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Obstetric and Parental Psychiatric Variables as Potential Predictors of Autism Severity

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Abstract Associations between obstetric and parental psychiatric variables and subjects' Autism Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic Observation Schedule (ADOS) domain scores were examined using linear mixed effects models. Data for the 228 families studied were provided by the Autism Genetic Resource Exchange. Hypertension ($P = 0.002$), preeclampsia ($P = 0.021$) and generalized edema ($P = 0.011$) were associated with higher ADI-R communication scores. Hypertension ($P = 0.011$), albuminuria ($P = 0.039$) and generalized edema ($P = 0.009$) were associated with higher ADI-R repetitive behaviors scores. Parent depression was associated with higher ADI-R repetitive behaviors scores ($P = 0.005$), and parent anxiety with lower ADOS social/communication composite scores ($P = 0.025$). The associations between hypertension-related obstetric conditions and autistic severity warrant further investigation and raise intriguing questions regarding potential causal and modifying factors in autism.

Keywords Autism · Preeclampsia · Psychiatric · Obstetric · Hypertension · Depression

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The CDC has recently released prevalence estimates for Autism Spectrum Disorders (ASD) assessed in an ongoing multi-site autism surveillance project. The data collected for the observation years 2000–2002 show that on average 6.6–6.7 out of 1,000 children have been diagnosed with an ASD by 8 years of age (CDC 2007). The public health impact of autism is clear given the debilitating nature of the disorder, which varies with the severity of autistic symptoms and other comorbid conditions such as mental retardation and seizure disorders. A number of different obstetric and parental psychiatric variables have been previously reported to be associated with the diagnosis of autism. The goal of the present study was to determine if specific obstetric and parental psychiatric variables predict the severity of autistic symptoms. The study's purposes raise basic issues regarding the nature of autism and about how to assess severity of autistic behavior. In addition, the present undertaking needs to be considered in the context of prior investigations of prenatal/perinatal factors in autism and of psychiatric symptoms in families of autistic individuals.

Autism is defined as a neurodevelopmental disorder that emerges before the age of three and is characterized by three core features: abnormal social interactions; impairments in verbal and nonverbal communication; and restricted, repetitive or stereotyped behaviors (APA 1994). The expression of these core features varies among individuals and it is likely that much of this heterogeneity is due to underlying genetic differences. While the etiology of autism is unknown, current evidence suggests a complex model in which multiple genes interact to cause susceptibility for autism, and several different subsets of these genes can lead to autism. It is unlikely that any single genetic variant is necessary to cause autism, as a large number of genome-wide scans of multiplex families have

failed to yield a single variant common to all or most cases (Ma et al. 2005). Several genetic disorders are known to be associated with the diagnosis of autism including phenylketonuria, adenylosuccinate lyase defect, Down syndrome, etc. (Feinstein et al. 2007) While the presence of these specific disorders has been linked to autism in a small percentage of cases, the ultimate picture is one of multiple etiologies. Although the contribution of genetics is well-established based on twin studies (Bailey et al. 1995; Steffenburg et al. 1989), environmental exposures (Stromland et al. 1994; Williams et al. 2001), gene-environment interactions and "experientially-influenced brain development processes" may also play important roles in the etiology of autism (Rubenstein and Merzenich 2003).

Autism is only one of a spectrum of pervasive developmental disorders (PDD), which are often lumped together under the classification autism spectrum disorders (ASD). The expression of autistic behavior across all autism spectrum disorders is necessarily more heterogeneous than within the more well-defined autism classification alone. The boundaries of autism spectrum disorders are unclear and appear to overlap to some degree with normal behavior (Micali et al. 2004). Studies that examine ASDs as a single entity may ultimately be biased toward the null hypothesis because of this overwhelming heterogeneity. Yet, the heterogeneity of autism is itself of interest and may offer insight into the potential modifiability of autistic symptoms. Therefore, it may be useful to take a more endophenotypic approach by exploring as endpoints the degree of impairment within each of the core features of autism (McBride et al. 1996; Szatmari et al. 2002).

Previous studies of the associations between prenatal and perinatal factors and the diagnosis of autism have found inconsistent results; however suboptimal conditions of pregnancy and delivery have been shown to be associated with autism in a number of studies (Bryson et al. 1988; Gillberg and Gillberg 1983; Stein et al. 2006). It is unclear why a range of complications or suboptimal obstetric conditions would be associated with autism such that combining these conditions into a single variable would help explain autism. Yet recent evidence suggests that subtle abnormalities of fetal development may be associated with autism (Anderson et al. 2007) and it is therefore not surprising that obstetric suboptimality may be observed. Although not causally related in this model, using optimality scales to predict the severity of autistic symptoms may still be informative and lead to improved methods or approaches to screening and early risk assessment.

An analysis based on optimality scores could not be accomplished as part of the present study due to a large amount of missing data on several variables included in prior optimality scales. Instead, an analysis of individual prenatal, perinatal, and neonatal characteristics and their associations with the ADI-R and ADOS domain scores will be undertaken to explore this general area. Additionally, one obstetric variable is of particular interest. A recent study by Connors et al. (2005) suggested an association between maternal use of beta-2 adrenergic receptor agonists during pregnancy and increased concordance for autism among dyzygotic twins. A potential mechanism for the increase in concordance may be an increase in severity because unaffected siblings, including unaffected twins in discordant pairs, often express some autistic symptoms or other developmental delays but do not meet criteria for autism (Micali et al. 2004; Murphy et al. 2000; Piven and Palmer 1999). The hypothesis that maternal use of beta-2 adrenergic agonists during pregnancy is associated with greater severity of autistic symptoms will be explored in the analysis.

The aggregation of psychiatric disorders among relatives of individuals with autism is another area of interest. Several studies have found an association between familial psychiatric disorders, most commonly anxiety and depression, and autism. Affective disorders were significantly more common among relatives of autistic probands than among relatives of Down syndrome probands in studies by Bolton et al. (1998) and Piven and Palmer (1999). Micali et al. (2004) found that depression and anxiety were more prevalent among mothers of autistic probands than mothers of controls. The relationship between autism and familial psychiatric disorders is unclear, although it is reasonable to consider a situation in which genetic loading for psychiatric disorders is also related to susceptibility for autism among offspring, and perhaps to the severity of autistic symptoms as well. However, it is possible that causality acts in the opposite direction, such that parents may experience depression or anxiety in response to the difficulties of parenting a child with autism. Finally, parents with depression may have particular difficulty managing the autistic symptoms of their children (leading to greater actual or perceived symptomatology), and may report these symptoms differently than non-depressed parents during the ADI-R interview. Associations between parental psychiatric histories and severity of autistic symptoms will be tested, and potential explanations for any significant associations will be considered.

The study was intended to accomplish three aims. The first was an exploratory analysis of prenatal and perinatal conditions as predictors of the severity of autistic symptoms. The second was specifically to test whether one obstetric variable, maternal use of beta-2 adrenergic receptor agonists during pregnancy, is associated with greater severity of autistic symptoms. The third was to test whether lifetime diagnoses of psychiatric disorders in parents of children with autism were associated with more severe autistic symptoms.

Method

Participants

The data for this study were provided by the Autism Genetic Resource Exchange (AGRE), and the study was approved by the Yale Human Investigation Committee in accordance with AGRE requirements.

At the time the data were acquired from AGRE for this study, 682 families were enrolled. (Recruitment methods are available from AGRE). However, medical histories were only available for 242 nuclear families, including: 467 affected children, 237 mothers and 231 fathers. The families recruited into the AGRE study were primarily multiplex, that is, families with more than one affected individual. Pedigrees were examined for this study to ensure that the appropriate parents were assigned to each affected child as some families included affected children from different sets of parents. A list of potentially non-idiopathic cases of autism was provided by AGRE. Twelve subjects from this list were excluded: 7 with full or intermediate fragile X, 2 with SNRPN duplication, 2 with a known chromosomal abnormality and 1 with a known neurogenic disorder. Five cases with missing data for all outcome measures were excluded, and for reasons to be explained in the statistical analysis section, one twin from each of six sets of twins with unknown zygosity was randomly excluded. The final sample size was 444 subjects in 228 families. In this sample, 28 families had 1 affected child, 185 had two affected children, 14 had three affected children, and 1 had four affected children. Fifty-nine children were from a multiple pregnancy and 27 of these were from a monozygotic twinship.

Instruments

Autism Diagnostic Interview-Revised (ADI-R). The ADI-R is a standardized, semi-structured interview used for diagnostic purposes. The interview includes 111 items which measure behaviors in the areas of social interaction, communication, and repetitive, restricted and stereotyped behaviors and interests (Lord et al. 1994). Items include questions about early childhood development and ages 4 to 5 (a period of particular aberration), and qualitatively abnormal behaviors currently exhibited and ever exhibited (Lord et al. 1994). Only the algorithm domain scores were used in this study.

Autism Diagnostic Observation Schedule–Generic (ADOS–G).The ADOS-G is a semi-structured observational examination (Lord et al. 2000). The ADOS provides information about current functioning within the social and communication domains of autism by providing subjects with the opportunity to engage in interactions with the trained examiners (Lord et al. 2000). Due to the brevity of the observation period of the ADOS, the severity of repetitive and sensory behaviors may not be fully captured during the examination. The diagnosis of autism is defined by cutoffs for each domain and a cutoff for the social/communication composite (Lord et al. 2000). Only the social/communication composite score was used in this study.

Affected Child Medical History. The Affected Child Medical History questionnaire is administered by trained AGRE interviewers. Most questionnaires were administered in the home. Two were administered in residential facilities and three were administered in a clinic setting. Information was collected from mothers, fathers or both parents. The questionnaire includes questions about the prenatal, perinatal and neonatal periods, as well as the mother's prior obstetric history with respect to each child. Additional information about childhood was available but not included in any analyses.

Maternal/Paternal Medical History. The Maternal/Paternal Medical History questionnaire collects information about seizures, tic/movement disorders, other neurological disorders, cancer, endocrine disorders, allergies, immune disorders, asthma, respiratory disorders, cardiovascular disorders, gastrointestinal disorders, genitourinary disorders, current medications, psychiatric history, substance use, and parental developmental history. Maternal and paternal medical histories, including psychiatric histories, were linked with the child data by family identification number. All medical history data were based on self-report.

Variables

Outcome variables were the ADI-R domain scores and the ADOS social/communication composite score. These are continuous variables that can be used to characterize the degree of impairment in each of the three core features of autism. From the ADI-R, the "B total" score measures impairment in social function, the "C total" score measures impairment in communication (scores for verbal and non-verbal subjects have been combined), and the "D total" score measures restricted, repetitive and stereotyped behaviors or circumscribed interests. The ADI-R domain scores are intended to be measures of lifetime impairment.

From the ADOS, the social/communication composite score is a combined measure of current impairment in social interaction and communication. The ADOS social and communication domains measure the quality of interactions as they are observed by the examiner. These domains are interrelated in the context of interpersonal interactions; therefore the composite was chosen as a measure of current severity.

Predictor variables were obstetric variables (including beta-2 adrenergic receptor agonist use during gestation) and parental psychiatric histories, all obtained via self-report. The following obstetric variables were included in the analyses: vaginal bleeding first trimester, vaginal bleeding any trimester, severe infection first trimester, severe infection any trimester, hypertension, gestational diabetes, eclampsia/preeclampsia, gestational age at delivery, epidural, cesarean section, cord problems, placenta problems, birth weight, hyperbilirubinemia, albuminuria, generalized edema, forceps delivery, prescription medications during pregnancy, maternal use of beta-2 adrenergic receptor agonists, maternal use of antibiotics, and maternal use of thyroid medication. Vaginal bleeding any trimester was created using the variables vaginal bleeding first trimester, vaginal bleeding second trimester, and vaginal bleeding third trimester. Severe infection any trimester was created using the variables severe infection first trimester, severe infection second trimester and severe infection third trimester. Eclampsia/preeclampsia was a combined measure within the medical history questionnaire. While it was not possible to separate preeclampsia from eclampsia, it was decided that this variable primarily represented cases of preeclampsia because no seizures or coma were reported elsewhere in the questionnaire. This variable will be called preeclampsia from this point forward. Four additional variables were collapsed into two variables, including cord problems created from nucchal cord and cord prolapse, and placenta problems created from placenta praevia and abruptio placenta. The following obstetric variables were not included because they were missing in more than 5% of subjects: gravida, spontaneous abortions, elective abortions, amniocentesis, chorionic villus sampling, vaginal bleeding second trimester, vaginal bleeding third trimester, severe infection second trimester, severe infection third trimester, maternal fever, hyperemesis, pitocin, presentation, vacuum extraction, meconium staining, Apgar score at 1 min, Apgar score at 5 min, resuscitation required, NICU admission, and mechanical ventilation. Three variables, sepsis, neonatal seizures and anemia, were not missing in more than 5% of subjects, but were too infrequent to include in analyses.

The following psychiatric variables were included in the analyses: maternal depression, maternal anxiety, paternal depression, paternal anxiety, parent depression and parent anxiety. Parent depression (a variable that captures whether 0 or at least 1 parent had been diagnosed with depression) and parent anxiety (a variable that captures whether 0 or at least 1 parent had been diagnosed with anxiety) were created using the variables maternal depression, paternal depression, maternal anxiety and paternal anxiety. These six variables were included in the analyses even though some were missing for more than 5% of subjects because there is evidence that these variables are strongly associated with autism. The following psychiatric variables were not included due to small frequencies: maternal bipolar, maternal ADHD, maternal OCD, paternal bipolar, paternal ADHD, and paternal OCD.

Age of the child at ADI-R, age of the child at ADOS, child's gender, maternal age at parturition and paternal age at parturition were included as covariates.

Statistical Analyses

Means, standard deviations and medians were calculated for the four outcome variables, and correlations between covariates and all predictor and outcome variables were calculated using Pearson correlation coefficients. The correlations between predictor variables were calculated in order to inform the selection of variables, and thereby avoid the problem of multicollinearity, in the multivariate models.

Correlations among siblings are a concern when including multiplex families, particularly those with identical twins, in the analyses. In order to adjust for the correlations between identical twins and between non-identical twins and siblings, a nested linear mixed effects model was used. This allowed for the random effects of twins and families to be included in the model. In effect, subjects were first nested within twinship (identical twin: yes/no), which was then nested within nuclear family. One twin from each of six pairs with unknown zygosity was randomly excluded because including both twins from each set may have resulted in biased parameter estimates due to misspecification of correlations.

The linear mixed effects model was used to assess whether the obstetric variables, including maternal use of beta-2 adrenergic receptor agonists during pregnancy, are useful in predicting the ADI-R and ADOS scores. The obstetric variables were dichotomous (present/absent). The least square (LS) means for the outcome variables, stratified by the presence or absence of each obstetric variable, were obtained. Gender, maternal age at parturition, and age at ADI-R or ADOS were included as covariates.

The linear mixed effects model was also used to assess whether parent psychiatric histories are useful in predicting the ADI-R and ADOS scores. The psychiatric variables for the mothers and fathers were dichotomous for lifetime diagnoses (present/absent). Much like the obstetric variables, LS means were obtained for each outcome, stratified by the status of the parent. LS means for maternal variables were adjusted for gender, maternal age at parturition, age at ADI-R or ADOS and other psychiatric disorders in both parents. For example, in testing an association with depression in mothers, the estimates were adjusted for anxiety in mothers, anxiety in fathers and depression in fathers. LS means for paternal variables were adjusted for

gender, paternal age at parturition, age at ADI-R or ADOS and other psychiatric disorders in both parents. LS means for parent variables were adjusted for gender, maternal age at parturition, paternal age at parturition, age at ADI-R or ADOS and the other parent psychiatric disorder. Comorbidity among psychiatric disorders is common (Kessler et al. 2005) and controlling for other disorders may be necessary in order to obtain unbiased estimates.

Non-parametric tests were also used to confirm the results of the mixed-effects modeling. Non-normally distributed outcome variables may cause the misspecification of predictive models, and ultimately, flawed conclusions. Therefore, the Wilcoxon two-sample test was performed for each of the comparisons tested with mixed-effects models and the *P*-values obtained from each two-sided t approximation were compared with the *P*-values from the mixed-effects models.

The a priori hypotheses to be tested were (1) maternal used of beta-2 adrenergic agonists during pregnancy will be associated with increased ADI-R/ADOS scores; (2) the presence of maternal and/or paternal depression will be associated with increased ADI-R/ADOS scores; and (3) the presence of maternal and/or paternal anxiety will be associated with increased ADI-R/ADOS scores. *T*-tests for the difference between LS means obtained using multivariate linear mixed effects models were considered significant if $|t| > 1.96$ ($P < 0.05$).

Results

The sample included 444 children with 220 fathers and 225 mothers. The male to female ratio for affected children was 3.5:1. Age at ADI-R ranged from 2 to 44 with a mean of 7.12 and a median of 6. Age at ADOS ranged from 2 to 45 with a mean of 8.68 and a median of 8. ADI-R domain scores were available for 443 children and the ADOS social/communication composite was available for 379 children. Analyses reflected these sample sizes.

This sample included subjects with autism as well as other ASDs. The scores on the ADI-R and ADOS domains appeared to follow fairly normal distributions, and the means and medians were similar for each of the domain scores. Means, standard deviations and medians for the four outcome variables were as follows: ADI-R social domain (19.66, 6.99, 20.00); ADI-R communication domain (13.82, 4.69, 14.00); ADI-R repetitive behaviors domain (5.52, 2.53, 5.00); and ADOS social/communication Composite (14.03, 5.11, 15.00). However, the Kolmogorov–Smirnov test suggested that the scores deviated significantly from normality ($P < 0.01$ for all domain scores).

Correlations (Pearson's *r*) between covariates and all predictor and outcome variables were calculated. Correlations between the covariates and the predictor variables were weak and usually not significant. Gender was significantly correlated with the ADI-R repetitive behaviors domain ($r = -0.11$, $P = 0.023$) such that male gender was associated with higher scores. Maternal age at parturition was significantly correlated with the ADI-R repetitive behaviors domain ($r = -0.13$, $P = 0.008$) and albuminuria ($r = -0.12$, $P = 0.014$). Age of subject at ADI-R was significantly correlated with epidural ($r = -0.14$, $P = 0.004$) and cesarean section ($r = -0.10$, $P = 0.040$), and age of subject at ADOS was significantly correlated with epidural ($r = -0.19$, $P < 0.001$). The subjects' ages at ADI-R and ADOS were significantly correlated with all outcome variables. Age at ADI-R was significantly correlated with the ADI-R social domain ($r = 0.18$, $P < 0.001$); ADI-R communication domain ($r = 0.14$, $P = 0.003$); ADI-R repetitive behaviors domain ($r = 0.22$, $P < 0.001$); and ADOS social/communication composite ($r = -0.15$, $P = 0.004$). Age at ADOS was significantly correlated with the ADI-R social domain ($r = 0.14$, $P = 0.007$); ADI-R communication domain ($r = 0.10$, $P = 0.046$); ADI-R repetitive behaviors domain ($r = 0.16$, $P = 0.001$); and ADOS social/communication composite ($r = -0.16$, $P = 0.002$).

All possible inter-correlations between the parental psychiatric variables (maternal depression, maternal anxiety, paternal depression, paternal anxiety) were found to be significant. Correlation values (Pearson's *r*) ranged from 0.10 to 0.30, with *P* values ranging from 0.039 to <0.001. The strongest correlations were observed between maternal depression and paternal depression ($r = 0.30$, $P < 0.001$) and between paternal depression and paternal anxiety ($r = 0.30$, $P < 0.001$).

There were no significant differences between LS means for the ADI-R social domain score on any obstetric variable or any parental psychiatric variable (Table 1). However, hypertension ($t = 3.22$, $P = 0.002$), pre-eclampsia ($t = 2.33$, $P = 0.021$) and generalized edema ($t = 2.58$, $P = 0.011$) were significantly associated with the ADI-R communication domain (Table 2). None of the parental psychiatric variables were significantly associated with the communication domain (Table 2).

As seen in Table 3, hypertension ($t = 2.58$, $P = 0.011$), albuminuria ($t = 2.08$, $P = 0.039$) and generalized edema ($t = 2.65$, $P = 0.009$) were all significantly associated with the ADI-R repetitive behaviors domain (Table 3). Parent depression was also significantly associated with the ADI-R repetitive behaviors domain ($t = 2.88$, $P = 0.005$) (Table 3).

None of the obstetric variables were significantly associated with the ADOS social/communication composite (Table 4). Of the psychiatric variables, the presence of parent anxiety was significantly associated with a smaller

Table 1 Adjusted associations between obstetrical and parental psychiatric variables and the ADI-R social domain

Predictor variables	LS means		Difference	Standard error	d.f.	t-Value[a]	*P*
	Absent	Present					
Vaginal Bleeding 1st Trimester[b]	19.57	20.20	0.63	0.94	176	0.67	0.501
Vaginal Bleeding Any Trimester[b]	19.62	19.94	0.32	0.86	173	0.38	0.707
Severe Infection 1st Trimester[b]	19.61	19.74	0.13	1.82	173	0.07	0.944
Severe Infection Any Trimester[b]	19.55	19.52	−0.03	1.21	172	−0.03	0.978
Hypertension[b]	19.60	19.68	0.08	1.11	178	0.07	0.942
Gestational Diabetes[b]	19.47	20.17	1.69	1.36	177	1.24	0.215
Preeclampsia[b]	19.50	20.60	1.10	1.71	173	0.64	0.521
Epidural[b]	19.68	19.59	−0.08	0.70	175	−0.12	0.905
Cesarean Section[b]	19.55	19.94	0.39	0.77	177	0.50	0.615
Cord Problems[b]	19.48	21.20	1.73	1.16	176	1.49	0.138
Placenta Problems[b]	19.70	18.00	−1.70	2.00	177	−0.85	0.396
Hyperbilirubinemia[b]	19.86	19.41	−0.45	0.70	178	−0.64	0.525
Albuminuria[b]	19.63	19.76	0.13	1.75	172	0.07	0.941
Generalized Edema[b]	19.55	20.13	0.59	1.28	177	0.46	0.643
Forceps Delivery[b]	19.59	19.67	0.08	1.22	175	0.07	0.946
Prescription Medication[b]	19.67	19.08	−0.59	0.99	168	−0.60	0.549
Beta-2 Adrenergic Agonists[b]	19.64	18.92	−0.72	1.36	168	−0.53	0.597
Antibiotics[b]	19.38	20.97	1.59	1.06	168	1.50	0.136
Thyroid Medication[b]	19.69	17.58	−2.11	1.68	168	−1.25	0.212
Maternal Depression[c]	19.73	20.14	0.41	0.93	157	0.44	0.661
Maternal Anxiety[d]	19.93	19.20	−0.73	1.28	157	−0.57	0.570
Paternal Depression[e]	19.84	19.91	0.07	1.13	157	0.07	0.947
Paternal Anxiety[f]	20.06	17.77	−2.29	1.39	157	−1.64	0.103
Parent Depression[g]	19.83	19.80	−0.03	0.81	159	−0.03	0.977
Parent Anxiety[h]	20.13	18.53	−1.60	0.99	159	−1.61	0.108

[a] t-Values are for differences between least square means

[b] Adjusted for gender, maternal age at parturition and age of the child when the ADI-R was conducted

[c] Adjusted for gender, maternal age at parturition, age of the child when the ADI-R was conducted, maternal anxiety, paternal depression, and paternal anxiety

[d] Adjusted for gender, maternal age at parturition, age of the child when the ADI-R was conducted, maternal depression, paternal depression, and paternal anxiety

[e] Adjusted for gender, paternal age at parturition, age of the child when the ADI-R was conducted, maternal depression, maternal anxiety, and paternal anxiety

[f] Adjusted for gender, paternal age at parturition, age of the child when the ADI-R was conducted, maternal depression, maternal anxiety, and paternal depression

[g] Adjusted for gender, age when the ADI-R was conducted, maternal age at parturition, paternal age at parturition, and parent anxiety

[h] Adjusted for gender, age when the ADI-R was conducted, maternal age at parturition, paternal age at parturition, and parent depression

LS mean (t = -2.26, $P = 0.025$) on the ADOS social/communication composite (Table 4).

Similar results were obtained from the non-parametric tests. Only the association between preeclampsia and the ADI-R repetitive behaviors domain differed from the results obtained with the mixed effects model. The non-parametric test found a significant association ($P = 0.046$), which was not observed in the mixed effect model ($P = 0.124$).

Discussion

Severity is seldom used as an outcome in autism studies, and to our knowledge, this is the first study to consider whether obstetric and psychiatric predictor variables are associated with the severity of autistic symptoms. Defining endpoints or phenotypes that capture severity is somewhat problematic because the severity of autism can be difficult to measure. A large amount of information can be extracted

Table 2 Adjusted associations between obstetrical and parental psychiatric variables and the ADI-R communication domain

Predictor variables	LS means		Difference	Standard error	d.f.	t-Value[a]	*P*
	Absent	Present					
Vaginal Bleeding 1st Trimester[b]	13.99	13.68	−0.31	0.64	176	−0.49	0.626
Vaginal Bleeding Any Trimester[b]	13.94	13.87	−0.07	0.59	173	−0.11	0.910
Severe Infection 1st Trimester[b]	14.00	13.02	−0.98	1.25	173	−0.79	0.432
Severe Infection Any Trimester[b]	14.02	13.12	−0.90	0.84	172	−1.07	0.285
Hypertension[b]	13.68	16.10	2.42	0.75	178	3.22	0.002
Gestational Diabetes[b]	13.91	14.31	0.40	0.94	177	0.43	0.669
Preeclampsia[b]	13.86	16.56	2.71	1.16	173	2.33	0.021
Epidural[b]	13.68	14.14	0.46	0.48	175	0.96	0.336
Cesarean Section[b]	14.04	13.73	−0.31	0.54	177	−0.57	0.572
Cord Problems[b]	13.90	13.84	−0.06	0.79	176	−0.07	0.948
Placenta Problems[b]	13.87	15.02	1.15	1.35	177	0.85	0.397
Hyperbilirubinemia[b]	14.02	14.01	−0.01	0.48	178	−0.04	0.969
Albuminuria[b]	13.87	15.59	1.71	1.19	172	1.44	0.153
Generalized Edema[b]	13.74	15.99	2.25	0.87	177	2.58	0.011
Forceps Delivery[b]	13.90	14.37	0.47	0.82	175	0.58	0.563
Prescription Medication[b]	13.78	14.80	1.02	0.69	168	1.48	0.141
Beta-2 Adrenergic Agonists[b]	13.85	14.86	1.01	0.95	168	1.06	0.292
Antibiotics[b]	13.80	14.71	0.91	0.73	168	1.25	0.214
Thyroid Medication[b]	13.88	14.73	0.85	1.18	168	0.72	0.472
Maternal Depression[c]	13.73	14.81	1.08	0.63	157	1.70	0.091
Maternal Anxiety[d]	13.99	14.58	0.59	0.87	157	0.68	0.497
Paternal Depression[e]	14.05	14.03	−0.02	0.77	157	−0.03	0.976
Paternal Anxiety[f]	14.16	12.94	−1.22	0.95	157	−1.29	0.200
Parent Depression[g]	13.80	14.55	0.75	0.55	159	1.36	0.176
Parent Anxiety[h]	14.13	13.88	−0.25	0.67	159	−0.37	0.709

[a] t-Values are for differences between least square means

[b] Adjusted for gender, maternal age at parturition and age of the child when the ADI-R was conducted

[c] Adjusted for gender, maternal age at parturition, age of the child when the ADI-R was conducted, maternal anxiety, paternal depression, and paternal anxiety

[d] Adjusted for gender, maternal age at parturition, age of the child when the ADI-R was conducted, maternal depression, paternal depression, and paternal anxiety

[e] Adjusted for gender, paternal age at parturition, age of the child when the ADI-R was conducted, maternal depression, maternal anxiety, and paternal anxiety

[f] Adjusted for gender, paternal age at parturition, age of the child when the ADI-R was conducted, maternal depression, maternal anxiety, and paternal depression

[g] Adjusted for gender, age when the ADI-R was conducted, maternal age at parturition, paternal age at parturition, and parent anxiety

[h] Adjusted for gender, age when the ADI-R was conducted, maternal age at parturition, paternal age at parturition, and parent depression

on each of the three features of autism using the ADI-R and the ADOS; however these instruments are primarily used to differentiate between autism and non-autism, or PDD and non-PDD, based on specified cutoffs (de Bildt et al. 2004). The utility of these tools to characterize the severity of autistic symptoms is unclear and there is some disagreement about the use of the ADI-R and ADOS scores as continuous variables. However, it should be noted that a number of studies have treated the domain scores as continuous measures of severity (Devlin et al. 2005; Lord et al. 2000; Spiker et al. 2002; Venter et al. 1992). While conceptually autism is defined by cutoffs that divide autism from non-autism, the symptoms that define autism appear to lie on a continuum of severity. Therefore, it is reasonable to suggest that any variable associated with behavioral impairment might also be associated with increasing severity, both above and below the cutoff.

This study found that of the obstetric conditions only hypertension, preeclampsia, albuminuria and generalized edema were significantly associated with the severity of

Table 3 Adjusted associations between obstetrical and parental psychiatric variables and the ADI-R repetitive behaviors domain

Predictor variables	LS means		Difference	Standard error	d.f.	t-Value[a]	*P*
	Absent	Present					
Vaginal Bleeding 1st Trimester[b]	5.37	5.30	−0.07	0.34	176	−0.19	0.847
Vaginal Bleeding Any Trimester[b]	5.39	5.21	−0.18	0.31	173	−0.58	0.565
Severe Infection 1st Trimester[b]	5.35	5.59	0.24	0.67	173	0.36	0.723
Severe Infection Any Trimester[b]	5.29	5.96	0.67	0.45	172	1.49	0.137
Hypertension[b]	5.26	6.29	1.04	0.40	178	2.58	0.011
Gestational Diabetes[b]	5.33	5.61	0.28	0.50	177	0.57	0.572
Preeclampsia[b]	5.33	6.28	0.95	0.61	173	1.54	0.124
Epidural[b]	5.55	5.19	−0.36	0.26	175	−1.39	0.165
Cesarean Section[b]	5.32	5.41	0.09	0.29	177	0.32	0.751
Cord Problems[b]	5.35	5.21	−0.14	0.41	176	−0.34	0.733
Placenta Problems[b]	5.36	5.43	0.07	0.70	177	0.11	0.915
Hyperbilirubinemia[b]	5.43	5.28	−0.15	0.25	173	−0.58	0.561
Albuminuria[b]	5.30	6.60	1.30	0.62	172	2.08	0.039
Generalized Edema[b]	5.25	6.47	1.22	0.46	177	2.65	0.009
Forceps Delivery[b]	5.33	5.45	0.12	0.43	175	0.30	0.766
Prescription Medication[b]	5.41	5.25	−0.16	0.38	168	−0.42	0.676
Beta-2 Adrenergic Agonists[b]	5.39	5.33	−0.06	0.52	168	−0.12	0.903
Antibiotics[b]	5.30	5.93	0.63	0.39	168	1.62	0.106
Thyroid Medication[b]	5.37	5.76	0.39	0.65	168	0.60	0.549
Maternal Depression[c]	5.25	5.63	0.38	0.35	157	1.08	0.280
Maternal Anxiety[d]	5.26	5.17	0.91	0.48	157	1.87	0.063
Paternal Depression[e]	5.24	5.94	0.70	0.43	157	1.60	0.113
Paternal Anxiety[f]	5.35	5.45	0.10	0.54	157	0.19	0.850
Parent Depression[g]	5.08	5.97	0.89	0.31	159	2.88	0.005
Parent Anxiety[h]	5.31	5.83	0.52	0.38	159	1.37	0.173

[a] t-Values are for differences between least square means

[b] Adjusted for gender, maternal age at parturition and age of the child when the ADI-R was conducted

[c] Adjusted for gender, maternal age at parturition, age of the child when the ADI-R was conducted, maternal anxiety, paternal depression, and paternal anxiety

[d] Adjusted for gender, maternal age at parturition, age of the child when the ADI-R was conducted, maternal depression, paternal depression, and paternal anxiety

[e] Adjusted for gender, paternal age at parturition, age of the child when the ADI-R was conducted, maternal depression, maternal anxiety, and paternal anxiety

[f] Adjusted for gender, paternal age at parturition, age of the child when the ADI-R was conducted, maternal depression, maternal anxiety, and paternal depression

[g] Adjusted for gender, age when the ADI-R was conducted, maternal age at parturition, paternal age at parturition, and parent anxiety

[h] Adjusted for gender, age when the ADI-R was conducted, maternal age at parturition, paternal age at parturition, and parent depression

autistic symptoms. Eight prior studies of predictors of autism included maternal hypertension or preeclampsia in their analyses (Deykin and MacMahon 1980; Gillberg and Gillberg 1983; Glasson et al. 2004; Hultman et al. 2002; Mason-Brothers et al. 1987, 1990; Matsuishi et al. 1999; Stein et al. 2006; Wilkerson et al. 2002), yet only one of these studies found a significant association between preeclampsia and the diagnosis of autism (Mason-Brothers et al. 1990).

Hypertension and co-occurring albuminuria, and often edema, are considered diagnostic of preeclampsia. There may be some misclassification of preeclampsia and uncomplicated hypertension of pregnancy in this sample as the data were obtained by self-report. Yet, the findings that albuminuria was associated with the repetitive behaviors domain, and generalized edema was associated with the communication and repetitive behaviors domains, are consistent with the associations seen between hypertension and these domains, as albuminuria and edema are presumed to be largely caused by hypertension. A possible explanation for the observed associations of autism

Table 4 Adjusted associations between obstetrical and parental psychiatric variables and the ADOS social/communication composite

Predictor variables	LS means		Difference	Standard error	d.f.	t-Value[a]	*P*
	Absent	Present					
Vaginal Bleeding 1st Trimester[b]	14.42	13.48	−0.94	0.75	149	−1.24	0.216
Vaginal Bleeding Any Trimester[b]	14.41	14.03	−0.38	0.69	146	−0.56	0.577
Severe Infection 1st Trimester[b]	14.39	11.99	−2.40	1.45	147	−1.65	0.100
Severe Infection Any Trimester[b]	14.47	12.96	−1.51	0.99	145	−1.53	0.128
Hypertension[b]	14.45	13.05	−1.40	0.90	151	−1.56	0.120
Gestational Diabetes[b]	14.35	14.03	−0.32	1.07	152	−0.30	0.766
Preeclampsia[b]	14.34	12.19	−2.15	1.37	147	−1.57	0.118
Epidural[b]	14.46	14.26	−0.20	0.56	148	−0.36	0.717
Cesarean Section[b]	14.16	14.54	0.38	0.62	150	0.61	0.540
Cord Problems[b]	14.25	14.55	0.30	0.86	150	0.35	0.726
Placenta Problems[b]	14.32	13.62	−0.70	1.58	150	−0.45	0.656
Hyperbilirubinemia[b]	14.49	14.09	−0.40	0.56	151	−0.73	0.469
Albuminuria[b]	14.44	11.86	−2.58	1.37	147	−1.89	0.061
Generalized Edema[b]	14.45	12.83	−1.62	1.02	151	−1.59	0.114
Forceps Delivery[b]	14.29	13.72	−0.57	0.92	148	−0.62	0.535
Prescription Medication[b]	14.49	13.60	−0.89	0.79	143	−1.14	0.257
Beta-2 Adrenergic Agonists[b]	14.48	12.92	−1.56	1.08	143	−1.44	0.152
Antibiotics[b]	14.42	14.01	−0.41	0.87	143	−0.47	0.639
Thyroid Medication[b]	14.41	13.35	−1.06	1.37	143	−0.78	0.438
Maternal Depression[c]	14.19	14.67	−0.48	0.76	133	−0.63	0.531
Maternal Anxiety[d]	14.50	12.79	−1.71	1.10	133	−1.55	0.123
Paternal Depression[e]	14.36	14.32	−0.04	0.91	133	−0.04	0.971
Paternal Anxiety[f]	14.52	12.28	−2.24	1.28	133	−1.75	0.083
Parent Depression[g]	14.43	13.95	−0.48	0.68	134	−0.71	0.479
Parent Anxiety[h]	14.58	12.61	−1.97	0.87	134	−2.26	0.025

[a] t-Values are for differences between least square means

[b] Adjusted for gender, maternal age at parturition and age of the child when the ADOS was conducted

[c] Adjusted for gender, maternal age at parturition, age of the child when the ADOS was conducted, maternal anxiety, paternal depression, and paternal anxiety

[d] Adjusted for gender, maternal age at parturition, age of the child when the ADOS was conducted, maternal depression, paternal depression, and paternal anxiety

[e] Adjusted for gender, paternal age at parturition, age of the child when the ADOS was conducted, maternal depression, maternal anxiety, and paternal anxiety

[f] Adjusted for gender, paternal age at parturition, age of the child when the ADOS was conducted, maternal depression, maternal anxiety, and paternal depression

[g] Adjusted for gender, age when the ADOS was conducted, maternal age at parturition, paternal age at parturition, and parent anxiety

[h] Adjusted for gender, age when the ADOS was conducted, maternal age at parturition, paternal age at parturition, and parent depression

severity with this constellation of symptoms is that factors related to the fetus are responsible for hypertension and preeclampsia in the mother. Poor placental perfusion and function are observed in pregnancies with preeclampsia, and recent evidence suggests that placental abnormalities may be more frequent in children who develop autism (Anderson et al. 2007).

Additionally, there may be a feedback loop in which fetally related abnormalities result in complications of pregnancy which cause further insult to the fetus. In a sample of children without autism, preeclampsia was associated with deficits in cognitive and motor performance in preschool and early school years (Kronenberg et al. 2006). In children with autism, similar impairments may be observed and it is possible that these deficits may influence the expression of autistic symptoms. Thus, the observed association between preeclampsia and severity of autistic symptoms may occur due to gene-environment correlation (the genetic makeup of the fetus causes a sub-optimal environment) and also by gene-environment

interaction (a suboptimal environment affects only the genetically vulnerable fetus). Future studies should explore the role of placental abnormalities among children with autism and whether hypertension and/or preeclampsia are associated with cognitive and motor impairments in autistic populations.

Maternal use of beta-2 adrenergic receptor agonists during pregnancy was not associated with greater severity of autism on any of the outcome variables. The Connors et al. (2005) study suggested an association between maternal use of beta-2 adrenergic receptor agonists during pregnancy and increased concordance for autism among dyzygotic twins. Fifteen of the thirty-seven twin-pairs were obtained from the same source as the current study, the Autism Genetic Resource Exchange (AGRE). However, the Connors et al. study only showed a significant association between maternal use of beta-2 adrenergic receptor agonists during pregnancy and increased concordance for autism in a subset of twin-pairs, excluding pairs with a female twin and pairs with other affected siblings (2005). If beta-2 adrenergic receptor agonists are in fact associated with higher concordance for autism among dyzygotic twins, this does not appear to occur through an increase in the severity of autistic symptoms.

Parent depression was significantly associated with the ADI-R repetitive behaviors domain. There are several potential mechanisms for this association. First, the more severe the autistic symptoms expressed by the child, the more likely it may be that a parent experiences depression. This explanation appears somewhat less likely when it is considered that the association was only observed within the repetitive behaviors domain. One might expect the severity of symptoms across all domains to affect parental mood. Additionally, previous studies have found that the prevalence of affective disorders, including depression, among parents of children with autism is elevated both before and after the birth of the child with autism (Bolton et al. 1998; Micali et al. 2004; Piven and Palmer 1999).

A second possibility is that the parents with depression reported on the symptoms of their children differently than the parents without depression. Again, this may be a less likely explanation because the association appears to be specific to the repetitive behaviors domain while it might be expected that parental mood would equally affect the reporting of symptoms across all domains. However, it is certainly possible that some autism-related behaviors (such as repetitive behavior) may be particularly noticeable to or able to affect parents prone to or suffering from mood problems.

A third potential mechanism for the association between parent depression and the repetitive behaviors domain is shared genetic susceptibility. Many studies have explored this area, and in particular much interest has been paid to serotonin (Conroy et al. 2004; Devlin et al. 2005; McCauley et al. 2004; Ozaki et al. 2003; Sugie et al. 2005; Sutcliffe et al. 2005). Although Mulder and colleagues (2005) did find an association between rigid and compulsive behavior in autism and the intron2 VNTR (variable number tandem repeat) of the serotonin transporter gene, in general, results have been inconsistent. Treatment studies provide further evidence that serotonin is involved in the severity of repetitive behaviors, as many studies have shown that SSRIs are effective in reducing autistic symptoms (DeLong et al. 1998; Moore et al. 2004; Sugie et al. 2005). Additionally, Coon et al. (2005) found a possible association between a variant of tryptophan hydroxylase (TPH2), a rate-limiting enzyme involved in controlling serotonin synthesis, and higher scores on the ADI-R repetitive behaviors domain. The true relationship between autism and depression may be complicated by the heterogeneity of each of these disorders, but future research should continue to explore this relationship.

Parent anxiety was significantly associated with the ADOS social/communication composite. Previous studies have reported an association between anxiety, social phobia and autism (Micali et al. 2004; Piven and Palmer 1999); however, the direction of the current finding, which suggests a protective effect, is unexpected. This finding is interesting because the ADOS does not rely on parent reporting of behaviors; rather the behaviors are observed by an examiner. Additional research should explore whether this association exists in other autistic populations and potential mechanisms to explain the association.

Several limitations of the present study need to be noted. The study was largely exploratory and included many comparisons, without use of a multiple-test correction. Yet, we believe the results are intriguing and can contribute to hypothesis generation for future studies. A second limitation was that the predictor variables were all based on self- or parent-report. However, self- or parent-report data is probably most concerning when it relates to recall bias. All of the parents included in this study were parents of children with autism, and it is unlikely that systematic reporting differences were a major problem. Although, as previously suggested, psychiatric disorders among parents may potentially lead to differential perception and reporting of symptoms, this did not seem to be the case as significant findings for associations with parental psychiatric disorders were not observed across all outcomes. Additionally, the use of self-report data, as opposed to obstetric records, may have led to some misclassification of hypertension and preeclampsia, making it difficult to interpret the associations observed between these variables and the ADI-R communication and repetitive behaviors domains. The data in this study were also limited as a result of being collected for the purpose of characterizing

phenotypes for genetic studies, not for assessing associations between predictor variables and the severity of autistic symptoms. Finally, due to the cross-sectional nature of the data, it was impossible to establish the temporality of the onset of parental psychiatric disorders with respect to the autism diagnoses of the children.

Follow-up studies may be able to avoid some of the limitations listed above. Importantly, it may be beneficial to rely on systematic clinical evaluations to assess psychiatric disorders among parents. In addition, the assessment of obstetric and pregnancy related variables might be significantly enhanced by the use of medical records and more extensive questionnaires. Furthermore, it may be critical to assess the severity of autism related behavioral symptoms and affected domains using instruments designed specifically for the quantification of relevant behaviors (e.g., Vineland Adaptive Behavior Scales (Paul et al. 2004), Broader Phenotype Autism Symptom Scale (Sung et al. 2005), Social Responsiveness Scale (Constantino et al. 2003)). Finally, it may be fruitful to compare predictor-outcome associations observed in different childhood disorders in order to understand better whether the factors are operating in related ways across disorders.

Autism is a complex disorder, and it is unlikely that any single variable will have much explanatory power in the face of potentially diverse etiologic factors. However, the expression of autism is quite heterogeneous, even among identical twins, which suggests that the symptoms of autism may be modifiable. From a public health perspective, the identification of factors that modify the severity of autistic symptoms might have a large impact on the high costs associated with the morbidity of autism. On the other hand, it is possible that pre/perinatal factors associated with autism, or autism severity, are largely epiphenomena (Bolton et al. 1998) and in this case their manipulation would probably have little effect on expression. However, whether primary and causative or merely associative, identification of factors associated with greater risk or severity may lead to improved methods for screening and early risk assessment, particularly among high risk children, such as younger siblings of children with autism. Future studies should attempt to establish the degree of association for specific factors while paying particular attention to hypertension-related obstetric conditions, and should work to determine to what extent clearly associated factors play a causative role.

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References

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington, DC.

Anderson, G. M., Jacobs-Stannard, A., Chawarska, K., Volkmar, F. R., & Kliman, H. J. (2007). Placental trophoblast inclusions in autism spectrum disorder. *Biological Psychiatry, 61*(4), 487–491.

Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E., et al. (1995). Autism as a strongly genetic disorder: Evidence from a british twin study. *Psychological Medicine, 25*(1), 63–77.

Bolton, P. F., Pickles, A., Murphy, M., & Rutter, M. (1998). Autism, affective and other psychiatric disorders: Patterns of familial aggregation. *Psychological Medicine, 28*(2), 385–395.

Bryson, S. E., Smith, I. M., & Eastwood, D. (1988). Obstetrical suboptimality in autistic children. *Journal of the American Academy of Child andAdolescent Psychiatry, 27*(4), 418–422.

CDC (Centers for Disease Control and Prevention). (2007). Prevalence of Autism Spectrum Disorders—Autism and Developmental Disabilities Monitoring Network, Six Sites, United States, 2000; Prevalence of Autism Spectrum Disorders—Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2002; and Evaluation of a Methodology for a Collaborative Multiple Source Surveillance Network for Autism Spectrum Disorders—Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2002. Surveillance Summaries. MMWR 2007;56(No. SS-1).

Connors, S. L., Crowell, D. E., Eberhart, C. G., Copeland, J., Newschaffer, C. J., Spence, S. J., et al. (2005). Beta2-adrenergic receptor activation and genetic polymorphisms in autism: Data from dizygotic twins. *Journal of Child Neurology, 20*(11), 876–884.

Conroy, J., Meally, E., Kearney, G., Fitzgerald, M., Gill, M., & Gallagher, L. (2004). Serotonin transporter gene and autism: A haplotype analysis in an Irish autistic population. *Molecular Psychiatry, 9*(6), 587–593.

Constantino, J. N., Davis, S. A., Todd, R. D., Schindler, M. K., Gross, M. M., Brophy, S. L., et al. (2003). Validation of a brief quantitative measure of autistic traits: comparison of the Social Responsiveness Scale with the Autism Diagnostic Interview-Revised. *Journal of Autism & Developmental Disorders, 33*(4), 427–433.

Coon, H., Dunn, D., Lainhart, J., Miller, J., Hamil, C., Battaglia, A., et al. (2005). Possible association between autism and variants in the brain-expressed tryptophan hydroxylase gene (TPH2). *American Journal of Medical Genetics B Neuropsychiatry Genetics, 135*(1), 42–46.

de Bildt, A., Sytema, S., Ketelaars, C., Kraijer, D., Mulder, E., Volkmar, F., et al. (2004). Interrelationship between Autism Diagnostic Observation Schedule-Generic (ADOS-G), Autism Diagnostic Interview-Revised (ADI-R), and the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) classification in children and adolescents with mental retardation. *Journal of Autism and Developmental Disorders, 34*(2), 129–137.

DeLong, G. R., Teague, L. A., & McSwain Kamran, M. (1998). Effects of fluoxetine treatment in young children with idiopathic autism. *Developmental Medicine and Child Neurology, 40*(8), 551–562.

Devlin, B., Cook, E. H., Coon, H., Dawson, G., Grigorenko, E. L., McMahon, W., et al. (2005). Autism and the serotonin transporter: The long and short of it. *Molecular Psychiatry, 10*(12), 1110–1116.

Deykin, E. Y., & MacMahon, B. (1980). Pregnancy, delivery, and neonatal complications among autistic children. *American Journal of Diseases of Children, 134*(9), 860–864.

Feinstein, C., & Singh, S. (2007). Social phenotypes in neurogenetic syndromes. *Child & Adolescent Psychiatric Clinics of North America, 16*(3), 631–647.

Gillberg, C., & Gillberg, I. C. (1983). Infantile autism: A total population study of reduced optimality in the pre-, peri-, and neonatal period. *Journal of Autism and Developmental Disorders, 13*(2), 153–166.

Glasson, E. J., Bower, C., Petterson, B., de Klerk, N., Chaney, G., & Hallmayer, J. F. (2004). Perinatal factors and the development of autism: A population study. *Archives of General Psychiatry, 61*(6), 618–627.

Hultman, C. M., Sparen, P., & Cnattingius, S. (2002). Perinatal risk factors for infantile autism. *Epidemiology, 13*(4), 417–423.

Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey replication. *Archives of General Psychiatry, 62*(6), 617–627.

Kronenberg, M. E., Raz, S., & Sander, C. J. (2006). Neurodevelopmental outcome in children born to mothers with hypertension in pregnancy: The significance of suboptimal intrauterine growth. *Developmental Medicine and Child Neurology, 48*(3), 200–206.

Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Leventhal, B. L., DiLavore, P. C., et al. (2000). The Autism Diagnostic Observation Schedule-Generic: A standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders, 30*(3), 205–223.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders, 24*(5), 659–685.

Ma, D. Q., Whitehead, P. L., Menold, M. M., Martin, E. R., Ashley-Koch, A. E., Mei, H., et al. (2005). Identification of significant association and gene-gene interaction of GABA receptor subunit genes in autism. *American Journal of Human Genetics, 77*(3), 377–388.

Mason-Brothers, A., Ritvo, E. R., Guze, B., Mo, A., Freeman, B. J., Funderburk, S. J., et al. (1987). Pre-, peri-, and postnatal factors in 181 autistic patients from single and multiple incidence families. *Journal of the American Academy of Child and Adolescent Psychiatry, 26*(1), 39–42.

Mason-Brothers, A., Ritvo, E. R., Pingree, C., Petersen, P. B., Jenson, W. R., McMahon, W. M., et al. (1990). The UCLA-University of Utah Epidemiologic Survey of Autism: Prenatal, perinatal, and postnatal factors. *Pediatrics, 86*(4), 514–519.

Matsuishi, T., Yamashita, Y., Ohtani, Y., Ornitz, E., Kuriya, N., Murakami, Y., et al. (1999). Brief report: Incidence of and risk factors for autistic disorder in neonatal intensive care unit survivors. *Journal of Autism and Developmental Disorders, 29*(2), 161–166.

McBride, P. A., Anderson, G. M., & Shapiro, T. (1996). Autism research. Bringing together approaches to pull apart the disorder. *Archives of General Psychiatry, 53*, 980–983.

McCauley, J. L., Olson, L. M., Dowd, M., Amin, T., Steele, A., Blakely, R. D., et al. (2004). Linkage and association analysis at the serotonin transporter (SLC6A4) locus in a rigid-compulsive subset of autism. *American Journal of Medical Genetics B Neuropsychiatry Genetics, 127*(1), 104–112.

Micali, N., Chakrabarti, S., & Fombonne, E. (2004). The broad autism phenotype: Findings from an epidemiological survey. *Autism, 8*(1), 21–37.

Moore, M. L., Eichner, S. F., & Jones, J. R. (2004). Treating functional impairment of autism with selective serotonin-reuptake inhibitors. *Annals of Pharmacotherapy, 38*(9), 1515–1519.

Mulder, E. J., Anderson, G. M., Kema, I. P., Brugman, A. M., Ketelaars, C. E., de Bildt, A., et al. (2005). Serotonin transporter intron 2 polymorphism associated with rigid-compulsive behaviors in Dutch individuals with pervasive developmental disorder. *American Journal of Medical Genetics B Neuropsychiatry Genetics, 133*(1), 93–96.

Murphy, M., Bolton, P. F., Pickles, A., Fombonne, E., Piven, J., & Rutter, M. (2000). Personality traits of the relatives of autistic probands. *Psychological Medicine, 30*(6), 1411–1424.

Ozaki, N., Goldman, D., Kaye, W. H., Plotnicov, K., Greenberg, B. D., Lappalainen, J., et al. (2003). Serotonin transporter missense mutation associated with a complex neuropsychiatric phenotype. *Molecular Psychiatry, 8*(11), 895, 933–936.

Paul, R., Miles, S., Cicchetti, D., Sparrow, S., Klin, A., Volkmar, F., et al. (2004). Adaptive behavior in autism and pervasive developmental disorder-not otherwise specified: microanalysis of scores on the Vineland Adaptive Behavior Scales. *Journal of Autism & Developmental Disorders, 34*(2), 223–228.

Piven, J., & Palmer, P. (1999). Psychiatric disorder and the broad autism phenotype: Evidence from a family study of multiple-incidence autism families. *The American Journal of Psychiatry, 156*(4), 557–563.

Rubenstein, J. L., & Merzenich, M. M. (2003). Model of autism: Increased ratio of excitation/inhibition in key neural systems. *Genes, Brain, and Behavior, 2*(5), 255–267.

Spiker, D., Lotspeich, L. J., Dimiceli, S., Myers, R. M., & Risch, N. (2002). Behavioral phenotypic variation in autism multiplex families: Evidence for a continuous severity gradient. *American Journal of Medical Genetics, 114*(2), 129–136.

Steffenburg, S., Gillberg, C., Hellgren, L., Andersson, L., Gillberg, I. C., Jakobsson, G., et al. (1989). A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 30*(3), 405–416.

Stein, D., Weizman, A., Ring, A., & Barak, Y. (2006). Obstetric complications in individuals diagnosed with autism and in healthy controls. *Comprehensive Psychiatry, 47*(1), 69–75.

Strömland, K., Nordin, V., Miller, M., Akerström, B., & Gillberg, C. (1994). Autism in thalidomide embryopathy: a population study. *Developmental Medicine and Child Neurology, 36*(4), 351–356.

Sugie, Y., Sugie, H., Fukuda, T., Ito, M., Sasada, Y., Nakabayashi, M., et al. (2005). Clinical efficacy of fluvoxamine and functional polymorphism in a serotonin transporter gene on childhood autism. *Journal of Autism and Developmental Disorders, 35*(3), 377–385.

Sung, Y. J., Dawson, G., Munson, J., Estes, A., Schellenberg, G. D., & Wijsman, E. M. (2005). Genetic investigation of quantitative traits related to autism: Use of multivariate polygenic models with ascertainment adjustment. *American Journal of Human Genetics, 76*(1), 68–81.

Sutcliffe, J. S., Delahanty, R. J., Prasad, H. C., McCauley, J. L., Han, Q., Jiang, L., et al. (2005). Allelic heterogeneity at the serotonin transporter locus (SLC6A4) confers susceptibility to autism and rigid-compulsive behaviors. *American Journal of Human Genetics, 77*(2), 265–279.

Szatmari, P., Merette, C., Bryson, S. E., Thivierge, J., Roy, M. A., Cayer, M., et al. (2002). Quantifying dimensions in autism: a factor-analytic study. *Journal of the American Academy of Child & Adolescent Psychiatry, 46*(4), 467–474.

Venter, A., Lord, C., & Schopler, E. (1992). A follow-up study of high-functioning autistic children. *Journal of Child Psychology and Psychiatry, and Allied Disciplines, 33*(3), 489–507.

Williams, G., King, J., Cunningham, M., Stephan, M., Kerr, B., & Hersh, J. H. (2001). Fetal valproate syndrome and autism: Additional evidence of an association. *Developmental Medicine and Child Neurology, 43*(3), 202–206.

Wilkerson, D. S., Volpe, A. G., Dean, R. S., & Titus, J. B. (2002). Perinatal complications as predictors of infantile autism. *The International Journal of Neuroscience, 112*(9), 1085–1098.