A LASSO Analysis of Maternal, Obstetric, and Perinatal Predictors of Autism

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Abstract

Background. While genetics is clearly implicated in the etiology of autism, other biological factors may also increase the likelihood of an autism diagnosis. Many of these pertain to obstetric history and maternal medical conditions, but as they tend to co-occur it is difficult to ascertain which ones are most predictive of child's autism.

Methods. Women reported regarding their medical history and the pregnancy history of their first-born (N=557, 34.5% of children diagnosed with autism) and second-born (N=374, 28.1% of children diagnosed with autism) children. In Study 1, the first-born and second-born data were analyzed separately using LASSO, which enables the selection of variables most associated with child's autism. In Study 2, obstetric course and perinatal factors were compared for siblings discordant for autism (N mothers= 132, 61.7% males), thus allowing to control for many genetic and environmental aspects.

Results. Study 1 revealed maternal medical conditions, obstetric course factors, and perinatal factors predictive of child's autism (15 and 20 predictors for the first and second child analyses, respectively). Factors that replicated across the two pregnancies were among others, infertility, vaginal bleeding during pregnancy, and Type II diabetes. Study 2 revealed that maternal vaginal bleeding differentiated the pregnancy course of children later diagnosed with autism from neurotypical children.

Limitations. The medical information analyzed in this study is based on participants' recollection. While this approach allows to collect information regarding medical symptoms that may not have been logged in official medical records, participants might have forgotten relevant information. Future studies would benefit from combining self-report and health-provider recorded data.

Conclusions. This is one of few studies to analyze a large set of predictors, spanning maternal medical conditions, obstetric course, and perinatal factors. Analyzing these together, using machine-learning methods, allows us to disentangle the specific factors associated with child's autism. Study 2 enabled us to examine this within families, thus passively controlling for genetic and non-specific environmental effects. Our findings highlight the pregnancy course and maternal fertility factors which are of importance for predicting child's autism.

Introduction

Autism spectrum conditions are a set of neurodevelopmental conditions characterized by difficulties in social communication and interaction, and by restricted and repetitive interests and behaviors (1). The prevalence of autism in the United States is 1 in 54, with a male bias of 4:1(2). The etiology of the condition is thought to be a combination of genetic and non-genetic factors (3, 4). However, the specific factors, both genetic and environmental, are yet mostly unknown (5).

Previous research has shown that for environmental/non-genetic factors the timing of the exposure is critical, and most studies focus on early exposure – in utero or immediately after. Thus, prenatal and
obstetric factors, such as maternal medical conditions during pregnancy and obstetric complications (6); and early perinatal factors, such as pre-term labor and infant characteristics (7), seem to be strongly related to autism in the developing child. Even though many of these factors are interconnected or co-occurring, most of the available studies focus on a few selected predictors (e.g., (8–11)), which makes it harder to understand the unique association of each one (beyond the others) with autism. To address this problem, we focus on specific medical symptoms and conditions that have been previously associated with child’s autism, and examine those within a single analysis using least absolute shrinkage and selection operator (12), which allows to choose variables most likely to be associated with the outcome from many potential predictors. We examined predictors of autism in the first and second-born children (Study 1) and compared siblings discordant for autism (Study 2). Our analyses focused on three groups of diagnoses; (1) Maternal medical factors include any diagnosis, typically chronic, the mother received in her lifetime. These may serve as proximal or distal factors in explaining child’s autism. For example, Polycystic Ovary Syndrome (PCOS) is a maternal condition associated with child’s autism (8, 13). This association has been interpreted as a consequence of maternal testosterone passing to the foetus and inducing neurological changes, which in turn, may trigger elevation of autistic traits and behavior (14, 15). (2) Obstetric factors include any diagnoses, conditions or symptoms that occur during and are related to pregnancy. For example, maternal infection during pregnancy (viral or bacterial) was previously associated with child’s autism (16–18). Another such condition is infertility, which has been reported with mixed results (19–22). These mixed findings can be the result of an indirect association of fertility treatment and autism, mainly through an increase in the likelihood of obstetric complications, pre-term labor, and low birth-weight; through an association with maternal medical conditions such as PCOS (8); or through parental age (23). All these factors have been independently associated with child’s autism (6, 7, 24). (3) Perinatal factors include characteristics of the child immediately after birth, such as gestational age. Gestational age was previously associated with autism (25), but also with various obstetric and maternal medical conditions (26).

The current study

As reviewed, many of the maternal medical conditions, obstetric conditions, and perinatal characteristics have been independently associated with autism, but are also interconnected or co-occurring (7, 25, 27–29). Only by examining a combination of predictors can we assess the relative contribution of each predictor above others and isolate the most relevant predictors. To this end, the current study utilizes information collected from a large group of women regarding their medical conditions and pregnancy course. We compare these factors for children later diagnosed with autism and children who are typically developing. Study 1 uses a data-driven, LASSO, approach to identify which conditions are associated with autism in the firstborn and second-born child, separately. Study 2 examines a sub-sample of mothers who reported on the obstetric course of births resulting in a typically developing child and a sibling later diagnosed with autism. We compare the siblings’ obstetric course to isolate pregnancy-course related predictors of autism, while passively controlling for many non-specific environmental and genetic factors.

Methods
Participants- A total of N = 1,230 women aged 15–77 years (mean = 38.42, SD = 12.4) were recruited for a study on health through two websites managed by the Autism Research Centre (ARC) at Cambridge University, UK; 721 women through a website that targets individuals diagnosed with autism and their family members (https://autismresearchcentre.net/) and 509 women through a website targeting the general population (affiliation to ARC is not mentioned; https://cambridgepsychology.com). Of all the participants enrolled in the study, only mothers of biological children were included in the current analyses. Participants were provided with information regarding the study and gave their consent before gaining access to the questionnaire. The study was approved by the Psychology Research Ethical Committee (PREC) at Cambridge University. In current analyses only women who reported regarding at least one pregnancy resulting in a live birth were included (N = 572). Births of multiples and children whose diagnosis status was missing were excluded from analyses.

Study 1. Two separate analyses were conducted, for the first and second live-born child of each mother. The final sample included: (1) N = 557 mothers and their first-born children (four mothers were removed due to missing data regarding child's autism diagnosis; and 11 due to multiple fetuses). (2) N = 374 mothers and their second-born children (31 mothers were removed due to missing data regarding child's diagnosis; and 13 due to multiple fetuses).

Study 2. Analyses were conducted on a sub-sample of 132 mothers (264 pregnancies) who reported on at least one child diagnosed with autism and at least one typically developing child (TD). For each mother we selected the first reported single birth of a TD child and the first reported single birth of a child later diagnosed with autism. The siblings share a biological father, as determined by the maternal report regarding father’s date of birth (see Table 1 for details).
### Table 1
Sample composition-

|                          | N mothers of a child diagnosed with autism | N mothers of a typically developing child | Total number of mothers | Parental mean age | N child male sex (% in sample) |
|--------------------------|------------------------------------------|------------------------------------------|-------------------------|------------------|-------------------------------|
| First child              | 192 (34.5%)                              | 365 (65.5%)                              | 557                     | Mothers-27.72 ± 5.7 | 306 (54.9%)                  |
|                          |                                          |                                          |                         | Fathers-30.61 ± 7  |                               |
| Second child             | 105 (28.1%)                              | 269 (71.9%)                              | 374                     | Mothers-30.28 ± 5.2 | 213 (57%)                    |
|                          |                                          |                                          |                         | Fathers-32.68 ± 6.1 |                               |
| Sub-sample analysis      | -                                        | -                                        | 132                     | Mothers-30.47 ± 5.3 | 163 (61.7%)                  |
|                          |                                          |                                          |                         | Fathers-32.71 ± 5.7 |                               |

**Measures**- Mothers filled out the following self-report measures:

1. Demographic questionnaire - including mother's and father's date of birth, BMI before pregnancy, number of pregnancies, and autism diagnoses in the family.

2. Health and pregnancy questionnaire (see Supplementary Information A) - maternal medical conditions (i.e. not specific to the pregnancy); conditions and complications during pregnancy; and infant characteristics. Only items relating to maternal medical conditions, obstetric course and perinatal factors were analyzed. See full list in Table 2 and Supplementary Information A.
### Table 2
List of the independent variables used in each analysis

|                        | First child | Second child | Sub sample analysis |
|------------------------|-------------|--------------|---------------------|
| **Demographic information** |             |              |                     |
| Mother autism diagnosis | X           | X            |                     |
| Family member autism diagnosis | X          | X            |                     |
| Mother's age at birth   | X           | X            | X                   |
| (0.4% imputed)          |             |              |                     |
| Father's age at birth   | X           | X            | X                   |
| (1.3% imputed)          |             |              | (0.5% imputed)      |
| Percentage of pregnancies ended in miscarriage | X          | X            |                     |
| **Maternal medical conditions** |             |              |                     |
| Ovarian cancer          | X           | X            |                     |
| Uterine cancer          | X           | X            |                     |
| Breast cancer           | X           | X            |                     |
| Chronic fatigue syndrome (CFS) | X      | X            |                     |
| Hyperthyroidism         | X           | X            |                     |
| Hypothyroidism          | X           | X            |                     |
| High cholesterol        | X           | X            |                     |
| Autoimmune disorder     | X           | X            |                     |
| Pre-menstrual syndrome (PMS) | X      | X            |                     |
| Polycystic ovary syndrome (PCOS) | X  | X            |                     |
| Cardiac conditions      | X           | X            |                     |
| High blood pressure     | X           | X            |                     |
| Anovulation (failure to ovulate) | X    | X            |                     |
| Type II diabetes        | X           | X            |                     |
| Epilepsy                | X           |              |                     |
| Infertility             | X           | X            |                     |
|                               | First child | Second child | Sub sample analysis |
|-------------------------------|-------------|--------------|---------------------|
| Sum of maternal medical conditions | X           | X            |                     |
| **Obstetric course factors**  |             |              |                     |
| Infertility treatments        | X           | X            |                     |
| Hormonal medications during pregnancy | X           | X            |                     |
| Infection                     | X           | X            | X                   |
| Maternal vaginal bleeding after second trimester | X           | X            | X                   |
| Blood sugar medications       | X           | X            |                     |
| Maternal BMI prior to the pregnancy | X           | X            | X                   |
|                               | (21.5% imputed) | (27% imputed) | 20.5% imputed)      |
| Maternal weight gain during pregnancy | X           | X            | X                   |
|                               | (36.4% imputed) | (39.9% imputed) | (34.5% imputed)     |
| Gestational diabetes          | X           | X            |                     |
| Preeclampsia                  | X           | X            |                     |
| Hyperemesis gravidarum        | X           | X            |                     |
| Hypertension                  | X           | X            |                     |
| Polyhydramnios                | X           | X            |                     |
| Placenta previa               | X           | X            |                     |
| Placental abruption           | X           |              |                     |
| Infection amniotic sac        | X           |              |                     |
| Other conditions              | X           | X            |                     |
| Sum maternal medical conditions during pregnancy | X           | X            | Pregnancy complication (yes/no) |
| Treatments for preterm labor  | X           | X            |                     |
| **Perinatal factors and infant's characteristics** |     |              |                     |
| Child gestational age         | X           | X            | X                   |
|                               | (8.6% imputed) | (7.2% imputed) | (5.3% imputed)      |
### Data Analysis

Most of the predictor variables were treated as binary variables (yes/no), and few as ordinal or quantitative variables (see Supplementary Information A for more information).

Missing data imputation-Variables with less than 40% missing data (quantitative variables) or less than 5 cases (binary variables) were included in the analyses, and the missing data was imputed using “MICE” (Multivariate Imputation via Chained Equations) package in R, and the Predictive Mean Matching (PMM) technique, which is appropriate for numeric variables. MICE assumes missing at random, meaning that the probability that a value is missing depends only on other observed values, and therefore observed values can be used to predict the missing value. We used the MICE procedure to create 1,000 imputed data sets (30), and averaged out the imputed variables across the data sets.

### Study 1

The main aim of the study was to examine the association between maternal medical conditions, obstetric factors and perinatal factors, with child’s autism. To select the predictors which show a unique contribution to autism we analyzed the data using the procedure LASSO (in R, "glmnet" package; (31)).

LASSO (Least Absolute Shrinkage and Selection Operator) is a technique for regression analysis, which performs both variable selection and regularization; and can be used for binary outcomes (12). The technique uses L1 penalty, which minimizes the absolute value of each predictor by shrinking all estimates toward zero (31, 32). In the context of this research, the technique assigns positive and negative weights to variables associated with child’s autism. Variables unrelated to the diagnosis are assigned a weight of zero, which effectively excludes them from the final model. LASSO yields a regularization parameter (a penalty; i.e "\( \lambda \)”) that creates a parsimonious model which contains the maximum number of parameters and minimum cross-validation errors. The final model contains the selected predictive variables that were associated with the outcome. Despite some limitations (31, 33), LASSO has high accuracy, and the chosen variables are highly likely to represent true contributions (33), and therefore is a reliable method for predictors selection. Although the LASSO yields coefficient value for each predictive variables, one cannot interpret the relative magnitude of the coefficients, because LASSO...
creates bias in the estimation of the parameters (due the shrinking toward zero), and the coefficients are not necessarily accurate. Therefore, an estimator was calculated for every coefficient by training LASSO classifiers on 1,000 bootstrapped samples of the data using the glmnet R package. To ensure a coefficient of zero for unrelated variables, the median of the coefficients for every variable, rather than the mean, was calculated as the final estimator. Additionally, a 95% confidence interval for the medians was calculated using scipy.stats.binom (34). To calculate the P value for every estimator, 10,000 equally-distributed random datasets were generated by independently bootstrapping each column of the original dataset. Then, a one-sided p-value was calculated for each estimator as the number of times the respective random coefficients were higher than or equal to the actual estimator (for positive estimators), or lower than or equal to the actual estimator for negative estimators, divided by the total number of random datasets (10,000). P values were not calculated for coefficients estimated to be 0.

**Study 2**

Ten predictors, which could differ between pregnancies, were included, negating the need for predictor selection. We conducted bootstrapped (5,000 repetition) paired t-test analysis to compare the rate of each condition for TD vs autistic siblings. A Bonferroni correction was applied to control for multiple testing (.05/10 = .005).

**Results**

**Study 1:**

A LASSO analysis was conducted for the first and second-born children separately. See descriptive statistics for each sample in Table 3. See summary of the predictors of child’s autism in Table 4. For the first-born, the final model included 15 predictors associated with increased likelihood for autism diagnosis in the child. For the second-born, the final model included 20 predictors of child’s autism.
Table 3  
Descriptive statistic by birth order group:

|                  | Autism | TD  |
|------------------|--------|-----|
| **First child**  |        |     |
| N                | 192    | 365 |
| Male             | 145 (75.5%) | 161 (44.1%) |
| Female           | 47 (24.5%) | 204 (55.9%) |
| Mother's age at birth | 28.72 ± 5.8 | 27.18 ± 5.6 |
| Father's age at birth | 31.83 ± 7.6 | 29.96 ± 6.6 |
| Mother's autism diagnosis | 41 (21.4%) | 72 (19.7%) |
| **Second child** |        |     |
| N                | 105    | 269 |
| Male             | 73 (69.5%) | 140 (52%) |
| Female           | 32 (30.5%) | 129 (48%) |
| Mother's age at birth | 30.75 ± 4.9 | 30.09 ± 5.4 |
| Father's age at birth | 33.01 ± 5.6 | 32.56 ± 6.2 |
| Mother's autism diagnosis | 21 (20.0%) | 46 (17.1%) |
Table 4

Results of the bootstrapped LASSO analysis, including the bootstrapped median coefficient and confidence intervals. Top panel – older child, bottom panel – younger child.

| Condition                                      | Median coefficient | P value | 95% C.I. Lower | 95% C.I. Upper |
|------------------------------------------------|--------------------|---------|----------------|---------------|
| **First child**                                |                    |         |                |               |
| Maternal autism diagnosis                      | 0                  | 0.8360  | -              | -             |
| Family member autism diagnosis                 | 1.428              | 0.0010  | 1.401          | 1.448         |
| Mother's age at birth                          | 0.034              | 0.0620  | 0.032          | 0.036         |
| Father's age at birth                          | 0.020              | 0.1040  | 0.019          | 0.022         |
| Percentage of pregnancies ended in miscarriage | -0.846             | 0.0130  | -0.904         | -0.783        |
| Ovarian cancer                                 | 0                  | 0.9830  | -              | -             |
| Uterine cancer                                 | 0                  | 0.9440  | -              | -             |
| Breast cancer                                  | 0                  | 0.9350  | -              | -             |
| Chronic fatigue syndrome                       | 0                  | 0.9210  | -              | -             |
| Hyperthyroidism                                | 0                  | 0.9880  | -              | -             |
| Hypothyroidism                                 | -1.147             | 0.0020  | -1.192         | -1.088        |
| High cholesterol                               | 0                  | 0.9380  | 0              | 0.053         |
| Autoimmune disorder                           | -0.228             | 0.0220  | -0.286         | -0.183        |
| Pre-menstrual syndrome                         | 0.113              | 0.0750  | 0.085          | 0.146         |
| Polycystic ovary syndrome                      | 0                  | 0.8630  | -              | -             |
| Cardiac conditions                             | 0                  | 0.9150  | -              | -             |
| High blood pressure                            | 0                  | 0.9170  | -              | -             |
| Anovulation                                    | 0.020              | 0.0160  | 0              | 0.085         |
| Type ii diabetes                               | -2.684             | 0.0010  | -2.828         | -2.565        |
| Epilepsy                                       | 0.958              | 0.0020  | 0.854          | 1.060         |
| Infertility treatments                         | 0                  | 0.9090  | -              | -             |
| Sum of maternal medical conditions             | 0.201              | 0.0270  | 0.186          | 0.216         |
| Maternal bmi prior to the pregnancy            | 0.007              | 0.2380  | 0.005          | 0.010         |
| Maternal weight gain during pregnancy          | -0.002             | 0.3020  | -0.004         | -0.001        |
| Condition                                         | Median coefficient | P value  | 95% C.I.   |       |
|--------------------------------------------------|--------------------|----------|------------|-------|
|                                                  |                    |          | Lower      | Upper |
| Infection                                        | 0.648              | 0.0030   | 0.618      | 0.677 |
| Maternal vaginal bleeding after second trimester | 0.066              | 0.0350   | 0.031      | 0.098 |
| Blood sugar medications                         | 0                  | 0.9890   | -          | -     |
| Hormonal medications during pregnancy           | 0.589              | 0.0030   | 0.528      | 0.648 |
| Gestational diabetes                             | 0                  | 0.9450   | -          | -     |
| Preeclampsia                                     | 0.042              | 0.0540   | 0          | 0.118 |
| Hyperemesis gravidarum                           | 0                  | 0.9820   | -          | -     |
| Hypertension                                     | 0                  | 0.9320   | -          | -     |
| Polyhydramnios                                   | 0                  | 0.9650   | -          | -     |
| Placenta previa                                  | 0                  | 0.9560   | -          | -     |
| Placental abruption                              | 0                  | 0.9670   | -          | -     |
| Infection amniotic sac                           | 0                  | 0.9640   | -          | -     |
| Other conditions                                 | 0.008              | 0.0660   | 0          | 0.052 |
| Treatments for preterm labor                    | 0                  | 0.9510   | -          | -     |
| Sum maternal medical conditions during pregnancy | 0.303              | 0.0220   | 0.276      | 0.327 |
| Child gestational age                            | -0.032             | 0.1390   | -0.037     | -0.028|
| Child sex                                        | 1.479              | 0.0010   | 1.456      | 1.502 |
| Infertility (none)                               | -0.242             | 0.0280   | -0.299     | -0.185|
| Primary infertility                              | 0                  | 0.9000   | -          | -     |
| Secondary infertility                            | 0                  | 0.8800   | -          | -     |
| Second child                                     |                     |          |            |       |
| Mother autism diagnosis                          | 0.135              | 0.0650   | 0.102      | 0.181 |
| Family member autism diagnosis                   | 1.587              | 0.0010   | 1.554      | 1.614 |
| Mother's age at birth                            | 0.026              | 0.1460   | 0.023      | 0.029 |
| Father's age at birth                            | 0.009              | 0.2070   | 0.006      | 0.012 |
| Percentage of pregnancies ended in miscarriage   | -1.091             | 0.0250   | -1.176     | -0.955|
| Condition                                      | Median Coefficient | P Value | 95% C.I.   |
|-----------------------------------------------|--------------------|---------|------------|
|                                               |                    |         | Lower      |
| Ovarian cancer                                | 0                  | 0.9930  | -          |
| Uterine cancer                                | -0.157             | 0.0180  | -0.259     |
| Breast cancer                                 | 0.271              | 0.0140  | 0.200      |
| Chronic fatigue syndrome                      | 0.037              | 0.0230  | 0          |
| Hyperthyroidism                               | 0                  | 0.9380  | -          |
| Hypothyroidism                                | 0                  | 0.8520  | -          |
| High cholesterol                              | 0                  | 0.9470  | -          |
| Autoimmune disorder                           | 0                  | 0.9480  | -          |
| Pre-menstrual syndrome                        | 0                  | 0.8970  | -          |
| Polycystic ovary syndrome                     | 0                  | 0.9420  | -          |
| Cardiac conditions                            | -1.369             | 0.0020  | -1.450     |
| High blood pressure                           | 0                  | 0.8530  | -          |
| Anovulation                                   | 1.645              | 0.0000  | 1.563      |
| Type ii diabetes                              | 1.392              | 0.0010  | 1.198      |
| Epilepsy                                      | 0                  | 0.9690  | -          |
| Sum of maternal medical conditions            | 0.080              | 0.1000  | 0.060      |
| Infertility treatments                        | 1.587              | 0.0010  | 1.459      |
| Maternal bmi prior to pregnancy               | -0.002             | 0.2850  | -0.007     |
| Maternal weight gain during pregnancy         | 0.032              | 0.1080  | 0.030      |
| Infection                                     | 0                  | 0.8880  | -          |
| Maternal vaginal bleeding after second trimester | -0.819          | 0.0040  | -0.884     |
| Blood sugar medications                       | 0                  | 0.9830  | 0          |
| Hormonal medications during pregnancy         | 0.712              | 0.0040  | 0.627      |
| Gestational diabetes                          | -0.834             | 0.0050  | -0.965     |
| Preeclampsia                                  | 0                  | 0.9530  | -          |
| Hyperemesis gravidarum                        | 0                  | 0.9830  | -          |
| Hypertension                                  | -3.313             | 0.0000  | -3.487     |
|                              | Median coefficient | P value | 95% C.I. Lower | 95% C.I. Upper |
|------------------------------|--------------------|---------|----------------|---------------|
| Polyhydramnios               | 2.645              | 0.0000  | 2.535          | 2.783         |
| Placenta previa              | -0.230             | 0.0110  | -0.339         | -0.098        |
| Other condition              | 0.586              | 0.0060  | 0.533          | 0.647         |
| Sum of pregnancy conditions  | 0                  | 0.7980  | -              | -             |
| Treatments for preterm labor | -0.397             | 0.0070  | -0.526         | -0.281        |
| Child's gestational age      | 0.064              | 0.0950  | 0.059          | 0.070         |
| Child sex                    | 1.029              | 0.0030  | 0.998          | 1.053         |
| Infertility (none)           | 0.729              | 0.0060  | 0.575          | 0.843         |
| Primary infertility          | -1.003             | 0.0030  | -1.136         | -0.852        |
| Secondary infertility        | 0                  | 0.9590  | -              | -             |

*Note:* Significant findings are in bold.

**Study 2:**

132 parents and their 264 children were included in the analysis. See results in Table 5. In this analysis, only child-related variables were included (obstetric and perinatal factors), as maternal factors are shared by both children.
Table 5
Bootstrapped paired t-test analyses

|                              | Mean (SD)    | T   | P  value | Lower   | Upper   |
|------------------------------|--------------|-----|----------|---------|---------|
| Birth order                  | .106 (.998)  | 1.221 | .224     | -.066   | .278    |
| Maternal age at birth        | .341 (4.472) | .876 | .383     | -.429   | 1.111   |
| Paternal age at birth        | .318 (4.525) | .808 | .421     | -.461   | 1.097   |
| Maternal pre-pregnancy BMI   | .308 (3.725) | .949 | .345     | -.334   | .949    |
| Weight gain during pregnancy | .726 (12.405)| .672 | .503     | -1.410  | 2.862   |
| Maternal vaginal bleeding after second trimester | .091 (.359) | 2.907 | .004     | .029    | .153    |
| Infection during pregnancy  | -.083 (.479) | -1.998 | .048    | -.166   | -.001   |
| Pregnancy complications     | -.015 (.302) | -.576 | .566     | -.067   | .037    |
| Child's gestational age      | .091 (2.100) | .495 | .621     | -.271   | .452    |
| Child's sex                  | -.295 (.662) | -5.125 | .000    | -.410   | -.181   |

**Discussion**

Studies on maternal medical factors predicting child's autism tend to focus on few factors within the same analysis. This can be problematic as some of these factors are etiologically or phenomenologically dependent, which makes it harder to determine which factors can have the largest contribution to predicting child's diagnosis. In Study 1, a case-control approach revealed 15 predictors of autism for the first-born, and eight of those were replicated in the second-born along with 12 additional predictors. In Study 2, we compared a subset of predictors, relating to pregnancy course and perinatal factors for siblings discordant for diagnosis. In this analysis two factors were significant, replicating those found in Study 1. Our analyses broadly showed that obstetric factors and maternal medical conditions were most predictive of child's autism. Below we will discuss the main findings.

*Gestational bleeding* during the second or third trimesters was associated with child's autism in all analyses (yet the effect is small in the first-born), replicating previous studies (6). Gestational bleeding is a non-specific condition, and can indicate a host of other pregnancy complications, such as placental insufficiency and preeclampsia, which have also been associated with autism (26) (but were of low prevalence in the current study and should be studied further). Interestingly, a cumulative factor of pregnancy complications was significant only for the first, but not second-born, which may reflect an
indirect association through maternal age, as pregnancy complications are associated with older maternal age (29).

The current findings suggest that infertility, rather than infertility treatments and other obstetric conditions, is related to autism, as we find this effect after controlling for infertility treatments and multiple obstetric complications, some of which are independently related to autism. Previous findings regarding the association between autism and infertility and fertility treatments were mixed. One study (27) found a higher incidence of autism among children born after infertility treatments, as compared to natural conception. The association remained statistically significant even after adjusting for parity, infant gender, and parental age, all factors that are independently associated with both infertility treatments and autism. In contrast, Hvidtjørn and colleagues (2010) found no association between autism in children and infertility treatments, in a large cohort study. Other studies (22, 35) also didn’t find any association between infertility treatments and autism diagnosis, in two separate studies conducted in California, USA. One hypothesis to explain the mixed findings is that infertility treatments are indirectly related to autism through an association with obstetric complications, pre-term labor, and low birth-weight, which are all factors that have been independently associated with an child's autism (6, 7, 24), and with older parental age (21, 36, 37). Parner and colleagues (2012) analyzed a cohort of all singleton births between 1980 to 2003 in Denmark and found that older parental age is associated with child's autism(38). Similarly, a cohort study of singleton births between 1989 to 2002 conducted in California, USA, also found that advanced parental age was associated with child's autism (39). Older maternal age may be associated with autism not only because of the increased risk of chromosomal abnormalities in the ova (6), but also due to the relationship between mature age and increased risk for pregnancy complications (29).

The current findings suggest that gestational diabetes and type II diabetes are both related to autism, but not BMI prior to pregnancy or weight gain during pregnancy. Previous studies found a relationship between various metabolic conditions such as maternal obesity prior to pregnancy, increased weight gain during pregnancy and gestational diabetes (defined as glucose intolerance with onset or first recognition during pregnancy) with autism and with general poor neurodevelopmental outcomes (40, 41). Krakowiak and colleagues found that mothers of children diagnosed with autism had a higher rate of gestational diabetes and obesity (defined as BMI > 30), compared to mothers of TD children (28). In addition, Xiang and colleagues found higher rates of child's autism among mothers diagnosed with type II diabetes before pregnancy, and among women diagnosed with gestational diabetes by 26 weeks of pregnancy (42). The current findings emphasize the importance of these metabolic conditions in autism.

The non-significant findings also shed light on the relative importance of various factors. Here we found no evidence for an association between child’s gestational age and autism. Pre-term labor and low birth-weight were previously found to be non-specific risk-factors for neurodevelopmental and intellectual disabilities such as learning disabilities, attention problems, and poor executive function (25). According to one study, low birth-weight infants (< 2500 g) have a 60% increased likelihood of autism, and pre-term infants (< 32 weeks) had twice the chance of developing autism, for both sexes (31). In addition, Burstyn
and colleagues (2010) found that low birth-weight (< 2500 g) increased the likelihood for autism diagnosis by 33%. However, pre-term labor and low birth-weight often can be the consequents of obstetric complications (26), and the current findings emphasize the importance of obstetric complications over gestational age in predicting child’s autism.

Immune system activation during pregnancy is another suspected environmental mechanism for child’s autism. Animal studies show that immune activation during pregnancy is associated with abnormal brain development (16, 43). Specifically, Shi and colleagues found a reduction in social behavior and elevated anxiety behaviors among mice born to mothers infected by a virus during pregnancy (44). Studies in humans find similar associations. Lee and colleagues (2015) found that maternal infection (viral or bacterial) which leads to hospitalization, increased the likelihood for autism by 37%, in a cohort study of all live births born 1984–2007, in Sweden (17). However, another study found an association only for hospitalization due to bacterial infections(16, 45), and another found no association between infection and autism in a Danish population (46). We also do not find evidence for this effect in the current study. Only autoimmune disorders and only for the firstborn were related to child’s autism, similar to other findings in the field (e.g., (9, 47, 48), but see (49, 50)).

Importantly, some maternal medical conditions which were found in previous research as associated with autism, were not found to be significant or did not replicate across the analyses. For example, maternal PCOS was found to be related to child’s autism in multiple large-scale analyses (8, 13), but in our study was not significant.

Limitations

The main limitation of the study is the use of self-report, recollection data, as opposed to the use of medical records. It is possible that some conditions and diagnoses were misremembered or missed entirely. Therefore, the findings of the current study should be replicated using medical records in order to validate the findings. Another limitation of the study is the sample size, which did not allow to examine the relationship between child’s autism and relatively rare disorders and conditions. A larger sample, or a sample biased to include reports of mothers who suffered specific serious health and obstetric complications would allow to focus on these effects.

Conclusions

Our findings emphasize the role of infertility, including miscarriages, and obstetric complications as predictive factors of autism, beyond typically correlated factors such parental age and vaginal bleeding. Thus, these findings are important in guiding further research into the involvement of infertility and obstetric complications in the etiology of autism.

Declarations

Ethics approval and consent to participate
The study was approved by the Psychology Research Ethical Committee (PREC) at Cambridge University

**Consent for publication**

Not applicable

**Availability of data and materials**

The datasets generated and/or analysed during the current study are not publicly available as at the time of data collection such permission was not sought from the participants, but are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests

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**Authors' contributions**

MR designed the study, analyzed and interpreted the data, and wrote the paper. AP designed the study, collected the data, helped interpret the data and gave critical comments on the paper.

DA analyzed the data and wrote parts of the paper. TS helped interpret the data and provided critical comments on the paper. AR helped design the study and provided critical comments on the paper.

PS helped collect and curate the data. ML and CA helped interpret the data and provided critical comments on the paper. AE helped with data analysis and provided critical comments on the paper.

SBC designed the study, interpreted the data, and provided critical comments on the paper. FU designed the study, helped with data analysis, interpreted the data, and wrote the paper.
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References

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, (DSM-5®): American Psychiatric Pub; 2013.
2. Maenner MJ, Shaw KA, Baio J, EdS, Washington A, Patrick M, et al. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016. MMWR Surveill Summ. 2020;69(4):1-12.
3. Sandin S, Lichtenstein P, Kuja-Halkola R, Larsson H, Hultman CM, Reichenberg A. The familial risk of autism. Jama. 2014;311(17):1770-7.
4. Lai MC, Lombardo MV, Baron-Cohen S. Autism. Lancet. 2014;383(9920):896-910.
5. Gaugler T, Klei L, Sanders SJ, Bodea CA, Goldberg AP, Lee AB, et al. Most genetic risk for autism resides with common variation. Nature genetics. 2014;46(8):881-5.
6. Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. The British journal of psychiatry. 2009;195(1):7-14.
7. Gardener H, Spiegelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. Pediatrics. 2011;128(2):344-55.
8. Kosidou K, Dalman C, Widman L, Arver S, Lee B, Magnusson C, et al. Maternal polycystic ovary syndrome and the risk of autism spectrum disorders in the offspring: a population-based nationwide study in Sweden. Molecular psychiatry. 2016;21(10):1441-8.
9. Keil A, Daniels JL, Forssen U, Hultman C, Cnattingius S, Söderberg KC, et al. Parental autoimmune diseases associated with autism spectrum disorders in offspring. Epidemiology (Cambridge, Mass). 2010;21(6):805.
10. Rom AL, Wu CS, Olsen J, Jawaheer D, Hetland ML, Mørch LS. Parental Rheumatoid Arthritis and Autism Spectrum Disorders in Offspring: A Danish Nationwide Cohort Study. Journal of the American Academy of Child & Adolescent Psychiatry. 2018;57(1):28-32.e1.
11. Curran EA, O'Neill SM, Cryan JF, Kenny LC, Dinan TG, Khashan AS, et al. Research Review: Birth by caesarean section and development of autism spectrum disorder and attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. Journal of Child Psychology and Psychiatry. 2015;56(5):500-8.
12. Tibshirani R. Regression shrinkage and selection via the lasso. Journal of the Royal Statistical Society: Series B (Methodological). 1996;58(1):267-88.
13. Cherskov A, Pohl A, Allison C, Zhang H, Payne RA, Baron-Cohen S. Polycystic ovary syndrome and autism: A test of the prenatal sex steroid theory. Translational psychiatry. 2018;8(1):1-10.
14. Baron-Cohen S, Knickmeyer RC, Belmonte MK. Sex differences in the brain: implications for explaining autism. Science. 2005;310(5749):819-23.

15. Knickmeyer RC, Baron-Cohen S. Fetal testosterone and sex differences. Early human development. 2006;82(12):755-60.

16. Atladóttir HÓ, Henriksen TB, Schendel DE, Parner ET. Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. Pediatrics. 2012;130(6):e1447-e54.

17. Lee BK, Magnusson C, Gardner RM, Blomström Å, Newschaffer CJ, Burstyn I, et al. Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. Brain, behavior, and immunity. 2015;44:100-5.

18. Fatemi SH, Earle J, Kanodia R, Kist D, Emamian ES, Patterson PH, et al. Prenatal viral infection leads to pyramidal cell atrophy and macrocephaly in adulthood: implications for genesis of autism and schizophrenia. Cellular and molecular neurobiology. 2002;22(1):25-33.

19. Kissin DM, Zhang Y, Boulet SL, Fountain C, Bearman P, Schieve L, et al. Association of assisted reproductive technology (ART) treatment and parental infertility diagnosis with autism in ART-conceived children. Human Reproduction. 2015;30(2):454-65.

20. Gao J, He X, Cai Y, Wang L, Fan X. Association between assisted reproductive technology and the risk of autism spectrum disorders in the offspring: a meta-analysis. Scientific reports. 2017;7:46207.

21. Hvidtjørn D, Grove J, Schendel DE, Sværke C, Schieve L, Uldall P, et al. Multiplicity and early gestational age contribute to an increased risk of cerebral palsy from assisted conception: a population-based cohort study. Human Reproduction. 2010;25(8):2115-23.

22. Grether JK, Qian Y, Croughan MS, Wu YW, Schembri M, Camarano L, et al. Is infertility associated with childhood autism? Journal of autism and developmental disorders. 2013;43(3):663-72.

23. Wu S, Wu F, Ding Y, Hou J, Bi J, Zhang Z. Advanced parental age and autism risk in children: a systematic review and meta-analysis. Acta Psychiatr Scand. 2017;135(1):29-41.

24. Dupont C, Sifer C. A review of outcome data concerning children born following assisted reproductive technologies. ISRN obstetrics and gynecology. 2012;2012.

25. Lampi KM, Lehtonen L, Tran PL, Suominen A, Lehti V, Banerjee PN, et al. Risk of autism spectrum disorders in low birth weight and small for gestational age infants. The Journal of Pediatrics. 2012;161(5):830-6.

26. Walker CK, Krakowiak P, Baker A, Hansen RL, Ozonoff S, Hertz-Picciotto I. Preeclampsia, placental insufficiency, and autism spectrum disorder or developmental delay. JAMA pediatrics. 2015;169(2):154-62.

27. Fountain C, Zhang Y, Kissin DM, Schieve LA, Jamieson DJ, Rice C, et al. Association between assisted reproductive technology conception and autism in California, 1997–2007. American Journal of Public Health. 2015;105(5):963-71.

28. Krakowiak P, Walker CK, Bremer AA, Baker AS, Ozonoff S, Hansen RL, et al. Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. Pediatrics. 2012;129(5):e1121-e8.
29. Mann JR, McDermott S, Bao H, Hardin J, Gregg A. Pre-eclampsia, birth weight, and autism spectrum disorders. Journal of autism and developmental disorders. 2010;40(5):548-54.
30. Groothuis-Oudshoorn K, Van Buuren S. Mice: multivariate imputation by chained equations in R. J Stat Softw. 2011;45(3):1-67.
31. Friedman J, Hastie T, Tibshirani R. Regularization paths for generalized linear models via coordinate descent. Journal of statistical software. 2010;33(1):1.
32. Steyerberg E, Eijkemans M, Habbema J. Application of shrinkage techniques in logistic regression analysis: a case study. Statistica Neerlandica. 2001;55(1):76-88.
33. Epskamp S, Kruit J, Marsman M. Estimating psychopathological networks: Be careful what you wish for. PloS one. 2017;12(6):e0179891.
34. Le Boudec J-Y. Performance evaluation of computer and communication systems: Epfl Press; 2010.
35. Lyall K, Baker A, Hertz-Picciotto I, Walker CK. Infertility and its treatments in association with autism spectrum disorders: a review and results from the CHARGE study. International journal of environmental research and public health. 2013;10(8):3715-34.
36. Bilder D, Pinborough-Zimmerman J, Miller J, McMahon W. Prenatal, perinatal, and neonatal factors associated with autism spectrum disorders. Pediatrics. 2009;123(5):1293-300.
37. Glasson EJ, Bower C, Pettersson B, de Klerk N, Chaney G, Hallmayer JF. Perinatal factors and the development of autism: a population study. Archives of general Psychiatry. 2004;61(6):618-27.
38. Parner ET, Baron-Cohen S, Lauritsen MB, Jørgensen M, Schieve LA, Yeargin-Allsopp M, et al. Parental age and autism spectrum disorders. Annals of epidemiology. 2012;22(3):143-50.
39. Grether JK, Anderson MC, Croen LA, Smith D, Windham GC. Risk of autism and increasing maternal and paternal age in a large north American population. American Journal of Epidemiology. 2009;170(9):1118-26.
40. Dodds L, Fell DB, Shea S, Armson BA, Allen AC, Bryson S. The role of prenatal, obstetric and neonatal factors in the development of autism. Journal of autism and developmental disorders. 2011;41(7):891-902.
41. Xu G, Jing J, Bowers K, Liu B, Bao W. Maternal diabetes and the risk of autism spectrum disorders in the offspring: a systematic review and meta-analysis. Journal of autism and developmental disorders. 2014;44(4):766-75.
42. Xiang AH, Wang X, Martinez MP, Walthall JC, Curry ES, Page K, et al. Association of maternal diabetes with autism in offspring. Jama. 2015;313(14):1425-34.
43. Patterson PH. Maternal infection: window on neuroimmune interactions in fetal brain development and mental illness. Current opinion in neurobiology. 2002;12(1):115-8.
44. Shi L, Fatemi SH, Sidwell RW, Patterson PH. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. Journal of Neuroscience. 2003;23(1):297-302.
45. Zerbo O, Qian Y, Yoshida C, Grether JK, Van de Water J, Croen LA. Maternal infection during pregnancy and autism spectrum disorders. Journal of autism and developmental disorders.
46. Maimburg RD, Væth M. Perinatal risk factors and infantile autism. Acta Psychiatrica Scandinavica. 2006;114(4):257-64.

47. Sweeten TL, Bowyer SL, Posey DJ, Halberstadt GM, McDougle CJ. Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. Pediatrics. 2003;112(5):e420-e.

48. Spann MN, Timonen-Soivio L, Suominen A, Cheslack-Postava K, McKeague IW, Sourander A, et al. Proband and Familial Autoimmune Diseases Are Associated With Proband Diagnosis of Autism Spectrum Disorders. Journal of the American Academy of Child & Adolescent Psychiatry. 2019;58(5):496-505.

49. Croen LA, Grether JK, Yoshida CK, Odouli R, Van de Water J. Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. Archives of pediatrics & adolescent medicine. 2005;159(2):151-7.

50. Mouridsen SE, Rich B, Isager T, Nedergaard NJ. Autoimmune diseases in parents of children with infantile autism: a case—control study. Developmental Medicine & Child Neurology. 2007;49(6):429-32.

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