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Prenatal Medication Use And Autistic Behaviors In A South Korean Cohort

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Abstract

Autism spectrum disorder (ASD) is thought to result from a combination of genetic and environmental factors, yet a specific cause remains unknown. In this study, exposure and outcome data from a South Korean cohort (N=3,711) were analyzed to investigate the possibility of an environmental contribution to autism etiology. No significant association was observed between use of prenatal medications and autistic behaviors, as measured by Korean versions of both the Autism Spectrum Screening Questionnaire (ASSQ; OR 1.20, 95% CI 0.95–1.51) and the Social Responsiveness Scale (SRS; OR 0.98, 95% CI 0.78–1.25). Similarly, no significant associations were observed when use of prenatal vitamins, folic acid, or iron supplements was examined. There was also no dose-response relationship observed between number of medications taken during pregnancy and increased autistic behaviors. No evidence for a role of prenatal medication use in autism etiology is suggested by the data in this study. Future investigations focusing on specific subgroups of medications in this population are warranted.

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Table of Contents

Tables 5
Introduction 12
Materials and Methods 17
Results 20
Discussion 23
Conclusions 28
References 29
Appendix 32

Tables

Table 1. Characteristics of study participants according to prenatal medication status.

	Exposure status[a]		
Characteristic	**Took any prenatal medication (N=1,656)[b]**	**Did not take any prenatal medication (N=1,885)[b]**	**p[c]**
Maternal education			<0.001
<12 years	54 (3.7)	89 (5.9)	
12 years	789 (54.7)	906 (60.1)	
>12 years	600 (41.6)	512 (34.0)	
Paternal education			<0.001
<12 years	53 (3.7)	75 (4.9)	
12 years	608 (42.1)	745 (48.8)	
>12 years	785 (54.3)	708 (46.3)	
Monthly family income			<0.001
<1M KW	65 (4.1)	146 (8.4)	
1–<2M KW	237 (15.1)	328 (18.9)	
2–<3M KW	510 (32.5)	551 (31.8)	
3–<4M KW	367 (23.4)	380 (21.9)	
4–<5M KW	243 (15.5)	184 (10.6)	
≥5M KW	147 (9.4)	145 (8.4)	
Maternal smoking during pregnancy			0.145
Yes	5 (0.4)	12 (0.8)	
No	1433 (99.7)	1513 (99.2)	
Family history of ASD			0.122
Yes	33 (2.0)	25 (1.3)	
No	1613 (98.0)	1843 (98.7)	
Disease during pregnancy			0.003
Yes	148 (11.1)	116 (7.9)	
No	1183 (88.9)	1355 (92.1)	
Child's sex			0.121
Male	808 (48.8)	969 (51.4)	
Female	848 (51.2)	916 (48.6)	
Premature birth			0.692
Yes	68 (4.8)	67 (4.4)	
No	1364 (95.3)	1441 (95.6)	

Parity			<0.001
1st child	845 (54.7)	725 (42.5)	
2nd child	579 (37.5)	749 (43.9)	
≥3rd child	120 (7.7)	234 (13.7)	
Gestational age at birth			<0.001
≤31 weeks	9 (0.6)	40 (2.3)	
32-36 weeks	225 (14.0)	221 (12.7)	
37-41 weeks	1171 (73.0)	1211 (69.6)	
≥42 weeks	200 (12.5)	268 (15.4)	

a. Values are N (%) for categorical variables.
b. Column percentages may not sum to 100 due to missing data and rounding.
c. p-values are for the chi-square test or Fisher's exact test.

Table 2. Description of the sample population, by prenatal medication (N=3,711).

Prenatal medication	N (%)[a]
Any medication	
Yes	1656 (44.6)
No	1885 (50.8)
Hyperemesis	
Yes	172 (4.6)
No	3369 (90.8)
Hypertension	
Yes	9 (0.2)
No	3532 (95.2)
Folic acid	
Yes	85 (2.2)
No	3459 (93.2)
Iron	
Yes	1551 (41.8)
No	1990 (53.6)
Alternative	
Yes	77 (2.1)
No	3464 (93.3)
Pain	
Yes	32 (0.9)
No	3509 (94.6)
Prenatal vitamins	
Yes	106 (2.9)
No	3435 (92.6)
Other	
Yes	42 (1.1)
No	3669 (98.8)

a. Column percentages may not sum to 100 due to rounding and missing data

Table 3. Associations between ASSQ and SRS measures and prenatal medication use.

	No. (%) of Participants		
Outcome measure	**Used any prenatal medication (N = 1,656)[a]**	**Did not use any prenatal medication (N = 1,885)[a]**	**p[b]**
ASSQ total score			0.919
High ASD likelihood (ASSQ ≥ 15)	80 (4.9)	94 (5.0)	
Intermediate ASD likelihood (10 ≤ ASSQ < 15)	124 (7.5)	135 (7.2)	
Low ASD likelihood (ASSQ < 10)	1445 (87.6)	1645 (87.8)	
SRS T-score			0.237
High ASD likelihood (SRS ≥ 76)	19 (1.2)	27 (1.5)	
Intermediate ASD likelihood (60 ≤ SRS < 76)	167 (10.2)	218 (11.7)	
Low ASD likelihood (SRS < 60)	1456 (88.7)	1612 (86.8)	

a. Column percentages may not sum to 100 due to missing data and rounding.
b. p-values are for the chi-square test.

Table 4. Crude and adjusted effect of prenatal medication use on ASSQ and SRS scores, ordinal logistic regression.

	Odds Ratio (95% Confidence Interval)	
Outcome measure	**Used any prenatal medication**	**Did not use any prenatal medication**
ASSQ total score		
Crude	1.01 (0.83–1.24)	1.00
Adjusted[a]	1.20 (0.95–1.51)	1.00
Adjusted[b]	1.04 (0.85–1.28)	1.00
SRS T-score		
Crude	0.84 (0.69–1.03)	1.00
Adjusted[c]	0.98 (0.78–1.25)	1.00
Adjusted[d]	0.89 (0.72–1.10)	1.00

a. Adjusted for child's sex and maternal education.
b. Multiple imputation model; Adjusted for child's sex and maternal education.
c. Adjusted for child's sex, paternal education, and premature birth.
d. Multiple imputation model; Adjusted for child's sex, paternal education, and premature birth.

Table 5. Crude and adjusted effect of prenatal vitamin, folic acid, or iron supplement use on ASSQ and SRS scores, binary logistic regression.

	Odds Ratio (95% Confidence Interval)	
Outcome measure	**Used prenatal vitamins, folic acid, or iron**	**Did not use medication**
ASSQ total score ≥10		
Crude	0.98 (0.79–1.22)	1.00
Adjusted[a]	0.99 (0.80–1.22)	1.00
SRS T-score ≥60		
Crude	0.72 (0.58–0.90)	1.00
Adjusted[b]	0.87 (0.68–1.13)	1.00

a. Adjusted for child's sex.
b. Adjusted for paternal education and premature birth.

Table 6. Crude and adjusted effect of any prenatal medication use (excluding vitamins, folic acid, or iron supplements) on ASSQ and SRS scores, binary logistic regression.

	Odds Ratio (95% Confidence Interval)	
Outcome measure	**Used medication other than vitamins, folic acid, or iron**	**Did not use medication**
ASSQ total score ≥10		
Crude	1.72 (1.00–2.97)	1.00
Adjusted[a]	1.72 (1.00–2.97)	1.00
SRS T-score ≥60		
Crude	1.33 (1.42–1.82)	1.00
Adjusted[b]	1.55 (0.79–3.02)	1.00

a. All covariates were removed during backward elimination.
b. Adjusted for child's sex, paternal education, and premature birth.

Table 7. Crude and adjusted effect of number of prenatal medications taken on ASSQ and SRS scores, binary logistic regression.

	Odds Ratio (95% Confidence Interval)					
Outcome measure	**Zero prenatal medications**	**1 prenatal medication**	**2 prenatal medications**	**3 prenatal medications**	**4+ prenatal medications**	**p for trend[c]**
ASSQ score ≥10						0.352
Crude	1.00	0.99 (0.80–1.23)	1.15 (0.79–1.66)	0.90 (0.38–2.12)	1.80 (0.20–16.14)	
Adjusted[a]	1.00	1.19 (0.93–1.52)	1.36 (0.91–2.06)	1.00 (0.39–2.57)	1.80 (0.19–16.92)	
SRS T-score ≥60						0.249
Crude	1.00	0.78 (0.63–0.98)	1.08 (0.75–1.56)	0.98 (0.44–2.19)	1.65 (0.18–14.78)	
Adjusted[b]	1.00	0.91 (0.71–1.19)	1.29 (0.86–1.94)	1.25 (0.52–3.01)	1.70 (0.17–17.40)	

a. Adjusted for child's sex and maternal education.
b. Adjusted for child's sex, paternal education, and premature birth.
c. Cochran-Armitage test for trend

Introduction

Autism spectrum disorder is a complex neurodevelopmental disorder marked by deficits in social communication, as well as in restrictive and repetitive behaviors (APA, 2013). The prevalence of ASD has risen dramatically in recent years. According to the Centers for Disease Control and Prevention's (CDC) Autism and Developmental Disabilities Monitoring (ADDM) Network, analysis of data from 2010 reveals that ASD affects 1 in 68 (~1.5%) children in the United States (Developmental, 2014). Results from the National Survey of Children's Health (NSCH) reveal that the parent-reported prevalence of ASD in children ages 6 to 17 increased from 1.16% in 2007 to 2.00% in 2011–2012 (Blumberg et al., 2013). Observed increases in ASD prevalence have not been restricted to the United States. Idring and colleagues (2014) investigated the prevalence of ASD in the Stockholm Youth Cohort (SYC), finding a change in prevalence among children ages 0 to 17 from 0.42% in 2001 to 1.44% in 2011. In addition, ASD prevalence has been measured at greater than 2% in South Korean samples (Kim et al., 2011).

ASD is thought to be among the most genetic of neuropsychiatric disorders (Miles, 2011; O'Roak & State, 2008; Rutter et al., 1999). Early twin studies provided the foundation for this notion, calculating the disorder's heritability at above 90% and demonstrating a large difference in concordance rate between monozygotic and dizygotic twins (60%-90% vs. <5%; Rutter et al., 1999). The largest and most recent ASD twin study, from a group in Sweden, has also supported the idea of a strongly genetic component, calculating a relative recurrence risk (RRR) of ASD of 153.0 (95% confidence interval [CI] 56.7–412.8) among monozygotic twins and observing an

increasing RRR with increasing genetic relatedness (Sandin et al., 2014). However, to date only 20% of all cases of ASD have been explained by inherited genetic variants (Miles, 2011). The Swedish twin study recently estimated the heritability—the proportion of phenotypic variability attributable to genetic variation—of ASD to be 0.50 (95% CI 0.45–0.56), and the contribution of non-shared environmental influence was estimated at 0.50 (95% CI 0.44–0.55). Thus, while the cause of ASD is likely to have a strongly genetic component, there is still a possibility that environmental factors acting directly or through gene-environment interactions may contribute substantially to ASD etiology.

Over the years, several studies have attempted to elucidate the relationship between medication use during pregnancy and development of autism in offspring. In the first meta-analysis of the relationship between prenatal factors and ASD risk, Gardener and colleagues (2009) identified fifteen studies that examined prenatal medication use and determined that maternal use of any medication during pregnancy was associated with a 46% increased risk of ASD (95% CI 8%–96%). Maimburg and Vaeth (2006) conducted a case-control study examining the associations between a variety of exposures and infantile ASD. Data on prenatal medication use was obtained from Danish medical birth records for approximately 460 cases and 460 controls. After adjusting for parental ages, maternal citizenship, birth weight, gestational age, Apgar score, and birth defects, the authors found that children of mothers who took medications during pregnancy had an odds ratio (OR) of 1.5 (95% CI 1.1–2.1) compared to children of mothers who did not take medications, although the authors did not specify which classes of medication were included in their analyses (Maimburg & Vaeth, 2006). In contrast to

these studies, an earlier review of seven studies did not report an association between prenatal medication use and autism or autistic traits (Kolevzon et al., 2007).

Other studies have focused on various types of medications and supplements. In a rodent model of autism, a Korean group demonstrated that prenatal administration of Korean red ginseng extract—a traditional Korean medicine—inhibited valproate-induced hyperactivity and decreased sociability in the offspring (Kim et al., 2013). Several studies have investigated associations between prenatal folic acid use and ASD. Steenweg-de Graaff and colleagues (2014) observed in a prospective study that maternal folate plasma concentration during pregnancy was not associated with autistic traits in children. However, this group also observed that children of mothers who used prenatal folic acid supplements had lower autistic traits scores, as measured by the Social Responsiveness Scale (SRS), compared to children of mothers who did not use such supplements ($p<0.001$; Steenweg-de Graaff et al., 2014). In an analysis of over 97,000 individuals enrolled in the Norwegian Mother and Child Cohort (MoBa), Surén and colleagues (2013) examined use of folic acid supplements from four weeks before to eight weeks after the start of pregnancy. Exposure assessment occurred via a postal questionnaire administered at gestation week 18 and a food frequency questionnaire administered at gestation week 22. After adjusting for year of birth, maternal education level, and parity, the authors found that children of mothers who took folic acid had decreased odds of being autistic compared to children of mothers who did not take folic acid (OR 0.61, 95% CI 0.41–0.90; Surén et al., 2013). Schmidt et al. (2012) examined folic acid as an exposure in an analysis of participants from the Childhood Autism Risks from Genetics and Environment (CHARGE) study. Mean folic acid intake was significantly greater in

the first month of pregnancy for typically developing children (N=278) compared to children with ASD (N=429). In comparison with children whose mothers reported taking <600 μg/day of folic acid, children whose mothers took ≥600 μg/day had an ASD OR of 0.62 (95% CI 0.42–0.92). Folic acid intake was then categorized into quintiles, and a significant trend (p=0.01) was observed for decreasing risk of ASD as folic acid intake increased, controlling for maternal education level and child's birth year. However, this trend was rendered non-significant after also adjusting for intake of vitamins A, B6, C, and D.

The CHARGE study has also yielded insight into the relationship between prenatal vitamin use and ASD (Schmidt et al., 2011). In a 2011 study, prenatal vitamin exposure was assessed retrospectively through phone interviews. After adjusting for maternal education and the child's birth year, children of women who used prenatal vitamins three months before pregnancy through one month after conception had reduced odds of ASD compared to children whose mothers did not use prenatal vitamins during this time period (OR 0.62, 95% CI 0.42–0.93). In an analysis of data from the Health Outcomes and Measures of the Environment (HOME) study, mothers were interviewed during pregnancy about second trimester prenatal vitamin use, and autistic behaviors were later measured in enrolled children at ages 4 and 5 using the SRS (Braun et al., 2014). Compared to children of women who reported using prenatal vitamins weekly or daily, children of women who reported using them never or rarely had reduced odds of having a T-score ≥60 (reflecting autistic behaviors of mild to moderate severity) on the SRS after adjusting for a variety of prenatal and demographic variables.

Finally, prenatal use of antidepressant and antiepileptic drugs has received increased attention as an exposure that may be related to autism. A prospective study conducted with participants in the MoBa cohort reported that children exposed to antiepileptic drugs in utero yielded a 3.4-fold increased risk (95% CI 1.6–7.0) of the child having autistic traits, compared to unexposed children, as measured by the Social Communication Questionnaire (SCQ) and adjusting for several maternal and child factors (Veiby et al., 2013). Croen and colleagues (2011) observed that children of mothers in a Northern California population who took selective serotonin reuptake inhibitors (SSRIs) during the first trimester had a 3.5-fold increased risk (95% CI 1.5–7.9) of ASD compared to unexposed children after controlling for age, race, education, and other factors. Highlighting the lack of consensus regarding the role played by medication use in the etiology of ASD, numerous studies have failed to find an association between prenatal antidepressant use and ASD, some employing the same cohorts as studies that reported positive associations (Hviid et al., 2013; Sørensen et al., 2013; Clements et al., 2014).

Previous studies have yielded mixed findings regarding the association between prenatal medication use and ASD. Different methodologies have been used to assess differing exposures and outcomes in various populations. Thus, there remains a need for further investigation to build upon these earlier investigations in determining whether prenatal use of medications and supplements contributes to risk for ASD. If prenatal medication use were ultimately shown to impart ASD risk in several disparate populations, then there would be strong evidence for a true association between medication use and ASD occurrence. Further, if such an association exists, then there are

two important opportunities: 1) a potential role for gene-environment interactions (GxE) or fetal programming in the etiology of ASD, and 2) the possibility of a preventive intervention that alters the use of these medications during pregnancy. GxE are defined by genetic differences in vulnerability to specific environmental factors that contribute to a phenotype (Kim & Leventhal, 2015), while fetal programming of adult health states takes place when normal patterns of fetal growth are disrupted due to unfavorable conditions *in utero* (Pollard, 2007). Both of these influences on adult phenotype are important avenues through which medication use may affect autism risk if a true association exists.

This study is intended to inform future research investigating environmental factors *in utero* that may play a role in the etiology of autism. Equipped with a large sample size and the ability to incorporate a number of potential confounding variables, the present study is, to our knowledge, the first analysis of the association between prenatal medication use and autistic traits in a South Korean cohort.

Materials and Methods

Study population

This study is a secondary analysis of de-identified data from the Children's Health and Environmental Research (CHEER) study, an effort to examine associations between environmental agents and health outcomes affecting South Korean children. The CHEER study is sponsored by the Korean Ministry of the Environment, and a detailed description of this research effort is found in Ha et al., 2009. Briefly, from 2005 to 2007, 6,722 five- to fourteen-year-old students from 22 public schools in 10 South Korean locations (3 metropolitan, 4 industrialized, and 3 rural) were recruited for evaluation in the ongoing

CHEER study. From the original cohort, 5,293 students (79%) completed follow-up evaluations in 2007 and 2008, during which environmental exposure data were collected and blood and urine testing, perinatal risk evaluations, and physical exams were performed. The 40 cc of blood collected from each individual was stored at -70^{o}C in the biorepository at Dankook University. This follow-up sample was 51.5% male and had a mean age of 7.3±1.0 years. Of this sample, 3,804 agreed to participate in the CHEER gene-environment interaction study. After excluding 93 participants who self-reported use of unspecified "weight gain" medications, these individuals formed our study sample for the present analyses.

Exposure ascertainment

The primary exposure measure was use of any of seven classes of medication during pregnancy. The medications included were folic acid, hypertension medication, hyperemesis medication, iron supplements, alternative medicines, pain medication, or prenatal vitamins. In separate survey items, mothers of participating children were asked to recall whether they had taken these medications during pregnancy. Use of any prenatal medication was defined as responding "yes" to any of these items or writing in a different medication in a free-response field.

Outcome ascertainment

The two main outcome variables were parent-rated Autism Spectrum Screening Questionnaire (ASSQ) total score and parent-rated Social Responsiveness Scale (SRS) T-score. The ASSQ is a parent- or teacher-completed checklist useful for identification of Asperger's and other high-functioning ASD in children and adolescents with normal intelligence or mild intellectual disability (Ehlers et al., 1999). The questionnaire consists

of 27 items, each inquiring whether the child stands out as different from other children of his or her age in some way. Responses are rated on a scale from 0 (no) to 2 (yes). For the present analysis, it was decided that a cutoff score of ≥15 was reasonable for high likelihood of ASD based on the notion that this score represented 76% sensitivity and 81% specificity for detecting ASD (Ehlers et al., 1999). Consequently, ASSQ scores in the range of 10 to 14 were selected to indicate social impairment as a proxy for intermediate likelihood of having ASD. While the ASSQ has yet to be validated in South Korean populations, it has been translated into Korean and used in previous autism research in that country (Kim et al., 2011).

The SRS is a parent- or teacher-completed questionnaire designed to assess a child's ability to engage in reciprocal social interaction (Constantino et al., 2003). The questionnaire consists of 65 items, each rating an observed aspect of social behavior on a numeric scale from 1 (never true) to 4 (almost always true). Interpretation is based on a single score reflecting the sum of all 65 responses, which is converted to a standardized T-score. A T-score between 60 and 75 places the child within the mild to moderate range, suggesting that the child has mild or high-functioning ASD. A T-score of 76 or higher places the child within the severe range, as these scores are strongly associated with an ASD diagnosis (Constantino & Gruber, 2005). The CHEER study employed the Korean version of the SRS.

Statistical analysis

All statistical analyses were conducted using SAS, version 9.4 software via the SAS Studio interface, version 3.3 (SAS Institute Inc., Cary, NC). Crude associations between the exposure of interest and covariates were conducted with the chi-square test.

The crude and adjusted relationships between prenatal use of any medication and ASSQ and SRS scores were examined using ordinal logistic regression models. The following covariates were examined as potential confounders of these associations: parent-reported maternal education, paternal education, monthly family income, maternal smoking during pregnancy, family history of ASD, maternal disease during pregnancy, child's sex, premature birth, gestational age at birth, and parity. The majority of these covariates were included on the basis of having been incorporated into previous analyses of relationships between prenatal medication use and ASD (Braun et al., 2014; Schmidt et al., 2011; Schmidt et al., 2012; Surén et al., 2013). Disease during pregnancy was defined as having any of gestational diabetes, pregnancy-related hypertension, pre-eclampsia, thyroid disease, appendicitis, asthma, atopy, or any self-reported disease during the prenatal period. We arrived at the final logistic regression models via backward elimination of covariates. Covariates that remained significant at the 0.05 level for the Wald chi-square test were retained. In crude and adjusted analyses, complete case analysis was performed. Power was greater than 90% to detect a true odds ratio of 1.5 (Appendix). Sensitivity analyses using multiple imputation were constructed to assess the impact of missing data. In secondary analyses, the crude and adjusted impact of folic acid, prenatal vitamin, or iron supplement use vs. medication non-use, as well as the impact of the number of medications taken, were assessed using binary logistic regression models.

Results

Study participants

This study sample consisted of 3,711 mother-child pairs, which is approximately 55% of the original CHEER cohort. Data on the primary exposure of prenatal use of any

medication were missing for 170 (4.6%) participants. Mothers who took any prenatal medication tended to have had a disease during pregnancy, and they also tended to be more highly educated and wealthier (Table 1). Compared to mothers who took prenatal medications, mothers who did not take any prenatal medications did not differ significantly with respect to smoking status, family history of ASD, giving birth prematurely, or child's sex.

Prenatal medication intake

Use of any medication during pregnancy was reported by 1,656 mothers (44.6%) in the study sample (Table 2). Iron supplements were the most common medication reported, with over 40% of prenatal medication users reporting iron supplement use. By contrast, hypertension medication use was least prevalent in the sample, with only 0.2% of those who took medications reporting its use. As shown in Table 3, prenatal use of any medication did not have a significant association with either ASSQ total score or SRS T-score, as measured by the chi-square test. The distribution of ASD likelihood as measured by both parent-rated ASSQ and parent-rated SRS was similar between children of mothers who did and did not use medications during pregnancy.

The crude and adjusted effects of prenatal medication use on ASSQ and SRS scores are shown in Table 4. In the unadjusted analysis, use of any prenatal medication was not predictive of an increased ASSQ score compared to non-use of prenatal medication. After adjusting for child's sex and maternal education, the OR for this association increased slightly but remained non-significant at 0.05 level (OR 1.20, 95% CI 0.95–1.51). Similarly, use of any prenatal medication was not predictive of an increased SRS score compared to non-use of prenatal medications in the unadjusted

analysis. After adjusting for child's sex, paternal education, and premature birth, the association remained non-significant (OR 0.98, 95% CI 0.78–1.25). Sensitivity analyses with imputed values for all variables in the adjusted analyses were also non-significant, although these effect estimates did not pass the score test for the proportional odds assumption and may thus be inaccurate estimates.

In the secondary analyses, children of mothers who used prenatal vitamins, folic acid, or iron supplements were no more likely to have an ASSQ score of 10 or greater compared to children of mothers who did not report prenatal medication use (Table 5; OR 0.99, 95% CI 0.80–1.22). Despite the observation of a protective effect in the crude analysis, children of mothers who took prenatal vitamins, folic acid, or iron supplements were not significantly more likely to have an SRS T-score of 60 or more in comparison to children of mothers who did not use medications in the adjusted analysis (OR 0.87, 95% CI 0.68–1.13). Conversely, in crude analyses, children of women who used any medication other than vitamins, folic acid, or iron supplements were more likely to have both an ASSQ score ≥10 and an SRS T-score ≥60 (Table 6). Attempts to adjust for covariates did not alter the ASSQ OR, but the SRS OR was rendered non-significant following adjustment for child's sex, paternal education, and premature birth. Finally, no dose-response relationship between number of medications taken and risk of autistic behaviors was observed (Table 7). One odds ratio in the crude analysis was marginally significant at the 0.05 level, but no effect estimates in the adjusted analyses were significant. Slight dose-response trends were observed correlating higher scores of autistic behavior with increasing number of medications taken, particularly in the SRS

analysis, but these too were deemed non-significant by the Cochran-Armitage test for trend.

Discussion

Prenatal medication intake

In this study of a South Korean cohort, no significant associations were found between prenatal medication use and ASSQ total score, as well as between prenatal medication use and SRS T-score. To our knowledge, this is the first study that has investigated these associations in a South Korean population. Assuming that the prevalence of autism in this Korean population is similar to that calculated by Kim and colleagues (2.64%), this study had sufficient power to detect an OR of 1.5 for children of exposed vs. unexposed mothers (Appendix). However, a much larger study sample would be necessary to detect a significant effect estimate if the true OR is more moderate than 1.5. An OR of 1.5 was chosen for our power calculations based on the ORs of 1.46 and 1.50 observed by Gardener et al. (2009) and Maimburg & Vaeth (2006), respectively, in their assessments of the relationship between any prenatal medication use and ASD. Sensitivity analyses employing multiple imputation of the variables included in the adjusted analysis did not substantially impact the ORs reported in Table 4.

Several of the covariates used in the main analyses had non-trivial amounts of missing data. For the adjusted ASSQ analysis in Table 4, 799 (20.9%) subjects were excluded due to missing data, while 873 (23.5%) subjects were excluded from the adjusted SRS analysis. Assuming non-differential misclassification of missingness, this phenomenon would be expected to bias the results toward the null, which could explain in part our non-significant effect estimates. Additionally, if mothers who took prenatal

medications are biologically different from mothers who did not take such medications, our results could be impacted by confounding by indication. In other words, the child's risk of autism could be influenced by the conditions that caused the mothers to take medications, rather than the medications themselves. Small numbers of subjects reporting certain medications, such as hypertension and pain medications, necessitated the use of a composite primary exposure variable and precluded substantial secondary analyses investigating medication subtypes. If such subgroup analyses had been possible, it might have been clearer as to which medications may impart the greatest risk of autism to offspring in this population.

Previous studies have suggested that use of prenatal vitamins and folate may confer protective effects against autism development to the fetus (Braun et al., 2014; Schmidt et al., 2011; Schmidt et al., 2012). Our secondary analyses of this relationship did not agree with these studies, as we observed in our adjusted analyses no significant association between maternal prenatal vitamin, folic acid, or iron supplement use and both outcome measures (Table 5). Iron supplements were included in this composite exposure variable because, like prenatal vitamins and folic acid, these supplements are taken as part of routine prenatal care rather than for a specific condition requiring treatment. These analyses were also impacted by missing data to roughly the same extent as the primary analyses. For the adjusted ASSQ analysis in Table 5, 472 (12.7%) subjects were excluded due to missing data, and 1,107 (29.8%) subjects were excluded from the adjusted SRS analysis. The analyses in Table 6 show the converse of those in Table 5. Use of any medication other than vitamins, folic acid, or iron supplements was shown to be borderline significant with an increased ASSQ score, while a non-significant OR was

observed in the SRS analysis. We hypothesized that, in contrast to the medications in Table 5, these medications might place children at increased risk of autistic behaviors. While we did observe ORs above 1.0, they were not significant at the 0.05 level. It is possible that, with a larger sample size, a significant effect might have been observed.

In the final secondary analyses, no significant trend relating increased prenatal medication intake to higher ASSQ and SRS scores was observed (Table 7). The wide CIs for some cells are indicative of the imprecision of these calculations, as cell sizes for those reporting the highest usage of medication were small. Few people reported taking more than four medications during pregnancy, so it was necessary to collapse these individuals into a single category of high medication intake so that the cell sizes would be large enough to conduct these analyses. Similarly, binary logistic regression was chosen over ordinal logistic regression for all secondary analyses, as the cell sizes would have been too small to permit meaningful calculations.

Study strengths and limitations

The large sample size in this study allowed for precise effect estimates in the primary analyses. Also, substantial covariate data allowed for the inclusion of numerous potential confounders in the adjusted analyses. Use of multiple measurements of autistic behaviors permitted us to examine the effect of medication use on disparate components of the autism phenotype. However, retrospective self-report of exposure information invites the possibility of recall bias. Mothers in this study were asked to provide exposure information from several years in the past, and recall accuracy was likely an issue for many participants. In some cases, mothers could have been recalling exposure information after their children had already been formally diagnosed with ASD, and these

mothers might have been more inclined than mothers of children without autism to report prenatal medication use, thinking it a potential cause of autism. At the same time, the total number of children scoring in the high-risk ranges of the ASSQ and SRS is small, so any recall bias is likely to be non-differential, skewing our results toward the null. The results in our analyses were also plagued by substantial missing data for several covariates. For almost all of our complete case analyses, more than one-fifth of all subjects were excluded due to missing data. These missing data impair our ability to generalize the results of this study to the rest of the CHEER cohort, as our study sample may not be wholly representative of the study population.

We are also limited by a lack of information on the frequency and dosage of prenatal medications to which women in the cohort were exposed. It is possible that prenatal medication use could confer autism risk only during particular stages of pregnancy, and previous studies have observed significant associations between use of specific medications during particular trimesters and ASD risk (Croen et al., 2011; Gidaya et al., 2014). However, women in the current study only self-reported any use of prenatal medications rather than use during specific intervals, so stratification by trimester was not possible. The lack of dosage information precluded the ability to test whether higher doses of specific medications might confer higher risk of ASD to the offspring. For almost all of the seven broad classes of medication examined in this study, the prevalence of use was too small to permit subgroup analyses that would have allowed us to examine the separate effects of different classes of medication. It is likely that the dosage of specific types of medication taken during pregnancy, rather than the total number of different medications taken, is of great importance in elucidating the

relationship between medication use and ASD risk, yet we were unable to investigate this area further in this study. This consideration is especially important in relation to potential opportunities for GxE as well as fetal programming. Different subtypes of prenatal medication, and even different medications within those subtypes, can exert disparate effects on the developing fetus. An inability to study medication subtypes makes it difficult to form plausible biological mechanisms for the potential effect of prenatal medications on autism development via GxE or fetal programming.

Although a number of previous studies have examined use of any prenatal medication as a risk factor for ASD (Gardener et al., 2009; Maimburg & Vaeth, 2006), not all of these studies specified the precise subclasses of medications that were included under the catch-all of "any medication use." This lack of detailed information makes it difficult to directly compare the results of this study with previous work. For example, the CHEER cohort did not contain information about use of psychiatric medication such as antidepressants, whereas Gardener and colleagues in their review identified this subclass of medications as one that might put children at an increased risk of ASD compared to just "any medication use" (Gardener et al., 2009).

Finally, outcome assessment in this sample population was conducted using tools that screen for autistic behaviors rather than those that form the gold standard in autism diagnosis. Our analysis thus assessed the risk of having a high score on either the ASSQ or SRS rather than receiving a clinical ASD diagnosis via the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS).

Conclusions

Though subject to various limitations as noted above, our data do not suggest an association between prenatal medication use and risk of autistic behaviors in this South Korean population. Given the disagreement between these results and those of previous studies, especially with regard to prenatal vitamin and folic acid use, further study with improved exposure assessment is warranted in order to clarify any relationship between prenatal medication use and autism that may exist. Such a relationship would provide evidence for an environmental contribution to autism risk, which could enable an avenue for intervention aimed at reducing autism risk in the population.

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Appendix

Ordinal logistic regression power calculations

Adapted from:

Whitehead, J. (1993). Sample size calculations for ordered categorical data. *Statistics in Medicine*, *12*(24), 2257–2271. doi:10.1002/sim.4780122404

OR of 1.5

Table A1.

No prenatal meds	C_1 – low likelihood ASD (ASSQ or SRS)	C_2 – intermediate likelihood ASD (ASSQ or SRS)	C_3 – high likelihood ASD (ASSQ or SRS)
p(iC)	0.9236	0.05	0.0264[a]
Q(iC)	0.9236	0.9736	1

a. Prevalence estimate from Kim et al., 2011

$$\theta_R = \log\left\{\frac{Q_{2ER}(1-Q_{2C})}{Q_{2C}(1-Q_{2ER})}\right\}$$

Fig. A1. Equation for ln(OR), from Whitehead, 1993

$$\theta_R = \ln\frac{0.9604(1-0.9736)}{0.9736(1-0.9604)} = -0.419$$

$$Q_{iER} = \frac{Q_{iC}}{Q_{iC} + (1-Q_{iC})\exp(-\theta_R)},$$

Fig. A2. Equation for cumulative probabilities of exposed group, from Whitehead, 1993

$$Q_{1ER} = \frac{0.9236}{0.9236 + (1-0.9236)e^{0.419}} = 0.88828$$

Table A2.

Prenatal meds	C_1 – low likelihood	C_2 – int. likelihood	C_3 – high likelihood
p(iER)	0.88828	0.07212	0.0396[a]
Q(iER)	0.88828	0.9604	1

a. Assumes that, if prenatal use of any medication does increase the likelihood of having a child with ASD, among those exposed to any medication, probability of being in high likelihood group is 1.5 times that of the unexposed group.

Table A3.

	C_1 – low likelihood	C_2 – int. likelihood	C_3 – high likelihood
$\bar{p}_i$	$\frac{0.88828+0.9236}{2} = 0.9059$	$\frac{0.05+0.07212}{2} = 0.1221$	$\frac{0.0264+0.0396}{2} = 0.0660$

$$n = \frac{3(A+1)^2(u_{\alpha/2}+u_{\beta})^2}{A\theta_R^2\left(1-\sum_{i=1}^{k}\bar{p}_i^3\right)}.$$

Fig. A3. Equation for total N required

Where A = allocation ratio = $N_{exposed}$:$N_{unexposed}$ = 1

80% power

$$N = \frac{3(1+1)^2(1.96+0.84)^2}{(-0.419)^2(0.254)} = 2105.9 = 2106 = 1053\ per\ group$$

90% power

$$N = \frac{3(1+1)^2(1.96+1.28)^2}{(-0.419)^2(0.254)} = 2824.9 = 2825 = 1413\ per\ group$$

OR of 1.25

Table A4.

No prenatal meds	C_1 – low likelihood ASD (ASSQ or SRS)	C_2 – intermediate likelihood ASD (ASSQ or SRS)	C_3 – high likelihood ASD (ASSQ or SRS)
p(iC)	0.9236	0.05	0.0264[a]
Q(iC)	0.9236	0.9736	1

a. Prevalence estimate from Kim et al., 2011

$$\theta_R = \log\left\{\frac{Q_{2ER}(1-Q_{2C})}{Q_{2C}(1-Q_{2ER})}\right\}$$

Fig. A4. Equation for ln(OR), from Whitehead, 1993

$$\theta_R = \ln\frac{0.967(1-0.9736)}{0.9736(1-0.967)} = -0.230$$

$$Q_{iER} = \frac{Q_{iC}}{Q_{iC} + (1 - Q_{iC})\exp(-\theta_R)},$$

Fig. A5. Equation for cumulative probabilities of exposed group, from Whitehead, 1993

$$Q_{1ER} = \frac{0.9236}{0.9236 + (1-0.9236)e^{0.419}} = 0.9057$$

Table A5.

Prenatal meds	C_1 – low likelihood	C_2 – int. likelihood	C_3 – high likelihood
p(iER)	0.9057	0.0613	0.033[a]
Q(iER)	0.9057	0.967	1

a. Assumes that, if prenatal use of any medication does increase the likelihood of having a child with ASD, among those exposed to any medication, probability of being in high likelihood group is 1.25 times that of the unexposed group.

Table A6.

	C_1 – low likelihood	C_2 – int. likelihood	C_3 – high likelihood
$\bar{p}_i$	$\frac{0.9057+0.9236}{2} = 0.9147$	$\frac{0.05+0.0613}{2} = 0.05565$	$\frac{0.0264+0.033}{2} = 0.0297$

$$n = \frac{3(A+1)^2(u_{\alpha/2} + u_\beta)^2}{A\theta_R^2\left(1 - \sum_{i=1}^{k}\bar{p}_i^3\right)}.$$

Fig. A6. Equation for total N required

Where A = allocation ratio = $N_{exposed}$:$N_{unexposed}$ = 1

$$N = \frac{3(1+1)^2(1.96+0.84)^2}{(-0.230)^2(0.234)} = 7585 = 3793\ per\ group$$