depression, with median adjusted odds ratios and confidence intervals of 1.4 (95% CI 0.9-2.4) and 2.1 (1.5-3.0), respectively. These results suggest that the original restriction approach to controlling for confounding by indication may have been influenced by depression misclassification.

Analysis restricting the birth cohort to those born between 1998 and 2002 yielded effect estimates comparable to those observed using our full sample (Table 10). In the sensitivity analysis estimating trimester-specific effects based on considering only those with exclusive medication use in the trimester window as exposed results were comparable to those shown in Table 2 with similar magnitude effect estimates and 95% confidence intervals having substantial overlap (data not shown).
Discussion

In a large population-based case-control study we observed an increased risk of ASD associated with *in utero* exposure to SSRIs. The effect was present in all exposure windows considered and persisted after adjustment for SSRI indications. Results from Croen et al 2011 also observed the same general level of elevated effect estimates in each exposure period, which remained even after statistical adjustment for maternal depression [15]. They did report the strongest effect associated with exposure during the first trimester (adjusted OR, 3.8 [95% CI, 1.8-7.8]), however the confidence intervals of trimester-specific estimates overlapped considerably [15]. Unlike Croen et al 2011, we were able to also explore prenatal dose and duration of use and risk for ASD and we did observe larger effect estimates in the highest dose or duration categories, but these dose and duration response effects were not linear, nor particularly strong.

Adequate control of confounding by indication in treatment effectiveness studies is often a concern, and poses a particular challenge in studying the effects of prenatal SSRI use on ASD risk. Due to our large sample, in addition to adjusting for the indication, we were also able to conduct analyses stratified by the indication and to consider the impact of confounder misclassification. Analyses adjusting for the indication still suggested that there was an independent effect of prenatal SSRI use. Yet, our initial analyses restricted to women with register data indicating a depression diagnosis resulted in an attenuation of the prenatal SSRI effect, supporting the idea that confounding by indication drives the association. However, because there were only 55 cases (just 11 exposed) and 109 exposed controls in this analysis restricted to mothers
with a register diagnosis for depression, the effect estimates here were imprecise. In addition, the pooled (adjusted) estimate is dominated by the data in the much larger strata without maternal depression indication (where the effect was similar to the unadjusted estimate) and the estimate in the strata with maternal depression indication is subject to substantive random fluctuation.

More importantly, these analyses do not consider what may be fairly substantive misclassification of maternal depression. The impact of under-ascertainment of maternal depression, would lead to incomplete control for confounding by indication. Published literature indicates a higher rate of maternal depression in other Nordic countries compared to what we observed in our dataset [14, 22, 36-38] suggesting that depression misclassification may be nontrivial. Our simulation analyses considered the potential impact of this misclassification and the results suggested that prenatal SSRI effect might indeed persist, independent of indication. Furthermore, the attenuation observed in the initial stratified analysis that did not consider confounder misclassification maybe a result of imprecision.

There are a number of biologically plausible explanations for our findings of an association between prenatal SSRI exposure and ASD, which investigate the role of the serotoninergic pathways during development [39-42]. Abnormalities in serotonin metabolism are one of the few consistent biological observations associated with ASD [39] and several lines of evidence suggest that alterations in the serotoninergic neurotransmitter system might be a mechanistic pathway leading to ASD. For example, serotonin (5-hydroxytryptamine, 5HT) has been shown to play an important role in brain development by regulating both serotoninergic outgrowth and maturation of target regions
Elevated levels of serotonin in the blood (5-hydroxytryptamine, 5-HT) have been reported in patients with ASD [40]. In addition, animal models have suggested adverse neurodevelopmental outcomes in the offspring associated with prenatal SSRI exposure [44]. Chronic neonatal exposure to SSRIs in male offspring of timed-pregnant Long Evans rats resulted in reduced serotonin expression that persists into adulthood, which produces changes in the normal maturation of the serotonin system in the brain [45]. Rats exposed to SSRIs during the prenatal period had neuroautonomic alterations in somatosensory structures [44], as well as elevated serotonin levels and receptor binding site levels [46]. Together these findings suggest that mechanistically, prenatal exposure to SSRIs may operate directly on the brain as it develops.

Our study had a number of potential limitations related to information bias. Register case definition may be imperfect, although recent validation study efforts are encouraging [23]. There was also the possibility of exposure and confounder misclassification. In measuring prenatal SSRI use, we had to assume that all the drugs from prescriptions were actually taken and we were unable to capture data on any SSRIs that may have been prescribed during hospital admissions, though in-hospital prescription is infrequent compared to outpatient prescription. Further, maternal depression, a critical SSRI indication appears to be underreported in the register. However, we were able to simulate the impact of misclassification of both the exposure and this important confounder and these analyses suggested that the SSRI effect persisted.

Our analysis benefited considerably from a large study population and available data on pharmacologic exposure reported prospectively to the register. As the Danish registers are population-based, selection bias is not anticipated to be a major limitation in
these analyses. The prevalence of prenatal SSRI use in our data is rather low (0.7%) which at the observed effect size implies that population attributable risks associated with this exposure will be fairly small. With an exposure prevalence of 0.7% and an odds ratio of 1.9, the population attributable risk is only 0.6%. However, if this effect is real, this does suggest that serotonin-mediated biological pathways may be involved in at least some cases of ASD and would suggest further exploration of these pathways, which might reveal other modifiable factors other than prenatal SSRI exposure. For example, a recent animal model study has suggested that the prenatal stress and certain serotonin transporter genotypes may have the combined effect of producing changes in social interaction and social interest in the offspring consistent with those observed in individuals with ASD [47].

The implications of reported associations between prenatal SSRI use and ASD risk need to be weighed against the evidence on health consequences of discontinuation of SSRI treatment. Direct associations between depression and developmental psychopathology have been hypothesized as operating through mechanisms involving the changing intrauterine environment or maternal stress response. Maternal hypothalamo–pituitary–adrenal (HPA) activity in response to psychological stress, which can be caused by depression, has been linked to reduced birth weight [48] and adverse neurodevelopmental effects [49]. There has also been evidence suggesting that depressive mood during pregnancy may be associated with restricted fetal growth [50]. A recent study found that 68% of the women who discontinued antidepressant treatment during pregnancy relapsed in depression, with 50% experiencing recurrence of
depression in the first trimester of pregnancy and 90% by the end of the second trimester [51].

In light of this, the epidemiologic evidence from this and other studies of SSRI and ASD risk should still be cautiously interpreted. Further epidemiologic investigation of the association between prenatal SSRI use and ASD risk is warranted, especially in studies capitalizing on large registers or databases and involving more heterogeneous populations. These studies must explicitly account for confounding by indication and, as demonstrated here, it will likely be important also to consider misclassification of both the exposure and the indication.
References


## TABLES

Table 1. Study demographics for SSRI analysis

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Table 1 (continued). Study demographics for SSRI analysis (continued)

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| Maternal history of psychiatric disorder by rank | Rank 1 | 14 (0.3) | 84 (0.2) | 15 (0.3) | 76 (0.2) | 14 (0.3) | 60 (0.2) | 13 (0.3) | 80 (0.1) | 13 (0.3) | 60 (0.1) |
|                     | Rank 2 | 23 (0.4) | 108 (0.2) | 22 (0.4) | 104 (0.2) | 22 (0.4) | 97 (0.2) | 20 (0.4) | 84 (0.2) | 22 (0.4) | 78 (0.2) |
|                     | Rank 3 | 13 (0.3) | 72 (0.2) | 13 (0.3) | 81 (0.2) | 13 (0.3) | 81 (0.1) | 13 (0.3) | 61 (0.1) | 13 (0.3) | 73 (0.1) |
|                     | Rank 4 | 123 (2.4) | 716 (1.3) | 126 (2.4) | 749 (1.4) | 123 (2.4) | 679 (1.4) | 120 (2.3) | 697 (1.3) | 119 (2.3) | 695 (1.3) |
|                     | No disorder | 5042 (96.7) | 51170 (98.1) | 5052 (96.6) | 51270 (98.1) | 5038 (96.7) | 51183 (98.2) | 5016 (96.8) | 50908 (98.2) | 5011 (96.7) | 50875 (98.2) |

| Family SES | Low | 834 (16.0) | 6474 (12.5) | 835 (16.0) | 6539 (12.5) | 833 (16.0) | 6683 (12.8) | 824 (15.9) | 6523 (12.6) | 821 (15.8) | 6690 (12.9) |
|            | Medium | 2724 (52.2) | 26558 (50.6) | 2727 (52.2) | 26677 (51.0) | 2720 (52.2) | 26317 (50.5) | 2706 (52.2) | 26323 (50.8) | 2704 (52.2) | 25937 (50.1) |
|            | High | 1657 (31.8) | 19109 (36.5) | 1666 (31.9) | 19055 (36.5) | 1657 (31.8) | 19088 (36.6) | 1653 (31.9) | 19074 (36.6) | 1653 (31.9) | 19145 (37.0) |
|            | Missing | 0 (0.0) | 9 (0.0) | 0 (0.0) | 9 (0.0) | 0 (0.0) | 12 (0.0) | 0 (0.0) | 10 (0.0) | 0 (0.0) | 8 (0.0) |
### Table 2. Effect estimates for prenatal SSRI exposure and risk for ASD.

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<th>Outcome</th>
<th># Cases</th>
<th>Exposure Period</th>
<th>Exposed No. (%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted Model 1 OR (95% CI)</th>
<th>Adjusted Model 2 OR (95% CI)</th>
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<td>413 (0.8)</td>
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<td>Third Trimester</td>
<td>39 (0.8)</td>
<td>127 (0.3)</td>
<td>*3.1 (2.2-4.5)</td>
<td>*3.1 (2.1-4.5)</td>
<td>*2.7 (1.8-4.0)</td>
</tr>
</tbody>
</table>

Abbreviations: Autism spectrum disorders (ASD); Odds ratio (OR); Confidence Intervals (CI)
Reference: No exposure during any exposure period
a. Controls for matching variables of child year and month of birth through conditioning
b. Odds ratios adjusted for parental age and sex of the child (and conditions on matching variables of child birth month and year)
c. Odds ratios adjusted for parental age, sex of the child, history of maternal depression (and conditions on matching variables of child birth month and year)
d. Odds ratios adjusted for parental age, sex of the child, history of maternal depression, other SSRI indications (and conditions on matching variables of child birth month and year)

*P*-value < 0.05
## Table 3. Duration of prenatal exposure to SSRIs.

<table>
<thead>
<tr>
<th>Duration of Prenatal Exposure to SSRIs (days)</th>
<th>Any ASD</th>
<th>Controls</th>
<th>Any ASD</th>
<th>Controls</th>
<th>Any ASD</th>
<th>Controls</th>
<th>Any ASD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pregnancy</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>140.1</td>
<td>116.2</td>
<td>63.3</td>
<td>58.5</td>
<td>63.1</td>
<td>56.9</td>
<td>69.6</td>
<td>65.5</td>
</tr>
<tr>
<td>Variance</td>
<td>103.8</td>
<td>96.2</td>
<td>29.7</td>
<td>30.0</td>
<td>29.5</td>
<td>25.6</td>
<td>31.1</td>
<td>31.3</td>
</tr>
<tr>
<td>Mode</td>
<td>28.0</td>
<td>30.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
</tr>
<tr>
<td>Minimum</td>
<td>8.0</td>
<td>1.0</td>
<td>2.0</td>
<td>1.0</td>
<td>4.0</td>
<td>1.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>25(^{th}) percentile</td>
<td>32.5</td>
<td>30.0</td>
<td>36.0</td>
<td>30.0</td>
<td>30.0</td>
<td>28.0</td>
<td>45.2</td>
<td>36.0</td>
</tr>
<tr>
<td>Median</td>
<td>119.0</td>
<td>75.0</td>
<td>78.0</td>
<td>65.0</td>
<td>75.0</td>
<td>60.0</td>
<td>90.0</td>
<td>86.0</td>
</tr>
<tr>
<td>75(^{th}) percentile</td>
<td>259.0</td>
<td>200.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>296.0</td>
<td>299.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
<td>90.0</td>
</tr>
</tbody>
</table>

## Table 4. Effect estimates ASD associated with duration of prenatal SSRI use.

<table>
<thead>
<tr>
<th>Models</th>
<th>Exposure Period</th>
<th>Unadjusted OR(^a) (95% CI)</th>
<th>Model 1 OR(^b) (95% CI)</th>
<th>Adjusted Model 2 OR(^c) (95% CI)</th>
<th>Adjusted Model 3 OR(^d) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preconception</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-45 days</td>
<td>*1.8 (1.2-2.7)</td>
<td>*1.7 (1.1-2.6)</td>
<td>*1.5 (1.0-2.4)</td>
<td>*1.6 (1.0-2.5)</td>
<td></td>
</tr>
<tr>
<td>≥ 45 days</td>
<td>*2.4 (1.8-3.2)</td>
<td>*2.3 (1.7-3.0)</td>
<td>*2.1 (1.6-2.8)</td>
<td>*2.1 (1.6-2.8)</td>
<td></td>
</tr>
<tr>
<td>First Trimester</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-45 days</td>
<td>*1.8 (1.1-2.8)</td>
<td>*1.7 (1.1-2.7)</td>
<td>*1.6 (1.0-2.6)</td>
<td>*1.6 (1.0-2.6)</td>
<td></td>
</tr>
<tr>
<td>≥ 45 days</td>
<td>*2.7 (1.9-3.6)</td>
<td>*2.6 (1.8-3.5)</td>
<td>*2.4 (1.7-3.4)</td>
<td>*2.4 (1.7-3.3)</td>
<td></td>
</tr>
<tr>
<td>Second Trimester</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-45 days</td>
<td>*2.1 (1.1-4.0)</td>
<td>*2.2 (1.1-4.2)</td>
<td>*2.0 (1.0-4.0)</td>
<td>*2.0 (1.0-3.9)</td>
<td></td>
</tr>
<tr>
<td>≥ 45 days</td>
<td>*2.6 (1.7-3.8)</td>
<td>*2.5 (1.7-3.8)</td>
<td>*2.3 (1.6-3.5)</td>
<td>*2.4 (1.6-3.5)</td>
<td></td>
</tr>
<tr>
<td>Third Trimester</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-45 days</td>
<td>*3.8 (1.7-8.5)</td>
<td>*3.1 (1.3-7.3)</td>
<td>*2.8 (1.2-6.7)</td>
<td>*2.9 (1.2-6.9)</td>
<td></td>
</tr>
<tr>
<td>≥ 45 days</td>
<td>*3.0 (2.0-4.5)</td>
<td>*3.1 (2.0-4.7)</td>
<td>*2.7 (1.7-4.1)</td>
<td>*2.9 (1.9-4.4)</td>
<td></td>
</tr>
</tbody>
</table>

The reference group is unexposed

The cut point at 45 days was determined since it was median number of days in each 90-day exposure period for preconception and each trimester.

**a.** Controls for matching variables of child year and month of birth through conditioning

**b.** Odds ratios adjusted for parental age and sex of the child (and conditions on matching variables of child birth month and year)

**c.** Odds ratios adjusted for parental age, sex of the child, history of maternal depression (and conditions on matching variables of child birth month and year)

**d.** Odds ratios adjusted for parental age, sex of the child, history of maternal depression, and other SSRI indications (and conditions on matching variables of child birth month and year)

\(^*\)P-value < 0.05
Table 5. Cumulative dose of prenatal exposure to SSRIs.

<table>
<thead>
<tr>
<th>Dose of Prenatal Exposure to SSRIs (mg)</th>
<th>Any ASD</th>
<th>Controls</th>
<th>Any ASD</th>
<th>Controls</th>
<th>Any ASD</th>
<th>Controls</th>
<th>Any ASD</th>
<th>Controls</th>
<th>Any ASD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnancy</td>
<td>Preconception</td>
<td>1st Trimester</td>
<td>2nd Trimester</td>
<td>3rd Trimester</td>
<td>2nd Trimester</td>
<td>3rd Trimester</td>
<td>2nd Trimester</td>
<td>3rd Trimester</td>
<td>2nd Trimester</td>
</tr>
<tr>
<td>Mean</td>
<td>5694.1</td>
<td>3734.7</td>
<td>2397.8</td>
<td>2115.4</td>
<td>2444.7</td>
<td>2127.3</td>
<td>3278.6</td>
<td>2543.7</td>
<td>3323.9</td>
<td>2716.3</td>
</tr>
<tr>
<td>Variance</td>
<td>10732.0</td>
<td>4477.0</td>
<td>2948.9</td>
<td>2456.5</td>
<td>3200.9</td>
<td>2880.7</td>
<td>5432.1</td>
<td>3209.5</td>
<td>4454.8</td>
<td>2274.2</td>
</tr>
<tr>
<td>Mode</td>
<td>560.0</td>
<td>600.0</td>
<td>1620.0</td>
<td>560.0</td>
<td>1800.0</td>
<td>600.0</td>
<td>380.0</td>
<td>1720.0</td>
<td>1760.0</td>
<td>600.0</td>
</tr>
<tr>
<td>Minimum</td>
<td>180.0</td>
<td>40.0</td>
<td>80.0</td>
<td>10.0</td>
<td>80.0</td>
<td>20.0</td>
<td>40.0</td>
<td>80.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>25th percentile</td>
<td>1000.0</td>
<td>860.0</td>
<td>840.0</td>
<td>700.0</td>
<td>900.0</td>
<td>600.0</td>
<td>995.0</td>
<td>860.0</td>
<td>1180.0</td>
<td>1540.0</td>
</tr>
<tr>
<td>Median</td>
<td>2710.0</td>
<td>2000.0</td>
<td>1700.0</td>
<td>1520.0</td>
<td>1640.0</td>
<td>1400.0</td>
<td>2050.0</td>
<td>1820.0</td>
<td>2100.0</td>
<td>2060.0</td>
</tr>
<tr>
<td>75th percentile</td>
<td>6460.0</td>
<td>5240.0</td>
<td>2900.0</td>
<td>2600.0</td>
<td>2720.0</td>
<td>2680.0</td>
<td>3650.0</td>
<td>3020.0</td>
<td>4100.0</td>
<td>3360.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>82900.0</td>
<td>36950.0</td>
<td>23400.0</td>
<td>25300.0</td>
<td>22400.0</td>
<td>34000.0</td>
<td>34900.0</td>
<td>23500.0</td>
<td>26200.0</td>
<td>17450.0</td>
</tr>
</tbody>
</table>
Table 6. Effect estimates for any ASD associated with cumulative dose of prenatal exposure to SSRI.

<table>
<thead>
<tr>
<th>Exposure Period</th>
<th>Dose^a</th>
<th>Unadjusted OR^b (95% CI)</th>
<th>Adjusted Model 1 OR^c (95% CI)</th>
<th>Adjusted Model 1 OR^d (95% CI)</th>
<th>Adjusted Model 1 OR^e (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preconception</td>
<td>Quartile 1 (&lt;700 mg)</td>
<td>*1.8 (1.1-3.0)</td>
<td>1.6 (0.9-2.7)</td>
<td>1.5 (0.9-2.5)</td>
<td>1.5 (0.9-2.4)</td>
</tr>
<tr>
<td></td>
<td>Quartile 2 (701-1520 mg)</td>
<td>*1.8 (1.1-3.0)</td>
<td>1.6 (0.9-2.7)</td>
<td>1.5 (0.9-2.5)</td>
<td>1.4 (0.8-2.3)</td>
</tr>
<tr>
<td></td>
<td>Quartile 3 (1521-2600 mg)</td>
<td>*2.4 (1.5-3.7)</td>
<td>*2.4 (1.5-3.9)</td>
<td>*2.3 (1.4-3.6)</td>
<td>*2.1 (1.3-3.4)</td>
</tr>
<tr>
<td></td>
<td>Quartile 4 (&gt;2600 mg)</td>
<td>*2.8 (1.8-4.2)</td>
<td>*2.6 (1.7-4.1)</td>
<td>*2.4 (1.6-3.8)</td>
<td>*2.3 (1.5-3.6)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Quartile 1 (&lt;860 mg)</td>
<td>1.6 (1.0-2.8)</td>
<td>1.7 (1.0-3.0)</td>
<td>1.6 (0.9-2.9)</td>
<td>1.5 (0.9-2.7)</td>
</tr>
<tr>
<td></td>
<td>Quartile 2 (861-2000 mg)</td>
<td>*2.1 (1.3-3.4)</td>
<td>*2.0 (1.2-3.4)</td>
<td>*1.9 (1.1-3.2)</td>
<td>*1.8 (1.1-3.0)</td>
</tr>
<tr>
<td></td>
<td>Quartile 3 (2001-5240 mg)</td>
<td>*1.9 (1.1-3.2)</td>
<td>*1.7 (1.0-3.0)</td>
<td>1.6 (1.0-2.8)</td>
<td>1.5 (0.9-2.6)</td>
</tr>
<tr>
<td></td>
<td>Quartile 4 (&gt;5240 mg)</td>
<td>*2.8 (1.8-4.3)</td>
<td>*2.6 (1.6-4.0)</td>
<td>*2.4 (1.5-3.9)</td>
<td>*2.3 (1.4-3.7)</td>
</tr>
<tr>
<td>First Trimester</td>
<td>Quartile 1 (&lt;600 mg)</td>
<td>1.5 (0.8-2.8)</td>
<td>2.3 (1.1-4.7)</td>
<td>1.4 (0.7-2.5)</td>
<td>1.3 (0.7-2.3)</td>
</tr>
<tr>
<td></td>
<td>Quartile 2 (601-1400 mg)</td>
<td>*2.7 (1.7-4.5)</td>
<td>*2.9 (1.7-5.0)</td>
<td>2.8 (1.6-4.7)</td>
<td>*2.6 (1.5-4.4)</td>
</tr>
<tr>
<td></td>
<td>Quartile 3 (1401-2680 mg)</td>
<td>*2.6 (1.6-4.3)</td>
<td>*2.6 (1.5-4.4)</td>
<td>*2.4 (1.4-4.2)</td>
<td>*2.3 (1.4-3.9)</td>
</tr>
<tr>
<td></td>
<td>Quartile 4 (&gt;2680 mg)</td>
<td>*2.5 (1.5-4.2)</td>
<td>*2.2 (1.3-3.7)</td>
<td>*2.1 (1.2-3.5)</td>
<td>*2.0 (1.2-3.4)</td>
</tr>
<tr>
<td>Second Trimester</td>
<td>Quartile 1 (&lt;860 mg)</td>
<td>*2.1 (1.1-4.2)</td>
<td>2.3 (1.1-4.7)</td>
<td>*2.2 (1.1-4.4)</td>
<td>*2.0 (1.0-4.1)</td>
</tr>
<tr>
<td></td>
<td>Quartile 2 (861-1821 mg)</td>
<td>1.9 (0.9-3.9)</td>
<td>*1.9 (0.9-3.9)</td>
<td>1.8 (0.8-3.7)</td>
<td>1.6 (0.8-3.4)</td>
</tr>
<tr>
<td></td>
<td>Quartile 3 (1822-3020 mg)</td>
<td>*2.5 (1.3-4.8)</td>
<td>2.3 (1.2-4.6)</td>
<td>*2.2 (1.1-4.4)</td>
<td>*2.0 (1.0-4.1)</td>
</tr>
<tr>
<td></td>
<td>Quartile 4 (&gt;3020 mg)</td>
<td>*3.2 (1.8-5.9)</td>
<td>3.2 (1.7-6.0)</td>
<td>*2.9 (1.5-5.5)</td>
<td>*2.7 (1.5-5.2)</td>
</tr>
<tr>
<td>Third Trimester</td>
<td>Quartile 1 (&lt;1540 mg)</td>
<td>*3.6 (1.8-7.1)</td>
<td>*3.2 (1.5-6.5)</td>
<td>*2.8 (1.3-5.8)</td>
<td>*2.5 (1.2-5.1)</td>
</tr>
<tr>
<td></td>
<td>Quartile 2 (1541-2060 mg)</td>
<td>*2.5 (1.1-5.3)</td>
<td>*2.2 (1.0-4.8)</td>
<td>1.9 (0.9-4.3)</td>
<td>1.8 (0.8-4.0)</td>
</tr>
<tr>
<td></td>
<td>Quartile 3 (2061-3360 mg)</td>
<td>*2.6 (1.2-5.7)</td>
<td>*3.0 (1.3-6.7)</td>
<td>*2.7 (1.2-6.0)</td>
<td>*2.3 (1.0-5.3)</td>
</tr>
<tr>
<td></td>
<td>Quartile 4 (&gt;3360 mg)</td>
<td>*3.9 (2.0-7.7)</td>
<td>*4.3 (2.1-8.7)</td>
<td>*3.7 (1.8-7.5)</td>
<td>*3.5 (1.7-7.1)</td>
</tr>
</tbody>
</table>

The reference group is unexposed

a. Dose categories were based on the distribution of cumulative dose of exposed controls in the exposure period of interest.
b. Controls for matching variables of child year and month of birth through conditioning
c. Odds ratios adjusted for parental age and sex of the child (and conditions on matching variables of child birth month and year)
d. Odds ratios adjusted for parental age, sex of the child, history of maternal depression (and conditions on matching variables of child birth month and year)
e. Odds ratios adjusted for parental age, sex of the child, history of maternal depression, other SSRI indications (and conditions on matching variables of child birth month and year)

*p-value < 0.05
Table 7. Effect estimates for SSRIs use with any ASD, restricted to mothers with a history of depression

<table>
<thead>
<tr>
<th>Exposure Period</th>
<th>No. (%)</th>
<th>Cases</th>
<th>Controls</th>
<th>Unadjusted OR(^a) (95% CI)</th>
<th>Adjusted Model 1 OR(^b) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preconception (n = 583)</td>
<td></td>
<td>9 (17.0)</td>
<td>96 (18.0)</td>
<td>0.9 (0.4-2.0)</td>
<td>1.0 (0.5-2.3)</td>
</tr>
<tr>
<td>Pregnancy (n = 605)</td>
<td></td>
<td>11 (20.0)</td>
<td>109 (19.8)</td>
<td>1.0 (0.5-2.0)</td>
<td>0.9 (0.4-1.8)</td>
</tr>
<tr>
<td>First Trimester (n = 605)</td>
<td></td>
<td>7 (12.7)</td>
<td>90 (16.4)</td>
<td>0.7 (0.3-1.7)</td>
<td>0.7 (0.3-1.6)</td>
</tr>
<tr>
<td>Second Trimester (n = 605)</td>
<td></td>
<td>5 (9.1)</td>
<td>69 (12.6)</td>
<td>0.7 (0.3-1.8)</td>
<td>0.7 (0.3-1.9)</td>
</tr>
<tr>
<td>Third Trimester (n = 605)</td>
<td></td>
<td>6 (10.9)</td>
<td>68 (12.4)</td>
<td>0.9 (0.4-2.1)</td>
<td>0.7 (0.3-1.9)</td>
</tr>
</tbody>
</table>

The reference group is unexposed

\(a\). Controls for matching variables of child year and month of birth through conditioning

\(b\). Odds ratios adjusted for parental age and sex of the child (matching: child birth month and year)

Table 8. Effect estimates for SSRIs use with any ASD, restricted to mothers with history of depression and other indications for SSRIs

<table>
<thead>
<tr>
<th>Exposure Period</th>
<th>No. (%)</th>
<th>Cases</th>
<th>Controls</th>
<th>Unadjusted OR(^a) (95% CI)</th>
<th>Adjusted Model 1 OR(^b) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preconception (n = 1628)</td>
<td></td>
<td>21 (14.2)</td>
<td>189 (13.0)</td>
<td>1.1 (0.7-1.8)</td>
<td>1.1 (0.7-1.8)</td>
</tr>
<tr>
<td>Pregnancy (n = 1617)</td>
<td></td>
<td>20 (13.6)</td>
<td>164 (11.2)</td>
<td>1.3 (0.8-2.1)</td>
<td>1.1 (0.7-1.9)</td>
</tr>
<tr>
<td>First Trimester (n = 1573)</td>
<td></td>
<td>16 (11.2)</td>
<td>163 (11.4)</td>
<td>1.0 (0.6-1.7)</td>
<td>1.0 (0.6-1.8)</td>
</tr>
<tr>
<td>Second Trimester (n = 1518)</td>
<td></td>
<td>11 (8.0)</td>
<td>82 (5.9)</td>
<td>1.3 (0.7-2.7)</td>
<td>1.2 (0.6-2.4)</td>
</tr>
<tr>
<td>Third Trimester (n = 1518)</td>
<td></td>
<td>11 (8.0)</td>
<td>94 (6.8)</td>
<td>1.2 (0.6-2.3)</td>
<td>1.0 (0.5-1.9)</td>
</tr>
</tbody>
</table>

The reference group is unexposed

\(a\). Controls for matching variables of child year and month of birth through conditioning

\(b\). Odds ratios adjusted for parental age and sex of the child (matching: child birth month and year)
<table>
<thead>
<tr>
<th>Assumed SSRI Prevalence (3%)</th>
<th>Adjusted OR estimates (median) and confidence bounds (2.5 and 97.5 percentiles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assumed Depression Prevalence (5%)</td>
<td>1.9 (1.6-2.6)</td>
</tr>
<tr>
<td>Assumed Depression Prevalence (15%)</td>
<td>2.2 (1.9-2.4)</td>
</tr>
<tr>
<td>Restricted to those with depression</td>
<td>1.9 (1.5-2.4)</td>
</tr>
<tr>
<td>Restricted to those without depression</td>
<td>1.4 (0.9-2.4)</td>
</tr>
<tr>
<td>Adjusted OR estimates (median) and confidence bounds (2.5 and 97.5 percentiles)</td>
<td></td>
</tr>
<tr>
<td>Restricted to those without depression</td>
<td>2.1 (1.5-3.0)</td>
</tr>
</tbody>
</table>

a. Unconditional logistic regressions models adjusting for child birth year and month, parental age, sex of the child, and maternal history of depression.
b. Reference group is no exposure during pregnancy
c. Monte Carlo with simulating 1,000 datasets to obtain the median OR estimates and the 2.5% and 97.5% percentile OR estimates as the confidence interval.

<table>
<thead>
<tr>
<th></th>
<th>ASD Cases (N = 3249)</th>
<th>Controls (N = 32490)</th>
<th>Unadjusted OR$^a$ (95% CI)</th>
<th>Adjusted Model 1 OR$^a$ (95% CI)</th>
<th>Adjusted Model 2 OR$^b$ (95% CI)</th>
<th>Adjusted Model 3 OR$^c$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed No. (%)</td>
<td>40 (1.2)</td>
<td>172 (0.5)</td>
<td>*2.3 (1.7-3.3)</td>
<td>*2.5 (1.6-3.8)</td>
<td>*2.1 (1.5-3.0)</td>
<td>*2.0 (1.4-2.8)</td>
</tr>
</tbody>
</table>

Abbreviations: SSRI, selective serotonin reuptake inhibitors; ASD, autism spectrum disorder; OR, odds ratio; CI, confidence interval.

a. The reference group had no exposure during any exposure period.
b. Odds ratios were adjusted for parental age and child sex (matching on child birth month and year)
c. Odds ratios were adjusted for parental age, child sex, maternal depression (matching on child birth month and year)
d. Odds ratios were adjusted for parental age, child sex, maternal depression, and other SSRI indicators (matching on birth month and year)

*P-value < 0.05
CHAPTER 5: INTERACTION BETWEEN SPECIFIC CANDIDATE GENOTYPES AND IN UTERO B2AR AGONIST EXPOSURE ON THE RISK FOR AUTISM SPECTRUM DISORDERS
Abstract

Objective: There has been strong evidence of a heritable component in autism spectrum disorder (ASD) etiology, however no definitive gene has been identified. Similar to the complexities in identifying the genetic influence of ASD, environmental risk factors for the disorder is also largely unknown. We explored whether the effects of beta-2 adrenergic receptor (B2AR) agonists in utero are modified by susceptible genotypes - Gly16 and Glu27 polymorphisms in the ADBR2 gene, on the risk for ASD.

Methods: This gene-environment interaction analysis was based on unmatched case-control data from the Study to Explore Early Development (SEED). Genotype information came from planned genotyping using the Illumina Infinium HD Assay. Unconditional logistic regression was used to estimate adjusted odds ratios and 95% confidence intervals. Two statistical approaches to test for interaction were applied – multiplicative model and the multiplicative two-degree of freedom joint test.

Results: In models assessing the interaction between the any B2AR agonist use and presence of the Arg16Gly polymorphism we found that compared to children who were homozygous for Arg16 and were unexposed to B2AR agonist drugs in utero, children who were either heterozygous or homozygous for the Gly16 and exposed to the drug had an effect estimate of 1.1 [95% CI, 0.6-2.3]) associated with risk for ASD. In interaction models between exposure and the Gln27Glu genotype we found an unadjusted OR of 1.0 (95% CI 0.4-2.4) with adjusted models to be slightly higher. The likelihood ratio test for the unadjusted model for the joint effect of Gln27Gly polymorphism was significant (p=0.01), with adjusted models reaching marginal significance.
Conclusion: These preliminary results do not confirm a gene-environment interaction effect, however it does encourage further exploration to untangle modest risk estimates found in exposure to B2AR agonist use during pregnancy. Results from this study encourage further research on maternal pharmacological exposures and the potential for interaction with susceptible genotypes.
Introduction

Autism spectrum disorders (ASD) are a group of developmental disabilities characterized by core deficits in three domains: social interaction, communication, and repetitive or stereotypic behavior [1]. The disorder is largely of unknown cause, and there is variability of impairment among individuals with ASD [2]. Although ASD has moderate to high heritability [3], several reviews of autism genetics have suggested the possibility for an interaction between genetic mechanisms and environmental exposures in autism etiology [4, 5]. Particularly, recent studies have focused on exposures during the prenatal period such as hazardous air pollutants [6], other prescription drug exposures during pregnancy [7-9], and pesticides [10]. A recent, large population-based twin study reported found a dizygotic twin concordance rate that was 0.77 for 45 monozygotic pairs (95% CI, 0.65-0.86) and 0.31 for 45 dizygotic pairs [11], which is substantially larger than recently reported non-twin sibling recurrence risk (18.7%) [12]. This suggests not only that the environmental contribution to autism etiology is substantial but that shared environmental factors unique to twins as opposed to other siblings (eg, those occurring during prenatal period) may be of particular importance.

As briefly mentioned, prenatal pharmacologic exposures have been implicated as potential risk factors for ASD [7, 9, 13]. In particular, concerns have been expressed that exposure to B2AR agonist drugs during pregnancy may increase the risk for neurodevelopmental disorders in offspring[14-16]. Terbutaline has been used off-label as tocolytic agents since they may delay early contractions[17]. Along with B2AR agonists drugs used as a tocolytic, drugs such as salmeterol and formoterol are indicated to reduce asthma exacerbations and provide asthma control in adults [18]. For women with certain
chronic medical conditions such as asthma the use of drugs is essential. The prevalence of B2AR agonist drug exposure (including both tocolytic and asthma indication) during pregnancy in the U.S. is estimated to be 4%-20% [19, 20]. B2ARs within the catecholamine system are required for normal nervous system development and for the function of both neural and non-neural tissues in the adult. If used during pregnancy, these drugs can impact the fetal brain by crossing the placenta, resulting in the disruption of either replication or differentiation of the developing neurons [21].

Prior human studies of in utero B2AR agonist exposure have been small but suggest an increased risk associated with ASD [22]. Epidemiological findings are limited however indicate a moderate risk associated with ASD [22, 23]. Connors et al 2005 reported an increased concordance for ASD in dizygotic twins exposed to terbutaline [22]. In a more recent study, the findings from Croen et al 2011 also suggest a modest increased risk associated with ASD, with the highest effect estimated for exposure during the first and second trimester of pregnancy[23]. Moreover, Croen et al 2011 also found the preconception period to be associated with the highest risk overall, however all their estimates had wide confidence intervals.

The observed main effects described in the epidemiological studies presented above are subtle in pregnancies exposed to B2AR agonists. It is possible that if a subgroup is more susceptible to these exposures, effects in those groups will be more pronounced. The beta 2-adrenergic receptor gene (ADBR2), located on chromosome 5q31-32, have been shown to influence B2AR receptor sensitivity and consequently may enhance susceptibility to the neurodevelopment effects of B2AR exposure [24]. There are two common polymorphisms known to influence receptor function [24]. On codon
16 (rs1042713), a G for A substitution encodes the amino-acid glycine rather than arginine (Arg16Gly), and a G for C substitution at codon 27 (rs1042714) codes for glutamic acid rather than glutamine (Gln27Glu) [25]. A study by Cheslack-Postava et al 2007 investigated these known polymorphisms in this gene and autism risk using 331 autism case parent trios from the Autism Genetic Resource Exchange (AGRE). Cheslack-Postava et al found a Glu27 homozygous genotype to be associate with increased autism risk, while Gly16 did not reach statistical significance [26]. If the true etiologic mechanism is one of gene-environment interaction, estimated genetic main effects, like environmental main effects, may be attenuated.

The objective of this study is to directly explore interaction between the ADRB2 gene and B2AR exposure during pregnancy in a population-based case-control sample.

Methods

Population

The Study to Explore Early Development (SEED) is a population-based case-control study of children born between September 1, 2003-August 31, 2005 (ages 2-5) and currently residing in the six study areas (San Francisco Bay area, Denver metropolitan area, Philadelphia metropolitan area, northeast Maryland, central North Carolina, and the Atlanta metropolitan area). This analysis includes the first 337 confirmed cases. Cases were identified from multiple clinical and educational developmental service providers based on a recorded diagnosis of ASD, one or more select diagnoses associated with ASD, or an ASD/ASD-related exceptionality from early intervention/special education services. All potential cases that were identified through
these services were then given the Social Communication Questionnaire (SCQ) and were later administered diagnostic evaluation such as the Autism Diagnostic Observation Schedule using the new algorithms, and the Autism diagnostic Interview-Revised Cases. In addition these potential cases were clinically observed and further divided into several sub classifications to characterize them by degree of observed or reported, or both, ASD behavioral symptoms based on the standardize diagnostic assessments mentioned above [27]. Controls (n = 521) were randomly selected from birth certificates files. Controls were also given the SCQ, and those with negative SCQ scores were assigned the control group workflow. If during their evaluation, a clinician suspected them of having ASD, they were then further tested for the disorder [27].

**Genotyping**

DNA was obtained from blood samples collected from study visits completed at enrollment. In cases where children were unable or unwilling to give blood, buccal samples were collected. Blood samples are collected by trained phlebotomists, processed and packaged for shipment following standardized protocols, and shipped via overnight courier to the Central Lab. Buccal sample kits are mailed to participants, the collection is self-administered, and samples are mailed directly to the Central Lab.

Candidate genotypes of interest were elected from GWAS genotype. GWAS genotyping was carried out by the Johns Hopkins SNP Center (joint effort with the Center for Inherited Disease Research) using the Illumina HumanOmni1-Quad panel. One ug of genomic DNA was genotyped on the Illumina Omni 1M Quad platform. This is a highly accurate and reproducible platform that measures over 1 million loci in the
human genome. After genotyping, several quality control measures were applied including a sample call rate of 98% and SNP call rate threshold of 96%. SNPs that were monomorphic or had a minor allele frequency of <0.01 were removed. Samples with sex discrepancies, IBS relatedness pi-hat score that is >0.25, and SNPs deviating from the Hardy Weinberg Equilibrium in the control samples (p<10^{-8} by Fisher exact) were removed from the downstream analysis.

Beginning and end coordinates of the ADBR2 gene were initially identified based on the default provided in the UCSC Genome Browser website (genome.ucsc.edu). We then extended the 5’ and 3’ coordinates to include contiguously correlated SNPs that were typed in HapMap (hapmap.org), with the rationale being that important regulatory regions may exist beyond the traditional known promoter and UTR regions of the gene and that these regulatory regions would likely co-travel and therefore be highly correlated with the traditional gene coordinates. After we used HapMap information to revise the start and end coordinates of the ADRB2 gene (chr5:148184770-148193699), 13 known HapMap SNPs were available within this extended region, seven of which were present in the Human Omni-Quad1 Marker genome-wide panel. However, these seven SNPs provide 100% coverage of the ADRB2 gene when comparing to HapMap.

Indicator variables for candidate genotypes of interest were developed as follows. The Arg16Gly polymorphism was one of the seven SNPs available in the Illumina panel. So for that SNP, a dichotomous genotype exposure variable was created directly from the presence of one or two copies of Gly allele contrasted against homozygous Arg/Arg reference. For the Gln27Glu polymorphism, which was not included in the panel, an imputation procedure was performed using the IMPUTE2 software and 1000 Genomes.
on all individuals [28]. We extended the gene region by 10ks on each end of the gene to ensure that we had sufficient information to get the right haplotype phasing from each SEED individual. A dichotomous genotype exposure variable for this SNP was created contrasting the presence of one or two copies of the Glu allele. The reference group was homozygous for the Gln allele.

*Phenotypic Data and Covariates*

Pharmacological exposure data was self-reported, and came from the SEED Caregiver Interview. A trained SEED interviewer administered the questionnaire to the mother and asked her to recall events during her pregnancy. They were asked generally between three months prior to the estimated date of conception to the time she stopped breastfeeding. Questions regarding exposure to B2ARs medications indicated for asthma were separate from those used as a tocolytic. Questions related to exposure first required that the participant recall whether or not they had experienced any of the listed illness. Once she had indicated an illness the question then prompted the interviewee to ask what medications were used to treat the illness. Since there could be multiple conditions that indicated for B2AR agonist drug use, we reported the existing conditions a mother had for those that were taking B2AR agonist drugs. A single dichotomous variable was created as either exposed or unexposed to B2AR agonist drugs anytime during pregnancy. The limited sample size did not permit trimester specific analysis since there were very few exposed mothers in each group.

Covariates included demographic factors that were strongly associated with ASD risk (parental age and sex of the child) and self-reported indications for B2AR agonist
drug use. They were selected based on prior literature suggesting them as potential risk factors for ASD [29-32]. In regards to race and ethnicity, the decision to adjust for these covariates were motivated by the idea that common ancestry is related to genotypes and perhaps to exposure through culturally mediated mechanisms. Population stratification occurs if the gene under study shows marked variation in allele frequency across different groups of the population and if the groups also differ in their baseline risk of the disease [33]. To ensure that we were correcting for this, the CGI included self-reported data on race/ethnicity categorizing subjects into several categories, which enabled participants to include mixed races as well, and thus there were several categories a person could include. However, because race and ethnicity were self-reported we suspected problems with accuracy, misclassification that is often not at random, and missing data on this variable [34]. Therefore using this variable alone could potentially hinder our ability to adequately control for population stratification.

The principal component analysis can be used to account for the different proportional contributions from various ancestral lineage due to population structure [35]. We used the top two principal components values (PCV) as covariates derived using the software EIGENSTRAT. This software detects and corrects for population stratification in GWAS, and is based on the principal component analysis that models differences between cases and controls along a continuous axes which is specific to a candidate markers variation across ethnic differences [36]. By incorporating the values from this analysis as covariates into our models we were able to correct for large differences in allele frequency across ancestral populations. It was especially important to use this in our analysis since we do not have a homogenous population. Our population included
children with ancestors from East Asia, south Asia, Africa, and the Europeans. Without correcting for this, we could potentially have false positives that are due to the differences between cases and control groups that are actually related to ancestral background, and not disease status. Once computed, each sample has values that correspond to a position on a coordinate system that effectively clusters samples together by ethnic similarity. This was then used to identify ethnic outliers as well as the structure of the genetic data. These two PCV numbers were used as continuous variables were then incorporated in all the models as covariates to see if they changed the log odds ratio by 10%, and then retained in the final model.

Statistical Analyses

Descriptive analyses compared cases and controls on covariates. Unconditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI). Adjusted models were constructed by individually adding parental age, child sex, self-reported race ethnicity, indications for B2ARs agonist, and PCV from the principle component analysis to the unadjusted model that included terms for the genotype, B2AR agonist drug exposure, and interaction term(s). A covariate was retained in the final model if its addition generated a 10% change in any of the beta estimates for main or interaction effects. Models were also fit forcing in the PCV variables to provide adjustment for potential population stratification. Separate models were constructed for the Arg16Gly and Gln27Glu polymorphisms.

We explored gene-environment interaction two ways. First we used a conventional interaction term approach testing for multiplicative scale interaction in the
full case control data set. Next, we used the two-degrees of freedom joint test of marginal genetic effects and gene-environment interactions [37]. This approach is not an explicit test of gene-environment interaction per se, but can be more efficient than conventional approaches for detecting multiplicative interaction when interaction is quantitative rather than qualitative. The test uses the same likelihood ratio test as the test of for gene environment interaction but constrains both the beta coefficient for the interaction term and the gene under the null so that the degrees of freedom are two.

Results

We estimated the unadjusted odds ratio from the unconditional logistic regression for the marginal effect of the B2AR agonist drug exposure anytime during pregnancy, the marginal effect of each genotype separately (Arg16Gly and Gln27Glu), and the interaction of the gene and exposure. There were no covariates that individually resulted in a 10% change in the log odds ratios. The two models presented for exposure to B2AR agonist drugs anytime during pregnancy, the genotype, and the interaction form consisted of the unadjusted (no additional covariates), and the model adjusted for PCVs.

Description of study sample

There were a total of 858 children with complete case status. However for the genetic information, there was one child with missing Arg16Gly information, and 61 children with missing Gln27Glu information. Only complete case, exposure and genetic information were used in all models for interaction (ie Total N for Arg16Gly = 857; Total N for Gln27Glu = 797). Children with ASD (n = 337) were more likely than controls
(n=521) to be male (81.9% vs 53.7%) and have mother’s who have reported using a B2AR agonist drug during their pregnancy (6.5% vs 4.6%) (Table 1). The parental age of the child in both the case and control groups was similar. The genotype counts and frequencies for both polymorphisms are shown in Table 2 and are similar to population frequencies observed in the literature [26].

Associations of any B2AR agonist use, susceptible genotype and their interaction with ASD

Table 3 shows the marginal effects of B2AR agonist use and both susceptibility genotypes associated with ASD risk in unadjusted and PCV-adjusted models. Point estimates for B2AR effects are above one while estimated genotype effects are protective. However, all 95% confidence intervals around all adjusted odds ratio estimates include 1.0.

Table 4 displays the B2AR agonist drug effect stratified by candidate susceptibility genotype from unconditional logistic regression models including interaction terms. The pattern of the effect estimates suggests qualitative multiplicative scale interaction between Arg16Gly and B2AR agonist drug use with unadjusted and PCV-adjusted odds ratios below 1.0 among those with the homozygous reference genotype and above one among those with either AG or GG genotype. There does not appear to be any qualitative multiplicative scale interaction for the Gln27Gly and B2AR with unadjusted and PCV-adjusted odds ratio both being above 1 for both the homozygous reference genotype and heterozygous or homozygous variant type. In both the unadjusted and PCV adjusted models, the confidence intervals were wide and the
value on the interaction term was greater than 0.05. From the two-degree of freedom test, the unadjusted and PCV adjusted models had p-values that were consistently smaller than the traditional multiplicative models. The p-value was below 0.05 for the unadjusted interaction between B2AR and Gln27Gly only.

Discussion

This analysis was completed using genotyping on DNA collected from the first 331 cases and 551 non-impaired controls in the SEED study population. While these preliminary results do not confirm a gene-environment interaction effect; however, the findings encourage further exploration of interaction hypotheses with respect to B2AR agonist use during pregnancy. We did find estimates for the marginal effects of B2AR agonist use during pregnancy similar in magnitude to those from an analysis of a large Danish register-based sample reported elsewhere in this dissertation (Chapter 3) as well to those reported by Croen et al 2011 [23] in a case-control study of Kaiser Permanente members. In regards to the two SNPs of interest, the Arg16Gly and Gln27Glu, the marginal effect estimates for carriers suggested a protection – a finding counter to that reported by Cheslack-Postava et al 2007 [26]. The marginal estimates we observed in the present analysis (0.7 for Arg16Gly and 0.6 for Gln27Glu) were very close to the lower bound, but fell outside the confidence limits reported by Cheslack-Postava et al 2007 (0.85–2.07 and 0.97–3.47, respectively). However our marginal estimates had very wide confidence intervals themselves including both protective and risk conferring effects.
We results suggest that the ASD risk associated with prenatal B2AR exposure may be modified by ADRB2 genotype; however, these findings are very preliminary given the imprecision of effect estimates. Animal models have suggested the existence of a biological mechanism by which the ADRB2 gene polymorphisms and exposure to B2AR agonist during pregnancy may interact in a manner that influences neurodevelopment. Experiments have shown that β2- adrenergic receptors (B2AR) in the central nervous system are present during gestation, with neurotransmitters acting as growth factors [22, 38]. During gestation, overstimulation of the receptors may have harmful effects on the development of the brain and the peripheral nervous system [38, 39]. Mature receptors have mechanisms protecting against over-stimulation; however, fetal receptors do not have the ability to regulate imbalances and may in fact become sensitized to agonist exposure [40]. B2AR agonist drugs administered to pregnant rats during the second trimester of gestation alters neural cell replication and differentiation, synaptogenesis, and expression of synaptic proteins involved in neurotransmission [40]. At the same time, polymorphisms in the β 2-adrenergic receptor gene (ADBR2) have been shown to influence fetal B2AR sensitivity and consequently may enhance susceptibility to the neurodevelopment effects of B2AR exposure [24]. Cardiovascular disease research supports the notion that ADBR2 polymorphisms may influence the biologic response to B2AR agonists [41]. The Gly16 substitution has been associated with faster activation of the cAMP formation [42, 43] in humans with homozygous Gly16 have a greater cardiac function and vasodilation in response than those with Arg16 [41, 44]. The Glu27 allele has been shown to increase resting sympathetic nerve traffic [45]. Compared to subjects who are homozygous for the Glu27 allele, subjects with the Gln27
substitution had a lower baseline blood flow and had significantly attenuated increase in the forearm blood flow, especially in veins [46]. Studies investigating the possibility for interaction have found that compared to Arg16 homozygotes, Gly16 homozygotes demonstrate significantly greater blood flow responses to systemic infusions of beta 2-agonists [44].

Although biologically plausible, the suggestion of interaction here needs to be interpreted with a substantial measure of caution. The direction of the marginal genotype effects we observed suggest protection with respect to ASD risk – a finding that is counter to expectation but may be a byproduct of the small sample size (as confidence limits also included risk conferring estimates). A previously published study using of 331 autism case parent trios from the Autism Genetic Resource Exchange (AGRE) found Glu27 homozygous genotype to be associated with increased autism risk and, although the Gly16 effect did not reach statistical significance, the odds ratio estimate for that genotype was also above one [26]. While the marginal genetic effect was not consistent with this previous work, we did observe stronger B2AR effects in the strata of individuals with these higher activity genotypes. Though consistent with the a priori interaction hypothesis, as has been mentioned, our power to detect interaction in this small sample is limited. The p-values for the two-degrees of freedom test were smaller than the p-values from the conventional multiplicative interaction models, which is consistent with the expected performance of this test [37].

Other limitations in this study include the possibility of B2AR agonist drug exposure misclassification since these data came from a maternal interview. However, retrospective self-report of pharmacologic exposures during pregnancy are most accurate
when questions are designed around indications for use, as was done here, and when recall periods are not overly long [47]. The SEED study employed a rigorous case confirmation approach [48]; consequently, there is unlikely to be misclassification of outcome. Because of the limited sample size we were unable to consider other potential confounders of the association between B2AR agonist drug exposure and ASD (e.g., indicating diagnosis).

Nonetheless, this is the first direct exploration of gene-environment interaction with respect to a prenatal pharmacologic exposure and ASD risk. Although there have been general concerns raised surrounding the use of B2AR agonist drugs in pregnancy and ASD risk [14] the limited epidemiologic evidence is equivocal [23] and the investigation of the continued exploration of the existence of genetically susceptible subgroups appears warranted. This analysis capitalized on initial genotyping data available in the SEED sample and as additional genotyping is completed, this exploration of gene-environment interaction should be revisited.
References


### Table 1. Descriptive characteristics of cases and controls for gene-environment analysis

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>337 (39.3)</td>
<td>521 (60.7)</td>
</tr>
<tr>
<td>Maternal age (median)</td>
<td>32.1</td>
<td>32.9</td>
</tr>
<tr>
<td>≤25 years</td>
<td>44 (13.1)</td>
<td>47 (9.0)</td>
</tr>
<tr>
<td>26-30</td>
<td>83 (24.6)</td>
<td>102 (19.6)</td>
</tr>
<tr>
<td>31-35</td>
<td>115 (34.1)</td>
<td>195 (37.4)</td>
</tr>
<tr>
<td>≥36</td>
<td>19.9</td>
<td>129 (24.8)</td>
</tr>
<tr>
<td>Missing</td>
<td>28 (8.3)</td>
<td>48 (9.2)</td>
</tr>
<tr>
<td>Paternal age (median)</td>
<td>34.2</td>
<td>34.5</td>
</tr>
<tr>
<td>≤25 years</td>
<td>30 (8.9)</td>
<td>27 (5.2)</td>
</tr>
<tr>
<td>26-30</td>
<td>48 (14.2)</td>
<td>81 (15.6)</td>
</tr>
<tr>
<td>31-35</td>
<td>92 (27.3)</td>
<td>158 (30.3)</td>
</tr>
<tr>
<td>≥36</td>
<td>121 (35.9)</td>
<td>187 (35.9)</td>
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<td>Missing</td>
<td>46 (14.0)</td>
<td>68 (13.1)</td>
</tr>
<tr>
<td>Child sex</td>
<td></td>
<td></td>
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<tr>
<td>Boys</td>
<td>276 (81.9)</td>
<td>280 (53.7)</td>
</tr>
<tr>
<td>Girls</td>
<td>61 (18.1)</td>
<td>241 (46.3)</td>
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<tr>
<td>Self-reported Race and Ethnicity</td>
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</tr>
<tr>
<td>White</td>
<td>233 (69.1)</td>
<td>426 (81.8)</td>
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<tr>
<td>Black</td>
<td>60 (17.8)</td>
<td>49 (9.4)</td>
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<tr>
<td>Hispanic</td>
<td>40 (11.9)</td>
<td>48 (9.2)</td>
</tr>
<tr>
<td>Asian</td>
<td>32 (9.5)</td>
<td>30 (5.8)</td>
</tr>
<tr>
<td>Pacific Islander</td>
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<td>1 (0.2)</td>
</tr>
<tr>
<td>Native America</td>
<td>9 (2.7)</td>
<td>15 (2.9)</td>
</tr>
<tr>
<td>Alcohol (pregnancy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>37 (11.0)</td>
<td>76 (14.6)</td>
</tr>
<tr>
<td>Missing</td>
<td>7 (2.1)</td>
<td>4 (0.8)</td>
</tr>
<tr>
<td>Active Smoking (pregnancy)</td>
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<td>33 (9.8)</td>
</tr>
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<td>Passive Smoking (pregnancy)</td>
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<td>27 (5.2)</td>
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<tr>
<td>Maternal Asthma</td>
<td>Yes</td>
<td>25 (7.4)</td>
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<tr>
<td>Any B2 indications*</td>
<td>Yes</td>
<td>18 (5.3)</td>
</tr>
<tr>
<td></td>
<td>182 (54.0)</td>
<td>276 (53.0)</td>
</tr>
</tbody>
</table>

*Includes: Asthma, Cold or Cough, Early Labor, Allergy, Upper Respiratory Infection, Influenza or Flu, Pneumonia, and Respiratory conditions.
Table 2: Allele frequencies among cases and controls for Arg16Gly and Gln27Glu.

<table>
<thead>
<tr>
<th>Allele Configuration</th>
<th>Cases (N=337)</th>
<th>Controls (N=521)</th>
<th>Gln27Glu Cases (N=337)</th>
<th>Gln27Glu Controls (N=521)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg</td>
<td>70 (20.8)</td>
<td>79 (15.2)</td>
<td>160 (50.5)</td>
<td>190 (36.5)</td>
</tr>
<tr>
<td>Arg/Gly</td>
<td>151 (44.8)</td>
<td>225 (48.9)</td>
<td>117 (36.9)</td>
<td>210 (43.8)</td>
</tr>
<tr>
<td>Gly/Gly</td>
<td>116 (34.4)</td>
<td>186 (35.7)</td>
<td>40 (12.6)</td>
<td>80 (16.7)</td>
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<td>Missing</td>
<td>0</td>
<td>1</td>
<td>20</td>
<td>41</td>
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Table 3. Marginal effects of genotypes and prenatal B2AR exposure

<table>
<thead>
<tr>
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<th>Marginal Effects</th>
</tr>
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<tr>
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<tr>
<td>B2AR</td>
<td>pvalue</td>
</tr>
<tr>
<td>Arg16Gly (AG or GG)**</td>
<td>1.5 (0.8-2.6)</td>
</tr>
<tr>
<td>Gln27Glu (CG or GG)***</td>
<td>0.7 (0.5-1.0)</td>
</tr>
<tr>
<td></td>
<td>0.6 (0.5-0.9)</td>
</tr>
</tbody>
</table>

* PCV=Principal Component Value – the terms that are derived using the software EIGENSTRAT and used to adjust for ethnic differences.
**Compared to homozygous reference genotype AA
***Compared to homozygous reference genotype CC

Table 4. Heterogeneity of multiplicative effects analysis (unadjusted and PCV adjusted) of B2AR Odds Ratios stratified by Arg16Gly and Gln27Glu

<table>
<thead>
<tr>
<th>Homozygous Reference Genotype</th>
<th>Arg16Gly</th>
<th>Gln27Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>PCV* Adjusted</td>
<td>Unadjusted</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homozygous Reference Genotype</td>
<td>0.56 (0.1-3.2)</td>
<td>0.53 (0.2-3.1)</td>
</tr>
<tr>
<td>Heterozygous or Homozygous Variant</td>
<td>1.71 (0.3-9.9)</td>
<td>1.91 (0.3-11.2)</td>
</tr>
<tr>
<td></td>
<td>1.22 (0.5-3.0)</td>
<td>1.62 (0.7-3.7)</td>
</tr>
<tr>
<td></td>
<td>1.13 (0.5-2.9)</td>
<td>1.79 (0.8-4.1)</td>
</tr>
</tbody>
</table>

* PCV=Principal Component Value – the terms that are derived from the principle component analyses and used to adjust for ethnic differences.

Table 5. Summary of test statistics for interaction.

<table>
<thead>
<tr>
<th>Multiplicative: parameter estimate for interaction term (p-val)</th>
<th>Arg16Gly</th>
<th>Gln27Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>PCV* Adjusted</td>
<td>Unadjusted</td>
</tr>
<tr>
<td>Multiplicative: parameter estimate for interaction term (p-val)</td>
<td>1.1 (0.24)</td>
<td>1.20 (0.21)</td>
</tr>
<tr>
<td>Multiplicative: 2df $\chi^2$ (p-val)</td>
<td>5.249 (0.07)</td>
<td>3.741 (0.15)</td>
</tr>
<tr>
<td></td>
<td>0.28 (0.65)</td>
<td>8.75 (0.01)</td>
</tr>
<tr>
<td></td>
<td>0.47 (0.47)</td>
<td>2.86 (0.23)</td>
</tr>
</tbody>
</table>

* PCV=Principal Component Value – the terms that are derived from the principle component analyses and used to adjust for ethnic differences.
CHAPTER SIX: DISCUSSION
Summary of Findings

The objective of this dissertation was to investigate associations between two specific classes of drug exposures, beta-2 adrenergic receptor (B2AR) agonists and selective serotonin reuptake inhibitors (SSRIs), still commonly used during pregnancy that may pose a risk for ASD. This was accomplished through two specific aims: 1) estimate the main effects of these medications commonly used during the prenatal period on autism risk in a large population-based cohort; 2) explore whether effects of exposure to B2AR agonist drugs are modified by maternal susceptibility genotypes in the ADRB2 gene.

The first aim focused on estimation of exposure main effects capitalizing on the large sample constructed from Denmark’s population-registers. The results from the analysis investigating the effect of B2AR agonist drugs and ASD suggest that exposure during the preconception (90 days before estimated date of conception) and prenatal periods were associated with modest increases in the risk for ASD. Preconception and trimester specific results during the prenatal period resulted in estimates that were similar across all models, with the second and third trimester effects being only slightly higher. However, since the effect sizes in all of the models had confidence intervals that overlapped, it does not provide enough evidence to suggest a particular exposure window. Our findings were consistent with other epidemiological evidence looking at in utero exposure to B2AR agonist drugs and the risk associated with ASD in that modest effect estimates were also reported [1, 2].

This is the first study to look at the association of both dose and duration of prenatal use of B2AR agonist drugs and risk for ASD. Epidemiological studies thus far
have only looked at duration of use and report the highest risk associated with prolonged use (>2 days) [1], which is also consistent with our findings. There was notable overlap between confidence intervals of the observed estimates, which remained elevated throughout each dose category. However, this evidence does not suggest any particular cumulative dose range or trimester specific exposure for dose. These dose and duration analyses were able to detect the elevated effect estimates in the second trimester, which is also supported by animal models [3, 4].

A limitation of these analyses however, was that exposure data were restricted to outpatient prescriptions. Although most prescriptions for B2AR agonist drugs, including those used for asthma indications, are obtained in an outpatient setting, they are also prescribed as a tocolytic for in-hospital use. B2AR agonist drug exposure due to tocolytic use would most likely occur in the third trimester. Therefore, third trimester exposure may have been underestimated since drugs prescribed during hospital admission are not included. It would have been interesting to explore the effect of B2AR agonist drugs indicated solely as a tocolytic. Another limitation to this study was that diagnostic subgroups of ASD were not explored. It would have been interesting to investigate the association between our exposure and various outcome definitions. These outcome definitions include childhood autism alone and those diagnosed with atypical autism, Asperger’s syndrome, and pervasive developmental disorder, unspecified. However, the validity of diagnostic subgroup classification other than autistic disorder in the register has not been established and the use of an outcome definition that included all ASD subtypes is consistent with other register-based studies of autism from Denmark [5, 6].
In the second aim of the dissertation, I investigated the relationship between prenatal exposure to SSRIs and ASD, also using the same case-control population used in aim one. Here I also found increased ASD risk associated with *in utero* exposure to SSRIs. In this analysis, any exposure to SSRIs during pregnancy was associated with ASD (odds ratio (OR) 2.0 [95% confidence interval CI, 2.0 [1.6-2.6]) compared to the unexposed reference group. To our knowledge, this is the first study to explore prenatal dose and duration of use and the risk associated with ASD. Our findings suggest that compared to children unexposed to SSRIs *in utero*, children who were exposed to the highest cumulative dose had the highest risk associated with ASD. Although I did not observe a linear dose response effect, I observed the highest effect estimate in the largest dose category. In regards to duration, I also found prolonged use of SSRIs during the prenatal period had a larger measure of association for ASD.

The limitations of this analysis are similar to those mentioned for the first aim. Namely, I only had the ability to investigate SSRIs dispensed in outpatient prescriptions. We were unable to capture data on any SSRIs that may have been prescribed during hospital admissions, though in-hospital prescription is infrequent compared to outpatient prescription. In addition, I had to assume that all the drugs from prescriptions were actually taken by the mother prior to delivery as well as throughout her pregnancy. It is, however, also possible that part of the drugs was left unused or used by others, such that I may overestimate the exposure and thus underestimate the drug effect. Lastly, I was not able to observe effects estimates from other diagnostic subgroups, which may shed light on how exposure may affect severity of ASD.
Since confounding by indication is a major concern in pharmacoepidemiology, the use of Denmark’s national registers provided a large sample size sufficient to perform several statistical analyses to control for possible independent effects of the indicating condition on ASD. In the analyses for B2AR agonist drugs, statistical adjustment for the indication and a restriction analysis on a subset of the study sample with maternal asthma resulted in estimates that remained robust regardless of the various sensitivity analyses implemented. However, in our SSRI analyses, understanding the potential impact of confounding by indication proved to be more complicated. I observed attenuated effect estimates when I restricted to only mothers with a diagnosis for depression, however after I had first corrected for misclassification of depression and then restricted the sample to only mothers with a depression, I observed a risky effect estimate in the strata with maternal depression. This suggests that under-reporting of the confounder, maternal depression, may be a limitation in approaches previously used to consider the influence of confounding by indication. We were also able to explore the potential effect of exposure misclassification in our analysis and found that exposure misclassification did not appear to have a substantial impact on the effect estimates in both the B2AR agonist drug and SSRI analyses.

Given that the effect sizes observed were modest, in the last aim I explored the extent to which prenatal medication exposure effects might be larger among a genetically susceptible subgroup. For this candidate gene-environment interaction analysis the register-based data from Denmark was not used, but instead I capitalized on the availability of exposure and genotype data in a large ongoing US autism case-control study, the Study to Explore Early Development (SEED). This analysis was limited to the
possible interaction between B2AR agonist drugs and candidate genotypes only. Although it is also of interest to consider genetic susceptibility with respect to SSRI effects, the single-nucleotide polymorphisms (SNPs) of interest for the best candidate gene (the serotonin transporter gene, SLC6A4) were not included on the Illumina genotyping panel used in the SEED study. There are two specific SNPs of interest within this gene, and although both were present in the imputed data, there were high rates of missing data (~50-70%). Tag SNPs could not be used because these two particular SNPs were untaggable (using r² > 0.8 threshold) due to the mixed ancestral populations in the dataset (African decent, European decent, and African-European admixed). I considered tagging SNPs within each of these three ancestral populations, however the same tag SNP could be used for the European decent and admixed populations, but not for the African decent population. It would have been possible to complete the analysis using different tag SNPs per population, which could then be followed by a meta analysis to assess the effect across ancestral populations. Another option considered was to analyze the whole SLC6A4 gene, which would involve pruning SNPs. Although these methods would have yielded informative results, I was not comfortable with the amount of missing genetic information for this SNP given the small sample size. Perhaps it would be beneficial for the larger SEED sample to explore these various methods using a meta analysis for tag SNPs, but for the purposes of this dissertation I decided to focus my efforts on the genetic influence of B2AR agonist drug exposure. Thus, I concentrated our analysis on exploring whether effects of B2AR agonists used during pregnancy are modified by polymorphisms in the beta 2-adrenergic receptor gene (ADRB2).
I investigated whether the effect estimates associated with B2AR agonist drug exposure in utero on the risk for ASD was modified by two candidate susceptibility genotypes – the Gly16 and Glu27 polymorphisms in the ADRB2 gene. The ADRB2 gene, located on chromosome 5q31-32, encodes the beta-2 adrenergic receptor and has been shown to influence receptor sensitivity and consequently may enhance susceptibility to the neurodevelopment effects of B2AR exposure [7]. There are two common polymorphisms known to influence receptor function [7]. On codon 16 (rs1042713), a G for A substitution encodes the amino-acid glycine rather than arginine (Arg16Gly), and a G for C substitution at codon 27 (rs1042714) codes for glutamic acid rather than glutamine (Gln27Glu) [8]. Unconditional logistic regression was used to estimate adjusted odds ratios and 95% confidence intervals and two different approaches to test for interaction were applied – multiplicative model and 2-degree of freedom joint test.

These preliminary results do not confirm a gene-environment interaction effect however they do encourage the further exploration of gene-environment interaction in order determine if subgroups more susceptible to prenatal B2AR exposure do exist. We did find similar in magnitude marginal effects for B2AR agonist use during pregnancy in the SEED sample that were similar to those found in the Denmark sample reported in Chapter Three of this dissertation and to those reported by Croen et al 2011 [1]. In regards to the two SNPs of interest, the Arg16Gly and Gln27Glu, the marginal effects of these genes alone indicated a protective effect which differed from the one previous report [9]; however, our estimates had very wide confidence intervals. The pattern of the effect estimates suggests qualitative multiplicative scale interaction between Arg16Gly and B2AR agonist drug use, however there does not appear to be any qualitative
multiplicative scale interaction for the Gln27Gly and B2AR. While the marginal genetic effect was not consistent with this previous work, we did observe stronger B2AR effects in the strata of individuals with these higher activity genotypes. However, the results from the two-degrees of freedom joint test suggest no interaction on the multiplicative scale, but the unadjusted model for the Gln27Gly analyses was significant. This may be a symptom of the imprecision of the effect estimates. As expected the, p-values for the two-degrees of freedom joint test for all models were less than the p-values from the conventional multiplicative interaction models, which is consistent with literature [10]. It has been shown that the two-degrees of freedom test has more power to detect genetic influences of gene-environment interaction, and is therefore known to be more sensitive to these effects when compared to traditional multiplicative interaction tests [10].

The interaction assessments were complicated due to this marginal genotype effect, which was against expectation, and were also impacted by the limits of power due to the small sample size. However, since this study took advantage of the first round of genotyping in the SEED populations, our results when used in conjunction with those from the larger SEED analysis, can lead to insight into other possible mechanisms for gene environment interaction. More importantly, this is the first direct exploration of gene-environment interaction with respect to a prenatal pharmacologic exposure and ASD risk and will encourage further research endeavors in this area.
Future Directions

To date, in addition to the work reported here, there have been only two epidemiological studies that have investigated prenatal exposure to B2AR agonist and risk for ASD [2, 11], and one observational study on SSRIs and risk for ASD [12]. Although these studies were all population based, they had relatively small sample sizes. Further research is needed to replicate these few epidemiological findings and, as has been noted, these approaches need to address potential confounding by indication. Randomized study designs are not an option when a treatment has already diffused or may not be applied to certain subpopulations (i.e., children, pregnant women) commonly excluded from trials. Therefore, employing statistical adjustment and a restriction analysis in observational studies should continue to be used in efforts to control for confounding by indication when randomized trials cannot be used. Moreover, future studies should also consider matching on the propensity score, which would be similar to the expected balance achieved through a randomized trial [13]. In conjunction with controlling for confounding by indication, investigations moving forward should consider methods to account for potential misclassification of the indication. This would include Monte Carlo simulations such as those used throughout this dissertation and Bayesian approaches which have significant advantages [14]. The impact of under-ascertainment of indication, would lead to incomplete control for confounding.

The indicating conditions for these pharmacologic exposures, consisting of maternal asthma and depression, have also been suggested as autism risk factors [15, 16]. Since these associations have not been themselves firmly established, continued investigation of alternative pathways by which these exposures could influence ASD risk
should also continue to be explored. This is a complicated challenge in itself and involves a range of factors to be considered, such as genetic susceptibility and physiologic changes related to maternal stress, especially during the pregnancy period. Recent animal models have suggested that the prenatal stress and certain serotonin transporter genotypes may have the combined effect of producing changes in social interaction. Associations between maternal depression and developmental psychopathology in children have been suggested as potentially operating through mechanisms involving the changing intrauterine environment or maternal stress response. Maternal hypothalamo–pituitary–adrenal (HPA) activity in response to psychological stress, which can be caused by depression, has been linked to adverse neurodevelopmental effects. Maternal stress can lead to *in utero* glucocorticoid exposure and reduced birth weight. The resulting gestational stress and poor fetal growth has been linked to the development of emotional problems in children [17].

As mentioned previously, there is a possible association between the indication and ASD, especially if these indications are uncontrolled during the pregnancy period. Understanding the mechanisms behind drug exposure and risk for ASD is just as important. It has been shown that during gestation, overstimulation of the beta-2-adrenergic receptors from B2AR agonist drugs has harmful effects on the development of the brain and the peripheral nervous system, especially when the fetus does not have the ability to regulate any imbalance [4, 18, 19]. Research using animal models has revealed abnormalities within the central and peripheral nervous system and a decrease in the cell numbers in the fetal brain and liver of rats that were exposed to B2AR agonist drugs prenatally[18]. These findings were also gender selective with males experiencing more
abnormalities, similar to what is observed in male predominance of autism in humans [18]. Abnormalities in serotonin metabolism are one of the few consistent biological observations associated with ASD [20] and several lines of evidence suggest that alterations in the serotonergic neurotransmitter system might be a mechanistic pathway leading to ASD. In addition, animal models have suggested adverse neurodevelopmental outcomes in the offspring associated with prenatal SSRI exposure [21]. The few epidemiological studies on prenatal exposure to B2AR agonist drugs and SSRIs in conjunction with insight from research using animal models suggest that mechanistically, prenatal exposure to these drugs may operate directly on the brain as it develops leading to ASD.

Since there is increasing need to understand the etiology and mechanisms underlying the medication exposure and non-medication exposure pathways potentially connecting prenatal maternal asthma and depression to offspring ASD risk in order to make informed decisions regarding treatment during pregnancy. Furthermore, efforts should be made to include in-patient prescription data particularly in analysis focusing on B2AR agonist drugs solely indicated as a tocolytic. If terbutaline administered as a tocolytic does in fact increase ASD risk as previously reported [1] there may be other opportunities to focus on preterm birth and the consequence of this medication used off-label. This information should be coupled with identifying critical time periods of development for the fetus, and adequately balance the risks and benefits of medication use during pregnancy.

The observed main effects described in this dissertation as well as in other epidemiological studies are subtle in pregnancies exposed to these medications, which
indicate the possibility for gene-environment interaction effects. This suggests that the combined effect of exposure during pregnancy with a fetus that carries a genetically susceptible genotype may have an increased risk of ASD. Without fully understanding the casual pathway of these exposures, it is also possible that the uncontrolled indication could also be a risk factor in this gene environment interplay. To explore possible gene-environment interaction effects, I recommend a large sample size to be adequately powered to observe an association. Moreover, investigators exploring gene-environment interactions should be cognizant of the idea that common ancestry is related to genotypes and perhaps to exposure through culturally mediated mechanisms. Population stratification occurs if the gene under study shows marked variation in allele frequency across different groups of the population and if these groups also differ in their baseline risk of the disease [22]. Studies exploring the genetic effects on ASD should be correcting for this using a principle component analysis (PCA), as was done in this dissertation, to summarize large scale genomic surveys through covariates that correct for population structure in GWAS analyses, and therefore enable us to further explain the structure of genetic variation in large samples [23]. In addition employing various statistical approaches to test for interaction provides several benefits (ie multiplicative model, additive model, case-only multiplicative model, and the multiplicative 2-degree of freedom joint test). The value of applying different statistical methods comes from understanding the strengths of each test. For example, the traditional conventional multiplicative interaction test can be easily interpreted and are conventionally reported throughout the gene-environment literature. Having additive interaction does not ensure multiplicative interaction, so using the additive interaction will allow one to explore
expected joint effects based on the sum of the different measures. The two-degrees of freedom test has been shown to be more sensitive to the genetic effects in the presence of an exposure when compared to the traditional multiplicative interaction. Lastly, the case-only approach has more power to detect interaction effects. Taken together, these methods can yield informative results, especially when faced a small sample size such as in this dissertation.

**Public Health Significance**

Prenatal pharmacologic exposures are one of the few known environmental risks factors for ASD [24] although the few drugs linked to ASD are no longer prescribed in pregnancy. However, as the teratogenic potential of most drugs with respect to neurodevelopmental outcomes is generally understudied, it is important to consider further prescription drug use as ASD risk factors. For women with a number of common chronic medical conditions, including epilepsy, diabetes, depression, inflammatory bowel disease and asthma, the use of prescription drugs in pregnancy is still the recommended standard of care. In addition, since clinical trials during drug development commonly exclude pregnant women due to ethical reasons, there have been many questions regarding the effects of the drug on the developing human fetus.

The combination of low exposure prevalence during pregnancy and modest effect size implies that population attributable risks associated with both exposures, B2AR agonist drugs and SSRIs, will be fairly small. Our Denmark data suggest a prevalence of SSRIs used during pregnancy of 0.7% and an effect estimate of 1.9, which imply that the population attributable ASD risk associated with this exposure is only 0.6%. Prevalence
of asthma drugs used during pregnancy in our study was 2.9% and I observed a 1.3 odds ratio associated with exposure and outcome - consequently, the population attributable risk here was 0.9%. If these effects are in fact real, this still suggests that even a small proportion of autism cases in the population could be prevented if they were not exposed to either of these medications during pregnancy. However, decisions to avoid these medication exposures prenatally because of concerns over ASD must be balanced against reports of the health effects on the developing fetus and the mother posed by uncontrolled indications during pregnancy. Further consideration of the biological mechanisms underlying these exposure effects could also lead to an improved understanding of common etiologic pathways in ASD that, in turn, might then inform potential prevention or treatment strategies that would affect larger number of ASD cases.

There is still a need to continue to carefully explore prenatal prescription drug use as ASD risk factors. At the same time, while it is important to detect any such associations and consider their etiologic implications, the public health implications may not be straightforward. With respect to maternal B2AR agonist drug use, uncontrolled asthma in pregnancy has been associated with poor birth outcomes [25, 26]. During an asthma exacerbation in pregnancy, the prenatal maternal stress response may be elevated, which would be harmful during a time when the fetal limbic system is considered to be the most vulnerable to such a stress response, especially before 32 weeks of gestation [27]. Consequently, any ASD risk associated with maternal B2AR agonist drug use needs to be balanced against the benefits of indicated medication use by pregnant mothers. Additional studies need to replicate the present study before the implications of prenatal B2AR agonist drug exposure through maternal use of these agents for asthma
control on ASD risk be considered when making individual decisions about asthma control in pregnancy.

Similarly, it would be potentially dangerous for both the fetus and mother to recommend avoidance of medications such as antidepressants during pregnancy. The implications for these findings on prenatal SSRI exposure and risk of ASD reported in this dissertation should be tempered with those reports of uncontrolled depression during pregnancy and the growing concern over the potential risk for relapsed depression after discontinuing antidepressant medication during pregnancy. A recent study found that 68% of the women who discontinued antidepressant treatment during pregnancy relapsed in depression, with 50% experiencing recurrence of depression in the first trimester of pregnancy and 90% by the end of the second trimester [28]. Associations between maternal depression and developmental psychopathology in children have been suggested as potentially operating through mechanisms involving the changing intrauterine environment or maternal stress response. It should also be noted that postnatal depression are often preceded by antenatal depression; similarly, postnatal anxiety is most like preceded by antenatal anxiety [29]. Furthermore, antenatal anxiety occurs frequently, overlaps with depression, and also increases the likelihood of postnatal depression [29]. The consequences of depression during pregnancy and its transference into postpartum depression could have dangerous repercussions for infants. This can manifest itself physically and behaviorally, including less optimal interaction behaviors and developmental delays [30, 31]. In addition, women who were depressed during their pregnancy were more likely to experience increased life stress, decreased social support, poor weight gain, and the use of harmful substances such as cigarettes, alcohol, and
cocaine [32]. The impact of this undoubtedly has adverse effect on infant outcome and their development. Interventions to change health behaviors during pregnancy in light of the finding from this dissertation should consider a woman's affective state, social context, and mental health. More importantly, appropriate judgment for treatment for indications, maternal asthma and depression, should include communication regarding risks and benefits of treatment, and any adverse consequences to their well-being and their infants.
References


APPENDIX:
INTRODUCTION

In this chapter several tables, figures, and more detailed information on analyses presented in the dissertation are described. It provides more in-depth explanation of aspects of the study design and analyses discussed in the previous chapters and also include some additional analyses not shown elsewhere.

DENMARK’S NATIONAL REGISTERS

The Fertility Database

The Fertility Database, formally known as the Statistical Register for Fertility Research, relates to births (both live and stillbirths) and is used primarily for analyses of fertility trends, birth patterns, and number of children in specific population groups. The Danish Social Science Research Council and Statistics Denmark maintain this database. Currently it has become a statistical register that is now on par with other registers operated by Statistical Denmark. It contains information on both women and men, including parental and familial relationships such as mother-child, father-child, and parents of the same child. A range of information, which is collected annually, covers the education and employment of the parents, as well as family and housing conditions. Information within this register contains data from 1996 that is updated annually. The female population of Denmark comprises approximately 1.3 million women.

Danish Civil Registration System

The Danish Civil Registration System (CRS) was established in 1968 and includes all live persons living in Denmark. It includes a unique personal identification
number, name, gender, date of birth, place of birth, citizenship, and identity of parents. This register is updated continuously to include all information regarding vital status, place of residence and martial information. All Danish citizens are assigned a unique personally identification number (CPR number) enabling accurate linkage between all national registers. This registration system was established for administrative purposes, and it is generally accepted that the information recorded is of very high quality. It is continuously updated and corrects for errors as they are encountered. It is required by law that residents register for this system. For persons born in Denmark in 1960 or later the register contains complete information on maternal identity, and for women born in April of 1935 or later there is complete information on all children. In regards to immigration and emigrations, there is complete information from 1969 and onward, permanent residence in a Danish municipality from 1971 onward, and a full address in Demark from 1977 and onward.

**Register of Medicinal Products Statistics**

The Danish Medicines Agency owns and is responsible for the Register of Medicinal Product Statistics. The Statistics and Analysis Department in the Pharmacoeconomic Division is responsible for the day-to-day operations of the register. It was established in order to obtain statistics and price indices as well as monitor the consumption of medicinal products in order to aid in the decision making of the central health authorities. It is unique on a global scale because it is the only register that contains information regarding consumption of the medicinal products over the entire population of a country over several years, since 1994. Danish pharmacies, hospital
pharmacies, the National Central Laboratory of the Danish Health System (Statens Serum Institut) and the Danish Institute for Food and Veterinary Research report information to this register every month. Over the counter medication has been included since 2001 including their monthly sales. This encompasses 8 million reports per month and around 96 million a year. Aside from the personal identification number, additional information include the identification number of the prescriber, information about the individual packet of medicine distributed, time and place of the sale, the doctor’s recommendations regarding substitutions, reiteration number on the prescription, price, and reimbursement. In regards to over-the-counter sales, the information reported includes the individual packet of medication and the number of packets sold. Hospital registers only their consumption of the medication used under ward codes and not under the personal identification number of person. Therefore, only medication dispensed in pharmacies for outpatient use is only recorded.

**Danish Medical Birth Register**

The Danish Medical Birth Register was established in 1968 and has been computerized since 1973. The information in this register includes records from all live and stillbirths of women living in Denmark. In addition, it also includes socio-demographic information of the newborn and parents, data on parity, complications and procedures during pregnancy and delivery, date of perinatal death and cause of death. The purpose of this register is to monitor the health of newborns, and assess the quality of obstetric care. Gestational age, a common variable of interest in perinatal studies, is included in this register, extracted from birth records completed by midwives.
Gestational age is recorded as completed weeks since the self-reported last menstrual period, and corrected using an ultrasound if the last menstrual period is uncertain.

**Danish National Hospital Register**

The Danish National Hospital Register was initiated in 1977 and includes information on all hospital admissions for all Danish residents. The initiation of information regarding outpatient care was included into this register in 1995. The Danish version of the International Classification of Diseases (ICD-8) was used from 1977-1993, and ICD-10 codes since 1993. Nearly complete registration of somatic hospital events is included in this register. The National Board of Health validates this registrar by double entry of each administrative and clinical data and it is generally regarded as having a high reliability.

**Danish Psychiatric Central Register**

A nationwide collection of data from mental hospitals was initiated in 1938 from the eight psychiatric hospitals available. It was not until April of 1969 that the Psychiatric Central Register became an electronic database, which contained information on every psychiatric admission since 1969 and onwards. From then on, all mental hospitals and psychiatric wards reported information to this register. It was not until 1995 that data on outpatient treatment and emergency room contacts was included. This register then became integrated as part of the Danish National Hospital Register in 1977, and each month, the psychiatric data is passed on to the Psychiatric Central Register at the Centre for Psychiatric Research, Aarhus University Hospital, Risskov in Denmark.
The National Board of Health is the authority responsible for the data.

**PHARACOLOGIC INFORMATION FROM THE REGISTER OF MEDICINAL PRODUCTS STATISTICS**

Pharmacologic information used in analyses included in Chapter 3 and 4 was drawn from the Register of Medicinal Products Statistics. For dispatches of prescribed medicine from any pharmacy, except hospitals’ dispensaries, in Denmark this register captures the unique individual patient identification number, date of dispatch, name of drug, amount of drug, code from the WHO Anatomical Therapeutic Chemical (ATC) classification system, and the coded defined daily dosage (DDD).

The WHO Anatomical Therapeutic Chemical classification system (ATC) and the defined daily dosage (DDD) were designed to enable drug utilization research in order to improve the quality of drug use [1]. The DDD is defined as the assumed average maintenance dose per day for a drug used for its main indication in adults [2]. It was developed in response to the need to convert and standardize readily available volume data from pharmaceutical inventory data into medically meaningful units, to make crude estimates of the number of persons exposed to a particular medicine or class of medication [1]. Drug consumption data presented in DDDs only give a rough estimate of consumption and not an exact description of actual use. The DDD provide a fixed unit of measurement independent of price and dosage form (e.g. tablet strength) enabling the researcher to assess trends in drug consumption and to perform comparisons between population groups. It should be noted that the DDD is not a recommended or prescribed
dose, but a technical unit of comparison, and usually the result of literature review. Thus DDDs may be high or low relative to actual prescribed doses. However, a recent study evaluating the DDDs system found that it is a reliable tool for standardizing antipsychotic doses in drug-utilization research [3].

ATC codes were used to identify B2AR agonists and SSRIs in the analyses included in Chapters 2 and 3. The table below displays the codes used. The DDD information was used to create dose and duration variables as described in the Chapters.

The table below describes the medication codes used in Chapter 3 and 4.

<table>
<thead>
<tr>
<th>Medication Class</th>
<th>Medication Abbreviation</th>
<th>Medication Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>β2-adrenergic receptor agonists</td>
<td>B2AR agonists</td>
<td>R03AC02, R03AC03, R03AC04, R03AC05, R03AC12, R03AC13, R03CC02, R03CC03, R03CC12</td>
</tr>
<tr>
<td>Selective serotonin reuptake inhibitors</td>
<td>SSRI</td>
<td>N06AB03, N06AB04, N06AB05, N06AB06, N06AB08, N06AB10</td>
</tr>
</tbody>
</table>
REFERENCES


THE INTERNATIONAL STATISTICAL CLASSIFICATION OF DISEASE AND RELATED HEALTH PROBLEMS, 10TH REVISION (ICD-10)

The Danish version of the International Classification of Diseases (ICD-8) was used from 1977-1993, and ICD-10 codes since 1993. The table below lists all ICD-8 and ICD-10 codes to construct individual covariates and the outcome, ASD utilized in analyses described in Chapters 3 and 4 of the dissertation. These individual covariates and outcome definitions are only relevant for data from Denmark, and not utilized in the gene-environment interaction analyses.
<table>
<thead>
<tr>
<th>Variable</th>
<th>ICD-8 Code</th>
<th>ICD-10 Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental psychiatric disorder ranked as follows (in order of most to least severe)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rank 1: schizophrenia-like psychosis</td>
<td>295, 297, 298.39, 301.83</td>
<td>F20–F25, F28–F29</td>
</tr>
<tr>
<td>Rank 2: affective disorder</td>
<td>296, 298.09, 298.19, 300.4</td>
<td>F30–F39</td>
</tr>
<tr>
<td>Rank 3: substance abuse</td>
<td>303, 304</td>
<td>F10–F19.9 excluding F1x.0</td>
</tr>
<tr>
<td>Rank 4: Any other psychiatric disorder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal depression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bipolar disorders</td>
<td>296.19 and 296.39</td>
<td>F31</td>
</tr>
<tr>
<td>Unipolar disorders</td>
<td>296.09, 296.29, 296.89, 296.99, 298.09, 300.49, and 301.19</td>
<td>F32, F33, F34, F38</td>
</tr>
<tr>
<td>Maternal asthma</td>
<td>493.00, 493.01, 493.02, 493.08, 493.09</td>
<td>J45, J45.0, J45.1, J45.8, J45.9, J46, J46.9</td>
</tr>
<tr>
<td>Parental autoimmune disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1D</td>
<td>249</td>
<td>E10</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>242.00</td>
<td>E05.0</td>
</tr>
<tr>
<td>Autoimmune thyroiditis</td>
<td>245.03</td>
<td>E06.3</td>
</tr>
<tr>
<td>Primary adrenocortical insufficiency</td>
<td>255.1</td>
<td>E27.1</td>
</tr>
<tr>
<td>RA</td>
<td>712.19, 712.39, 712.59</td>
<td>M05, M06</td>
</tr>
<tr>
<td>Juvenile arthritis</td>
<td>712.09</td>
<td>M08</td>
</tr>
<tr>
<td>Dermatopolymyositis</td>
<td>716</td>
<td>M33</td>
</tr>
<tr>
<td>Polymyalgia rheumatica/temporal arteritis</td>
<td>446.3</td>
<td>M31.5, M31.6, M35.3</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>734.0</td>
<td>M34 (except M34.2)</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>734.19</td>
<td>M32.1, M32.9</td>
</tr>
<tr>
<td>Sjögren syndrome</td>
<td>734.90</td>
<td>M35.0</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>712.49</td>
<td>M35.1</td>
</tr>
<tr>
<td>Wegener granulomatosis</td>
<td>446.29</td>
<td>M31.3</td>
</tr>
<tr>
<td>Celiac disease</td>
<td>269.00</td>
<td>K00</td>
</tr>
<tr>
<td>Crohn disease</td>
<td>563.01</td>
<td>K50</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>563.19</td>
<td>K51</td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td>281.0</td>
<td>D51.0</td>
</tr>
<tr>
<td>Autoimmune hemolytic anemia</td>
<td>283.90, 283.91</td>
<td>D59.1</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura</td>
<td>446.49</td>
<td>D69.3</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>340</td>
<td>G35</td>
</tr>
<tr>
<td>Guillain-Barré syndrome</td>
<td>354</td>
<td>G61.0</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>733.09</td>
<td>G70.0</td>
</tr>
<tr>
<td>Pemphigus</td>
<td>694</td>
<td>L10 (except L10.5)</td>
</tr>
<tr>
<td>Psoriasis vulgaris</td>
<td>696.09, 696.10, 696.19</td>
<td>L40 (except L40.4)</td>
</tr>
<tr>
<td>Alopecia areata</td>
<td>704.00</td>
<td>L63</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>709.01</td>
<td>L80.9</td>
</tr>
<tr>
<td>Maternal infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any infection</td>
<td>000–136, 780.21, 788.89 and all listed below</td>
<td>A00–B99, G00–G09, R50.9, R56.0 and all listed below</td>
</tr>
<tr>
<td>Virus infection</td>
<td>008.8–008.9, 040–079, 381.00, 470–474, 480</td>
<td>A08, A80–A99, B00–B34, B97, G02.0, G05.1, H67.1, J10–J12, J17.1, J20.3–J20.7, J21.0, M01.4–M01.9</td>
</tr>
<tr>
<td>Bacterial infection</td>
<td>000–005, 008.0–008.3, 010–039, 079.84, 090–104, 320–324, 381.01, 390–391, 464.03, 481–482, 501, 508.00–508.03, 510, 513, 540–542, 590, 595, 599.00, 599.06, 612–614, 616.0, 620, 622, 630</td>
<td>A00–A05, A15–A59, A65–A79, B95–B96, G00, G01, G04.2, G05.0, G06–G09, H66, H67.0, I00–I01, J13–J15, J17.0, J20.0–J20.2, J36, J39.0–J39.1, J85–J86, K35–K37, L00–L08, M00, M01.0–M01.3, N10–N19</td>
</tr>
<tr>
<td>Condition</td>
<td>ICD-10 Codes</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>Respiratory infection</td>
<td>032–034, 460–474, 480–486, 491.01, 501, 503, 506, 508.00–508.05, 510–511, 513</td>
<td></td>
</tr>
<tr>
<td>Infectious enteritis</td>
<td>001–009</td>
<td></td>
</tr>
<tr>
<td>Skin infection</td>
<td>680–686</td>
<td></td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>590, 595, 599.00, 599.06, 635</td>
<td></td>
</tr>
<tr>
<td>Genital infection including STDs</td>
<td>054.02, 079.84, 090–099, 131, 612–614, 616.0, 620, 622, 630</td>
<td></td>
</tr>
<tr>
<td>Appendicitis</td>
<td>540–542</td>
<td></td>
</tr>
<tr>
<td>Birth complications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breeched presentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assisted labor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postpartum hemorrhage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Childhood autism</td>
<td>F840</td>
<td></td>
</tr>
<tr>
<td>Atypical autism</td>
<td>F841</td>
<td></td>
</tr>
<tr>
<td>Asperger’s syndrome</td>
<td>F845</td>
<td></td>
</tr>
<tr>
<td>Pervasive developmental disorder, unspecified (PDD-NOS)</td>
<td>F848, F849</td>
<td></td>
</tr>
<tr>
<td>Indications for SSRIs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety and Phobia</td>
<td>300.0, 300.2</td>
<td></td>
</tr>
<tr>
<td>Obsessive-compulsive disorder</td>
<td>300.3</td>
<td></td>
</tr>
<tr>
<td>Adjustment disorders</td>
<td>F42</td>
<td></td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>295</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F20, F29</td>
<td></td>
</tr>
</tbody>
</table>
DERIVED VARIABLES FROM THE DANISH REGISTRY USED IN CHAPTERS 3 and 4.

**Family Socioeconomic Status (SES)**

Family SES was measured as a standardized sum of father’s educational level, mother’s educational level, father income, and mother income. Education for each parent was scored from 1-3 and income from 1-4. Then a parent’s SES was a summation of income and education. Then to obtain family SES both the mother and father SES were added to obtain a scale from 1-14.

**Parental Psychiatric History**

Parental psychiatric history was categorized using an algorithm from a recent paper that explored the association between perinatal factors, parental psychiatric history, socioeconomic status, and risk of ASD [4]. A parent was defined as having a psychiatric history if a psychiatric diagnosis had been recorded before the date that autism was diagnosed in the child. Parental psychiatric history was categorized as either absent or falling into one of four severity categories following the algorithm of a prior study [4] developed by Larrson et al in 2005. A parent was defined as having a psychiatric history if a psychiatric diagnosis had been recorded before the date of birth of the child. Diagnosis considered in the severity score included schizophrenia-like psychosis followed by affective disorder in the second most severe category. Diagnoses were ranked by Larrson et al 2005 according to severity and each parent with a psychiatric
history received a score equal to the highest ranked condition. The score of the parent with the highest-ranking diagnosis determined the specific category assigned.

A ranking of psychiatric diagnosis will determine how the parents were categorized; the parent with the highest-ranking diagnosis will determine the specific category.

**CONFOUNDING BY INDICATION**

This section provides an in depth review of the concept of confounding by indication. Since this issue is frequently encountered in studies involving drug exposures and a range of outcomes, special attention to the methodology was explored. In addition, this overview demonstrates how confounding by indication can affect the findings of pharmacoepidemiologic studies if not properly accounted for in the analyses.

Due to the strong association between the indications described in the dissertation and exposures of interests and documented association between the indications and autism risk, special attention was paid to address potential confounding by indication. In observational studies of medical treatment, confounding by indication is a term used when confounding is introduced because a known or perceived indication (or contraindication) for the treatment under study and is also a risk factor for an outcome. It occurs when a variable is a risk factor for a disease and is associated with the exposure of interest in the population from which the cases derive, without being an intermediate step in the causal pathway between the exposure and the disease. The *Dictionary of Epidemiology* gives the following definition: “A distortion of the effect of the treatment on the outcome that is caused by the presence of a sign or symptom that is associated
with both with the treatment and with the outcome; or a distortion of the effect of
treatment that is caused by the presence of an indication or a contraindication for the
treatment that is associated with the outcome” [5]. Meittien describes confounding by
indication as “a commonly used term that refers to an extraneous determinant of the
outcome parameter that is present if a perceived high risk or poor prognosis is an
indication for intervention” [6].

Confounding by indication can occur in treatment studies when outcomes of
interest are measures of effectiveness (e.g., cure, symptom amelioration, longer survival
etc.) or when outcomes of interest are near-term adverse events or side effects. In
treatment studies confounding by indication is typically introduced by disease severity or
other prognostic factors (e.g., comorbidity). An example comes from a recent study of
combined antiretroviral therapy (cART) on AIDS-free survival [7]. cART is more likely
to be initiated in individuals with low CD4 cell count, an indicator of greater HIV
severity. Consequently, in this example, low CD4 cell count is expected to be both
associated with the outcome, AIDS-free survival, and the exposure without being on the
causal pathway.

Adequate control of confounding by indication in treatment effectiveness studies
is often a challenge. As with other confounding challenges, randomized designs can
offer distinct advantages. However randomized designs are not always an option when a
treatment has already diffused or may not be applied to certain subpopulations (i.e.,
children, pregnant women) commonly excluded from trials. Further, randomized trials
may not be adequately powered to study rare side effects. In observational studies, most
commonly, statistical adjustment is used in attempts to control for confounding by
indication. In the Philips et al HIV treatment study, the authors adjusted for CD4 cell count. Because this was a prospective study where CD4 count was affected by prior cART use (time-varying confounding), it was important that the authors appropriately adjusted for the time-dependent confounding by indication variables (e.g., due to CD4 cell count); in this case by using inverse probability weighting of marginal structural models. Each patient in the logistic models had received a time-varying weight inversely proportional to the estimated probability of having his/her own observed history of cART initiation.

Propensity score approaches are another analytic method that has more recently gained popularity as a way to control confounding by indication in observational designs [8, 9]. The propensity score approach uses a statistical model to estimate the conditional probability of exposure to a treatment given a range of predictor variables [10]. Propensity is then defined as an individual's predicted probability of being treated with the intervention of interest given the complete set of all information about that individual and provides a single metric that summarizes all the information from variables predicting treatment, typically including measures of disease severity [11]. Matching on the propensity score has been promoted as superior to conventional adjustment since the balance achieved between the exposed and unexposed groups has been viewed as being similar to the expected balance achieved through a randomized trial [12]. A limitation of matching on propensity scores is that there may be unmatched subjects which will then be excluded from analysis, leading to a loss of information and a decrease in the precision of the estimate association between the drug and the outcome [11]. Of course, if
propensity scores omit important confounders, propensity score approaches like other analytic strategies can be subject to residual confounding.

Residual confounding occurs when a confounder control approach does not completely remove the confounding effect. This can occur when confounders are omitted or when a confounder is measured with error. In the study discussed above focused on cART treatment and AIDS free survival, there was concern that using CD4 cell counts alone was an inadequate measure of disease severity and would result in residual confounding. The investigators chose to adjust for both CD4 cell count and viral load as a more complete measure of HIV severity. Misclassification of a confounding variable is another means by which confounder control may be incomplete and residual confounding introduced.

An alternative method of confounder control not yet discussed is restriction. In confronting confounding by indication in treatment studies where the confounder is indicating condition severity, restriction would involve limiting the analysis to a particular indicating condition severity level. The principal drawback to restriction approaches is the reduced number of subjects available for analysis. However, restriction can sometimes be used as a means to avoid residual confounding due to measurement error if there is one level of severity less subject to measurement error (e.g., if it is easier to identify those without comorbid disease than to distinguish between mild, moderate and severe comorbid disease).

Thus far, the discussion here has been about confounding by indication in its most common context, observational studies of treatment effectiveness. However confounding by indication can also be a challenge in risk factor investigations when an established
treatment for a particular indication is being investigated as a risk factor for another outcome. Here the temporal period between the exposure (the established treatment) and outcome is usually much longer than that seen in traditional treatment effectiveness studies. Another important feature distinguishing these studies from observational studies of treatment effectiveness is that confounding by indication can also be introduced in these investigations by the presence of an indicating condition, because the study population includes subjects with and without the indication, whereas in the typical observational study of treatment all subjects are affected (Salas et al; 1999). Salas et al (1999) offered the example of an investigation of antidepressant drugs and an increased risk of infertility. A large case control study (597 cases and 3,833 controls) reported a 3-fold increased risk of infertility in women self-reporting use of an antidepressant at least 6 months prior [13]. However, at the same time, a case-control study of 428 women nested in a prospective cohort of nearly 3,000 subjects found history of depression was associated with subsequent infertility; the hypothesized mechanism being elevated prolactin levels among women with depression [14]. Consequently, in the example provided by Salas (1999), the presence of the indication, depression, may be a confounder.

As illustrated in this example, the presence of an indicating condition can be a potential confounder when studying downstream effects of pharmacologic treatment. This was the case in this dissertation. The indicating conditions for the pharmacologic exposures, maternal asthma and depression, have also themselves been suggested as autism risk factors [15, 16]. While the association is not firmly established and mechanisms have not been well specified, should these indicating conditions be
associated with ASD risk through some way other than a direct pathway going through pharmacologic exposure, confounding by indication would be a concern. Associations between maternal depression and developmental psychopathology in children have been suggested as potentially operating through mechanisms involving the changing intrauterine environment or maternal stress response. Maternal hypothalamo–pituitary–adrenal (HPA) activity in response to psychological stress, which can be caused by depression, has been linked to adverse neurodevelopmental effects. Maternal stress can lead to \textit{in utero} glucocorticoid exposure and reduced birth weight. The resulting gestational stress and poor fetal growth has been linked to the development of emotional problems in children [17]. It has also been hypothesized that the presence of maternal asthma, could affect neurodevelopmental outcomes via altered levels of circulating inflammatory cytokine interleukin 6 (IL-6), which can cross the placenta and cause dysregulation of IL-6 during critical periods of development for the fetus [18]. In the dissertation, subjects in both case and control children groups have mothers \textit{with} and \textit{without} history of both these indicating conditions.

The directed acyclic graph (DAG) in Figure 1 was be used to guide exploration of confounding by indication in analyses of SSRI exposure and autism risk in the full study sample. It shows that controlling for the presence of maternal depression through statistical adjustment will remove confounding by indication as well as block the path

![Figure 1: DAG - Maternal SSRI use and risk for ASD. Statistical adjustment is used to control for maternal depression.](image)
between any antecedent genetic liability associated with both maternal depression and autism risk. In addition, controlling for the presence maternal depression does not appear to open any additional paths and introduce unexpected biases. There is however one major assumption made in this DAG – that there is no association between other maternal SSRI indicators and ASD, including a backdoor path going through a shared genetic liability with autism risk. Other known indications for SSRI use in this population include premenstrual syndrome and anxiety, neither of which to-date, has been associated with ASD. However, studies have reported family history of psychiatric conditions (including anxiety disorder) to be associated with autism risk [16, 19, 20], thus suggesting that there could be shared genetic determinants of maternal anxiety disorders and autism.

As discussed above, residual confounding is also a concern when adjustment does not completely remove the confounding effect. In my investigation, the presence of maternal depression is measured through ICD-10 codes (F32 to F34: depressive episode, recurrent depressive disorder, persistent mood [affective] disorders) as reported from episodes of both inpatient and outpatient care from psychiatric hospitals and psychiatric wards in Denmark. If these codes as captured during outpatient and inpatient visits are an imperfect measure of the presence of maternal depression, confounding by indication may not be removed. Consequently, I incorporated a sensitivity analysis to quantify likely effects of misclassification of depression (partial knowledge of exposure misclassification probabilities).

Confounding by indication can also be introduced in my study by depression severity. An example of this would be if only more serious depression lead to prescribed
doses or durations of SSRIs sufficient to increase risk of having a child with ASD. To approach this, analysis was conducted separately in women with and without indicators of depression. Here this stratification can be viewed as removing confounding by indication associated with the presence of the indicating condition. However, SSRI exposure prevalence in the group of women without depression will be fairly rare and effect estimates may have limited precision. In the group of women with depression, statistical adjustment for measures of depression can then be used to address what can be now thought of as residual confounding due to severity. Measures of depression severity will be constructed from available data on depression including: presence of an inpatient hospitalization for depression, recurrent mention of depressive disorder in outpatient claims; mention of only persistent mood [affective] disorders or dysthymia diagnoses without depression diagnoses.

The DAG shown in Figure 2 illustrates the approach in the restricted sample where all included women now have the indicating condition. Similar to that seen in Figure 1 in the full sample analysis, statistical adjustment will remove confounding by severity and block any path between a genetic variant associated with both depression severity and autism risk while not appearing to open any additional pathways in which maternal SSRI use may be associated with ASD. The depression severity measures available are also potentially susceptible to measurement error. The misclassification correction analyses mentioned
previously can also be used to quantify likely effects of misclassification of each depression subtype.

Confounding by indication is often found in epidemiological treatment studies focused on measures of treatment effectiveness and can also be found in studies of treatments as risk factors for more downstream health events. The presence or absence of an indicating (or contraindicating) condition or the severity of the indicating (contraindicating) condition can introduce this type of confounding. Unless treatment is assigned across subjects through a randomization process, treatment allocation will be based on indications – this is in fact what is desired in the thoughtful medical treatment of individual patients. For observational studies where treatments are risk factors, this poses real analytic challenges. Statistical adjustment, propensity scores, and restriction are techniques that can be utilized to control for confounding by indication in these types of studies. The potential for unmeasured or erroneously measured indicators leading to residual confounding is also a common concern. The investigator in observational studies of medical treatment must clearly be attentive to confounding by indication. Understanding the impact of confounding by indication and extent to which it may or may not be controlled is vital in the proper interpretation of pharmacoepidemiologic research findings.
REFERENCES


ADDITIONAL ANALYSIS

Imputation of Depression Severity ICD-10 Codes as a means of predicting ICD-10 Codes for mothers with only ICD-8 codes for depression in Chapter 4, that would have otherwise been dropped.

Severity measures for depression were explored in order to control for confounding by indication. The Danish version of the International Classification of Diseases (ICD-8) was used from 1977-1993, and ICD-10 codes since 1993. ICD-10 codes categorize depression as severe, moderate or high. For women who only had an ICD-8 code, we imputed ICD-10 codes using a regression model with maternal age and family SES as predictors and ICD-10 case the outcome. Below is the effect estimate for imputed ICD-10 severity codes and risk for ASD. This imputation analysis was not used in any of the Chapters since I did not explore the effect of depression severity on drug exposure and outcome. This was mainly due to the small numbers that resulted from stratifying by depression severity based solely on ICD-10 codes. In addition there were other definitions for depression that were not included in the severity measure (ie: major depressive episode) that would have been eliminated if I had only used ICD-10 depression severity scores.
Table C: Results from Imputation of Depression Severity Analysis in Chapter 4

<table>
<thead>
<tr>
<th>SSRI Exposure Period</th>
<th>Cases n= 53</th>
<th>Controls n=4326</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preconception</td>
<td>6</td>
<td>619</td>
<td>0.8 (0.3-1.9)</td>
<td>0.8 (0.3-1.9)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>7</td>
<td>730</td>
<td>0.9 (0.4-2.0)</td>
<td>0.9 (0.4-2.0)</td>
</tr>
<tr>
<td>First Trimester</td>
<td>4</td>
<td>524</td>
<td>0.7 (0.3-2.0)</td>
<td>0.7 (0.2-2.0)</td>
</tr>
<tr>
<td>Second Trimester</td>
<td>3</td>
<td>415</td>
<td>0.8 (0.2-2.5)</td>
<td>0.7 (0.2-2.4)</td>
</tr>
<tr>
<td>Third Trimester</td>
<td>3</td>
<td>444</td>
<td>0.6 (0.2-2.1)</td>
<td>0.6 (0.2-2.0)</td>
</tr>
</tbody>
</table>

Abbreviations: SSRI, Selective serotonin reuptake inhibitors; ASD, autism spectrum disorder; OR, odds ratio; CI, confidence interval.

The reference group had no exposure during the exposure period of interest.

a. Odds ratios were adjusted for month and year of birth of child and severity (reference is most severe)

Maternal Depression Severity

Mild: F32.0, 33.0, 32.8, 32.9, 33.4, 33.8, 33.9, 34.0, 34.1, 34.8, 34.9
Moderate: F32.1, 33.1
Severe: F32.2, 32.3, 33.2, 33.3
Multiplicative case-only approach in Chapter 5

The case-only approach to test for multiplicative interaction was also considered in the Chapter 5 analyses. This approach is more statistically efficient than conventional approaches of multiplicative interaction but requires a strong assumptions of independence between the exposure and genotype [1]. In implementing these analyses logistic regression models were limited to the ASD cases with predictors including exposure, the genotype alone, and their joint effects. In the models, only observations where ASD is equal to 1 were used (ie: cases only). The models were constructed by modeling the genotype of interested as the outcome, and the B2AR agonist exposure during pregnancy as the outcome, thus achieving the assumption that genotype and exposure are independent.

The odds ratio for this is a function of the odds ratios for the exposure alone, the genotype alone, and their joint effects in a standard case-control study:

\[
\text{OR}_{\text{from case-only}} = \frac{\text{OR}_{\text{from standard case-control}}}{(\text{OR}_e \times \text{OR}_g)} \times \text{OR}_{\text{from controls only}}
\]

Under the null hypothesis of no multiplicative effects the case-only odds ratio will be one. The results of this analysis are presented below.

The tables below summarize the results of these analyses. As can be seen, the case-only interaction odds ratio for the Arg16Gly genotype and B2AR agonist use during pregnancy was 2.7 (95% CI: 0.6-11.9) with adjusted odds ratios increasing after adjustment for potential indications and principle component values to adjust for ethnic differences (OR: 3.7; 95%CI 3.7 (0.8-16.6), Similarly, for associations for any B2AR agonist use, Gln27Gly, and their interaction we found a crude case-only interaction OR of 1.1 (95% CI 0.5-2.7) with little subsequent impact of adjustment. Possible indications
were adjusted in these models, but not elsewhere in the dissertation. The reason for this was that although it would have been interesting to see how these indications would effect the estimates, these variables were constructed based on all possible illnesses the mother mentioned while taking B2AR agonist drugs. Since there could be multiple conditions that indicated for B2AR agonist drug use, we reported the existing conditions a mother had for those that were taking B2AR agonist drugs.

Though the magnitudes of the case-only interaction odds ratio for Arg16Gly were above two, the confidence intervals were still quite wide and none of these estimates, nor those for Gln27Gly, were significantly different from the null at a conventional alpha error tolerance of 0.05. These estimates were not included in Chapter 5 since they did not add any addition information that was already presented in the traditional multiplicative interaction models and the two-degrees of freedom test. Furthermore, the confidence intervals for these estimates were rather wide due to the imprecision resulting form a small sample size.

Table D: Multiplicative effects analysis (unadjusted and PCV adjusted) of B2AR odds ratios from Arg16Gly case only models

<table>
<thead>
<tr>
<th>Arg16Gly</th>
<th>Crude OR (95% CI)</th>
<th>P value</th>
<th>Adjusted OR&lt;sup&gt;a&lt;/sup&gt; (95% CI)</th>
<th>P value</th>
<th>Adjusted OR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>2.7 (0.6-11.9)</td>
<td>0.1868</td>
<td>2.7 (0.6-11.9)</td>
<td>0.1901</td>
<td>3.7 (0.8-16.6)</td>
<td>0.0887</td>
</tr>
</tbody>
</table>

<sup>a</sup> Adjusted for principle component values
<sup>b</sup> Adjusted for principle component values and B2AR agonist drug indications (Indications: Asthma, Cold or Cough, Early Labor, Allergy, Upper Respiratory Infection, Influenza or Flu, Pneumonia, and Respiratory conditions.

Crude OR<sub>controls</sub> = 0.84 (0.3-2.5)

Table E: Multiplicative effects analysis (unadjusted and PCV adjusted) of B2AR odds ratios from Gln27Glu case only models

<table>
<thead>
<tr>
<th>Gln27Glu</th>
<th>Crude OR (95% CI)</th>
<th>P value</th>
<th>Adjusted OR&lt;sup&gt;a&lt;/sup&gt; (95% CI)</th>
<th>P value</th>
<th>Adjusted OR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>1.1 (0.5-2.7)</td>
<td>0.8093</td>
<td>1.1 (0.4-3.0)</td>
<td>0.7878</td>
<td>1.3 (0.5-3.5)</td>
<td>0.5790</td>
</tr>
</tbody>
</table>

<sup>a</sup> Adjusted for principle component values
<sup>b</sup> Adjusted for principle component values and B2AR agonist drug indications (Indications: Asthma, Cold or Cough, Early Labor, Allergy, Upper Respiratory Infection, Influenza or Flu, Pneumonia, and Respiratory conditions.

Crude OR<sub>controls</sub> = 0.84 (0.4-2.0)
ADDITIONAL NOTES ON SENSITIVITY ANALYSES USED IN CHAPTER 3 and 4

Below are additional notes that were used to formulate the results from the sensitivity analysis from Chapters 3 and 4. It provides additional calculations that were used to conceptualize the sensitivity analysis in these Chapters. To briefly summarize, misclassification was a potential challenge in this study since we were relying on registers data. The proportion of non-diseased or unexposed that is correctly classified can be assumed to be high. In other words, a person is less likely to be in the register if they do not have the disease or can be considered unexposed. While using Monte Carlo analysis we assumed a specificity of 100%. To correct for the misclassification of exposure and the confounder a Monte Carlo analysis was used to produce 1,000 simulated datasets, reclassifying exposure and indication status. For each simulation we present median OR estimates and the 2.5% and 97.5% percentile OR estimates as the confidence interval. Models using observed data are from conditional logistic regressions conditioning on the matching variables (child birth year and month) and adjusting for maternal depression while models using simulation were from unconditional logistic regressions models adjusting for the same variables as covariates.
**Exposure to SSRIs during pregnancy and risk for ASD**

Non-differential exposure misclassification with respect to outcome (equal sensitivity and
(specificity = 1) of exposure among cases and controls)

- Increase overall p(SSRI) from 0.8% to 3%

Observed

<table>
<thead>
<tr>
<th></th>
<th>ASD+</th>
<th>ASD-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSRI+</td>
<td>76</td>
<td>365</td>
<td>441</td>
</tr>
<tr>
<td>SSRI-</td>
<td>5139</td>
<td>51785</td>
<td>56924</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>5210</td>
<td>52150</td>
<td>57365</td>
</tr>
</tbody>
</table>

Truth

<table>
<thead>
<tr>
<th></th>
<th>ASD+</th>
<th>ASD-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSRI+</td>
<td>297  = x</td>
<td>1424 = y</td>
<td>1721</td>
</tr>
<tr>
<td>SSRI-</td>
<td>4918</td>
<td>50726</td>
<td>55644</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>5210</td>
<td>52150</td>
<td>57365</td>
</tr>
</tbody>
</table>

- \( (76/x) = (365/y) \)
- \( x + y = 1721 \)
- \( 76y = 365x \)
- \( y = 365x/76 \)
- \( x + 365x/76 = 1721 \)
- \( 5.8x = 1721 \)
- \( x = 297 \)
- Overall sensitivity = 0.26
- Sensitivity cases = 0.26
- Sensitivity controls = 0.26
- Reclassify 4.3% unexposed cases and 2.0% unexposed controls to exposed to SSRIs during pregnancy.
Differential exposure misclassification with respect to outcome (unequal sensitivity and (specificity = 1) of exposure among cases and controls)

- Increase overall p(SSRI) from 0.8% to 3%

Observe:

<table>
<thead>
<tr>
<th></th>
<th>ASD+</th>
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<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSRI+</td>
<td>76</td>
<td>365</td>
<td>441</td>
</tr>
<tr>
<td>SSRI-</td>
<td>5139</td>
<td>51785</td>
<td>56924</td>
</tr>
<tr>
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<td>5210</td>
<td>52150</td>
<td>57365</td>
</tr>
</tbody>
</table>

Truth:

<table>
<thead>
<tr>
<th></th>
<th>ASD+</th>
<th>ASD-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSRI+</td>
<td>194</td>
<td>1556</td>
<td>1750</td>
</tr>
<tr>
<td>SSRI-</td>
<td>5021</td>
<td>50594</td>
<td>55615</td>
</tr>
<tr>
<td>Total</td>
<td>5210</td>
<td>52150</td>
<td>57365</td>
</tr>
</tbody>
</table>

- 3% of 57365 = 1721
- 1721 – 441 = 1280 needs to be reclassified
- Overall sensitivity = 0.26
- Sensitivity cases = 0.39
- Sensitivity controls = 0.23
- Reclassify 2.3% unexposed cases and 2.3% unexposed controls to exposed to SSRIs during pregnancy
Non-differential misclassification of depression with respect to outcome (equal sensitivity and (specificity = 1) of maternal depression among cases and controls)

- Increase overall p(depression) from 0.7% to 15%

### Observed

<table>
<thead>
<tr>
<th></th>
<th>ASD+</th>
<th>ASD-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression+</td>
<td>55</td>
<td>347</td>
<td>402</td>
</tr>
<tr>
<td>Depression-</td>
<td>5160</td>
<td>51803</td>
<td>56963</td>
</tr>
<tr>
<td>Total</td>
<td>5210</td>
<td>52150</td>
<td>57365</td>
</tr>
</tbody>
</table>

### Truth

<table>
<thead>
<tr>
<th></th>
<th>ASD+</th>
<th>ASD-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression+</td>
<td>1177 = x</td>
<td>7428 = y</td>
<td>8605</td>
</tr>
<tr>
<td>Depression-</td>
<td>4038</td>
<td>44722</td>
<td>48760</td>
</tr>
<tr>
<td>Total</td>
<td>5210</td>
<td>52150</td>
<td>57365</td>
</tr>
</tbody>
</table>

- \( \frac{55}{x} = \frac{347}{y} \)
- \( x + y = 8605 \)
- \( 76y = 365x \)
- \( y = \frac{347x}{55} \)
- \( x + \frac{347x}{55} = 8605 \)
- \( 7.3x = 8605 \)
- \( x = 1177 \)
- Overall sensitivity = 0.047
- Sensitivity cases = 0.047
- Sensitivity controls = 0.047
- Reclassify 21.7% cases and 13.7% controls who did not have a mother with a history of depression as now having a diagnosis
Differential misclassification of depression with respect to outcome (unequal sensitivity and (specificity = 1) of maternal depression among cases and controls)

- Increase overall p(depression) from 0.7% to 15%

**Observed**

<table>
<thead>
<tr>
<th></th>
<th>ASD+</th>
<th>ASD-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression+</td>
<td>55</td>
<td>347</td>
<td>402</td>
</tr>
<tr>
<td>Depression-</td>
<td>5160</td>
<td>51803</td>
<td>56963</td>
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<tr>
<td>Total</td>
<td>5210</td>
<td>52150</td>
<td>57365</td>
</tr>
</tbody>
</table>

**Truth**

<table>
<thead>
<tr>
<th></th>
<th>ASD+</th>
<th>ASD-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression+</td>
<td>803</td>
<td>7858</td>
<td>8661</td>
</tr>
<tr>
<td>Depression-</td>
<td>4407</td>
<td>44292</td>
<td>48699</td>
</tr>
<tr>
<td>Total</td>
<td>5210</td>
<td>52150</td>
<td>57365</td>
</tr>
</tbody>
</table>

- 15% of 57365 = 8605
- 8605 – 402 = 8203 needs to be reclassified
- Overall sensitivity = 0.047
- Sensitivity cases = 0.068
- Sensitivity controls = 0.044
- Reclassify 14.5% cases and 14.5% controls who did not have a mother with a history of depression as now having a diagnosis
**Exposure to B2ARs during pregnancy and risk for ASD**

Correcting for non-differential exposure misclassification with respect to outcome (equal sensitivity and (specificity = 1) of exposure among cases and controls)

- Increase overall p(B2AR) from 3% to 4%

**Observed**

<table>
<thead>
<tr>
<th></th>
<th>ASD+</th>
<th>ASD-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2AR+</td>
<td>190</td>
<td>1489</td>
<td>1679</td>
</tr>
<tr>
<td>B2AR-</td>
<td>5010</td>
<td>50511</td>
<td>55521</td>
</tr>
<tr>
<td>Total</td>
<td>5200</td>
<td>52000</td>
<td>57200</td>
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</tbody>
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**Truth**

<table>
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<tr>
<th></th>
<th>ASD+</th>
<th>ASD-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2AR+</td>
<td>259 = x</td>
<td>1424 = y</td>
<td>2288</td>
</tr>
<tr>
<td>B2AR-</td>
<td>4941</td>
<td>49971</td>
<td>54912</td>
</tr>
<tr>
<td>Total</td>
<td>5200</td>
<td>52000</td>
<td>57200</td>
</tr>
</tbody>
</table>

- \( \frac{190}{x} = \frac{1489}{y} \)
- \( x + y = 2288 \)
- \( 190y = 1489x \)
- \( y = \frac{1489x}{190} \)
- \( x + \frac{1489x}{190} = 2288 \)
- \( 8.8x = 2288 \)
- \( x = 259 \)
- Overall sensitivity = 0.73
- Sensitivity cases = 0.73
- Sensitivity controls = 0.73
- Reclassify 1.38% unexposed cases and 1.07% unexposed controls to exposed to B2AR agonist drugs during pregnancy
Correcting for non-differential misclassification of maternal asthma with respect to outcome (equal sensitivity and (specificity = 1) asthma among cases and controls)

- Increase overall p(asthma) from 1.3% to 2%

### Observed

<table>
<thead>
<tr>
<th></th>
<th>ASD+</th>
<th>ASD-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma+</td>
<td>83</td>
<td>673</td>
<td>756</td>
</tr>
<tr>
<td>Asthma-</td>
<td>5117</td>
<td>51327</td>
<td>56444</td>
</tr>
<tr>
<td>Total</td>
<td>5200</td>
<td>52000</td>
<td>57200</td>
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</tbody>
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### Truth

<table>
<thead>
<tr>
<th></th>
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<th>ASD-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma+</td>
<td>126 = x</td>
<td>1018 = y</td>
<td>1144</td>
</tr>
<tr>
<td>Asthma-</td>
<td>5074</td>
<td>50982</td>
<td>56056</td>
</tr>
<tr>
<td>Total</td>
<td>5200</td>
<td>52000</td>
<td>57200</td>
</tr>
</tbody>
</table>

- \( \frac{83}{x} = \frac{673}{y} \)
- \( x + y = 1144 \)
- \( 83y = 673x \)
- \( y = \frac{673x}{83} \)
- \( x + \frac{673x}{83} = 1144 \)
- \( 9.1x = 1144 \)
- \( x = 126 \)
- Overall sensitivity = 0.66
- Sensitivity cases = 0.66
- Sensitivity controls = 0.66
- Reclassify .84% cases and .67% controls that did not have a mother with an asthma diagnosis in the register to having asthma.
CURRICULUM VITAE
Nicole B. Gidaya, Ph.D., M.P.H.

Education
2013  Drexel University
      Philadelphia, PA
      Doctorate of Philosophy Degree (Ph.D.): March 2013
      School: School of Public Health
      Department: Epidemiology and Biostatistics
      Advisor & Dissertation Chair: Craig Newschaffer, Ph.D.
      Dissertation Title: “The Effect of In Utero Exposure to B2AR Agonists and
      SSRIs on the Risk for Autism Spectrum Disorders”
      Current GPA: 3.89/4.00

2006  Drexel University School of Public Health
      Philadelphia, PA
      Master of Public Health (M.P.H.) Degree: May 18, 2006
      School: School of Public Health
      Department: Community Health and Prevention
      Advisor & Dissertation Chair: Michael Yudell, Ph.D., M.P.H.
      Dissertation Title: “Implementing an Early Intervention Strategy for
      Autism for Children in Norristown”
      Overall GPA: Pass (Excellent)

2004  University of California, Los Angeles
      Los Angeles, CA
      Graduated: June 2004
      Major: Psychobiology (B.S.)
      Overall GPA: 3.61/4.00

2002  University of California, Riverside
      Riverside, CA
      Attendance: September 2000–June 2002
      Major: Biomedical Studies

Professional Experience
2012–  HealthCore, Inc.
      Research Analyst
      Wilmington, DE

      –Performing prospective and retrospective health services, health outcomes, and
      health economic research activities
      –Supporting research activities for Health Outcomes project managers in the
      following areas: research designs, methodologies, analytical techniques, and
      reporting (with respect to statistical results)
      –Collaborating with the project managers to support the research functions that
      bring high quality reports, peer-reviewed publications, presentations to clients
      and at professional meetings, and other milestones to fruition
      –Utilizing industry standard as well as cutting-edge health economic analytical
      and statistical techniques
University of Copenhagen
“The Effect of In Utero Exposure to B2AR Agonists and SSRIs on the Risk for Autism Spectrum Disorders”
Copenhagen, Denmark (March 2011–May 2011)
-Designed a case-control study focused on estimation of exposure main effects capitalizing on a population-based sample constructed from Denmark’s national registries
-Constructed a large-scale dataset consisting of 763,474 children born in Denmark between 1996–2006, which included all maternal health information, maternal pharmacologic exposures during pregnancy, health outcomes, and psychiatric information
-Utilized the Danish Civil Registration System, Drug Prescription Registry, National Hospital Registry, Psychiatric Central Registry, and Medical Birth Registry to obtain the necessary variables for all study subjects

Early Autism Risk Longitudinal Investigation (EARLI)
Graduate Research Assistant; Network Clinical Coordinator
- Coordinated the multi-site clinical work group for a large-scale prospective project
- Collected environmental and biologic data through home and delivery visits
- Participated in study outreach to speak with potentially eligible families about the study protocol and eligibility criteria.
- Managed cross-site reliability, clinical protocols, and data collection instruments for the clinicians at each site
- Facilitated research efforts in the collaboration between the IBIS Network (Infant Brain Imaging Study) and EARLI
- Assisted with grant writing

University of Pennsylvania and Children’s Hospital of Philadelphia
Clinical Research Coordinator
- Organized scientific conferences
- Managed the database for the Center
- Assisted with grant writing
- Monitored budget and consortium agreement
- Provided an administrative link with collaborators

Maternity Care Coalition – Early Head Start
Norristown, PA (September 2005–May 2006)
Intern
- Created a program called “Implementing an Early Invention Strategy for Autism for Children in Norristown”
Conducted resource seminar on autism for parents and family advocates on early intervention
Established referral source for parents of autistic children

2005
Maternity Care Coalition – Cribs for Kids
Intern
-Educated families regarding sudden infant death syndrome (SIDS) reduction and healthy sleep habits
-Assisted in home visits
-Provided families with cribs
-Conducted SIDS reduction training program to health professionals

2005
University of California, Los Angeles Neuropsychiatric Institute – Child and Adolescent Partial Hospitalization Program
Los Angeles, CA (May 2005–September 2005)
Therapy Coordinator
-Coordinated activities for elementary and adolescent classrooms
-Assisted with group therapy sessions

2004-2006
Drexel University,
Graduate Research Assistant
-Conducted a literature review on Autism Spectrum Disorders
-Prepared presentations for conferences

2002–2005
University of California, Los Angeles Neuropsychiatric Institute – Early Childhood Partial Hospitalization Program
Los Angeles, CA (September 2002–September 2005)
Research Assistant
-Created an electronic database for Early Invention Project
-Administered four year follow-up assessments to children with autism
-Compiled a handbook for parents with autistic children regarding self help skills
-Performed intensive literature searches on autism spectrum disorders

2002–2005
Private Residences
Los Angeles, CA (September 2002–September 2005)
Behavioral Therapist
-Implemented early intervention in the home
-Administered behavioral therapy to toddlers diagnosed with autism
-Provided speech and play therapy
Editorial Activities
2011 Environmental Health Perspectives, October 2011
Manuscript reviewer

Professional Activities
2010– International Society for Autism Research
Member
2009– Society for Epidemiologic Research
Member
Organizer
2004– American Public Health Association
Member

Professional Presentations

2012 Drexel University School of Public Health Career Day
Philadelphia, PA (January 2012)
Selected Guest Speaker
-Provided insight to master level students on doctoral programs in the field of epidemiology
-Encouraged public health graduate students and undergraduates to make the most of their public health careers by considering a doctoral degree


Publications


**Teaching Experience**

2012–

Drexel University, Philadelphia, PA (January 2012–March 2012)
Teaching Assistant
PHBL 632 Applied Survey Research in Epidemiology

2010–2011

Drexel University School of Public Health: Epidemiology Department's ENRICH: Philadelphia Program

- Introduced public health concepts to students in Philadelphia high schools through seminars and open forums
- Taught public health and epidemiology principles to high school students and other community members.
- Stimulated critical thinking among high school students and actively engage them in learning in order to foster potential mentoring relationships

2010

Drexel University, Philadelphia, PA (May 2010)
Guest Lecturer
ENVS 451 Fundamentals of Epidemiology

2010

Drexel University, Philadelphia, PA (January 2010–March 2010)
Teaching Assistant
PBHL 530 Principles of Epidemiology

2009–2010

Drexel University, Philadelphia, PA (September 2009–June 2010)
Moderator
PBHL-Journal: Epidemiology and Biostatistics Journal Club

**Honors**

Dean’s List, University of California, Los Angeles (Aug 2002–June 2004)

**Awards**

Drexel University Student International Experience Award
“The Effect of In Utero Exposure to B2AR Agonists and SSRIs on the Risk for Autism Spectrum Disorders”
$6,000
- Supplement travel and housing cost to Copenhagen, Denmark