

## Original Article

**Cite this article:** Kodesh A *et al* (2022). Maternal health around pregnancy and autism risk: a diagnosis-wide, population-based study. *Psychological Medicine* **52**, 4076–4084. <https://doi.org/10.1017/S0033291721001021>

Received: 5 November 2020  
Revised: 4 February 2021  
Accepted: 4 March 2021  
First published online: 26 March 2021


**Key words:**

Autism; diagnosis-wide; maternal health; prenatal effects

**Author for correspondence:**

Magdalena Janecka,  
E-mail: [magdalena.janecka@mssm.edu](mailto:magdalena.janecka@mssm.edu)

# Maternal health around pregnancy and autism risk: a diagnosis-wide, population-based study

Arad Kodesh<sup>1,2</sup>, Stephen Z. Levine<sup>1</sup>, Vahe Khachadourian<sup>3,4</sup>, Rayees Rahman<sup>5</sup>, Avner Schlessinger<sup>5</sup>, Paul F. O'Reilly<sup>6</sup>, Jakob Grove<sup>7,8,9,10</sup>, Diana Schendel<sup>7,11</sup>, Joseph D. Buxbaum<sup>3,4,12,13</sup>, Lisa Croen<sup>14</sup>, Abraham Reichenberg<sup>3,4,12,15</sup>, Sven Sandin<sup>3,4,16</sup> and Magdalena Janecka<sup>3,4,9,12,13</sup> 

<sup>1</sup>Department of Community Mental Health, University of Haifa, Haifa, Israel; <sup>2</sup>Meuhedet Health Services, Tel Aviv, Israel; <sup>3</sup>Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, New York, USA; <sup>4</sup>Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, New York, USA; <sup>5</sup>Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, New York, New York, USA; <sup>6</sup>Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, New York, USA; <sup>7</sup>The Lundbeck Foundation Initiative for Integrative Psychiatric Research, iPSYCH, Aarhus, Denmark; <sup>8</sup>iSEQ, Centre for Integrative Sequencing, Aarhus University, Aarhus, Denmark; <sup>9</sup>Department of Biomedicine – Human Genetics, Aarhus University, Aarhus, Denmark; <sup>10</sup>Bioinformatics Research Centre, Aarhus University, Aarhus, Denmark; <sup>11</sup>Section for Epidemiology, National Centre for Register-Based Research, Aarhus University, Aarhus, Denmark; <sup>12</sup>Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, New York, USA; <sup>13</sup>Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, New York, USA; <sup>14</sup>Division of Research, Kaiser Permanente Northern California, Oakland, California, USA; <sup>15</sup>Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA and <sup>16</sup>Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

**Abstract**

**Background.** Many studies have reported an increased risk of autism spectrum disorder (ASD) associated with some maternal diagnoses in pregnancy. However, such associations have not been studied systematically, accounting for comorbidity between maternal disorders. Therefore our aim was to comprehensively test the associations between maternal diagnoses around pregnancy and ASD risk in offspring.

**Methods.** This exploratory case-cohort study included children born in Israel from 1997 to 2008, and followed up until 2015. We used information on all ICD-9 codes received by their mothers during pregnancy and the preceding year. ASD risk associated with each of those conditions was calculated using Cox proportional hazards regression, adjusted for the confounders (birth year, maternal age, socioeconomic status and number of ICD-9 diagnoses during the exposure period).

**Results.** The analytic sample consisted of 80 187 individuals (1132 cases, 79 055 controls), with 822 unique ICD-9 codes recorded in their mothers. After extensive quality control, 22 maternal diagnoses were nominally significantly associated with offspring ASD, with 16 of those surviving subsequent filtering steps (permutation testing, multiple testing correction, multiple regression). Among those, we recorded an increased risk of ASD associated with metabolic [e.g. hypertension; HR = 2.74 (1.92–3.90),  $p = 2.43 \times 10^{-8}$ ], genitourinary [e.g. non-inflammatory disorders of cervix; HR = 1.88 (1.38–2.57),  $p = 7.06 \times 10^{-5}$ ] and psychiatric [depressive disorder; HR = 2.11 (1.32–3.35),  $p = 1.70 \times 10^{-3}$ ] diagnoses. Meanwhile, mothers of children with ASD were less likely to attend prenatal care appointment [HR = 0.62 (0.54–0.71),  $p = 1.80 \times 10^{-11}$ ].

**Conclusions.** Sixteen maternal diagnoses were associated with ASD in the offspring, after rigorous filtering of potential false-positive associations. Replication in other cohorts and further research to understand the mechanisms underlying the observed associations with ASD are warranted.

**Introduction**

Autism spectrum disorder (ASD) is a neurodevelopmental disorder affecting ~1.5% of children in the USA (Wiggins, Baio, & Rice, 2006), and characterized by difficulties in social communication and rigid, repetitive behaviors. While the disorder likely arises due to a combination of genetic and environmental factors (Bai *et al.*, 2019; Sandin *et al.*, 2014), potential modifiable risk factors for this disorder remain unknown.

As the pathology underlying ASD likely originates *in utero*, a search of the actionable, etiological risk factors for the disorder has focused on maternal exposures in pregnancy. Although the fetus is protected from many such exposures due to the placental barrier – which ensures lack of direct mixing of maternal and fetal blood – certain hormones (Chan, Vasilopoulou, &

Kilby, 2009; Dancis, Jansen, & Levitz, 1980), immunoglobulins (Kohler & Farr, 1966; Palmeira, Quinello, Ana, Zago, & Carneiro-sampaio, 2012), nutrients (Acosta et al., 2015), viruses (Castanha et al., 2016) and toxins (Mahadevan et al., 2013) can still pass through this barrier, potentially exposing the fetus to a number of external insults from maternal circulation. Furthermore, some of those factors can affect placental function without having to cross it (Walker, Filis, Shaughnessy, Bellingham, & Fowler, 2019), potentially affecting the fetus in spite of the mechanical shield provided by the placenta.

Fetal exposure to factors in maternal circulation provides therefore a plausible mechanism for how maternal health factors in pregnancy may impact the fetus. To date, some of the maternal diagnoses linked with offspring ASD risk include e.g. depression (Rai et al., 2013; Viktorin, Uher, Reichenberg, Levine, & Sandin, 2017), diabetes (Xiang et al., 2019), autoimmune diseases (Atladóttir et al., 2009), asthma (Croen, Grether, Yoshida, Odouli, & Van de Water, 2005), and recurrent infections (Atladóttir et al., 2010). Nevertheless, pregnant women receive many other diagnoses around pregnancy, most of which have not been evaluated for an association with offspring ASD. We have previously shown (Janecka et al., 2018) that mothers of children with ASD have on average four more distinct diagnoses around the pregnancy than mothers of control children – suggesting that the associations between maternal health and ASD may be far more pervasive than just the diagnoses identified to date.

Here we conduct an exploratory study to investigate the association between offspring ASD and the full spectrum of maternal diagnoses around pregnancy. Using a large, population-based cohort from Israel, we test the associations between the full range of recorded maternal diagnoses and offspring ASD, applying a series of rigorous screens for potential false-positive findings and controlling for the correlation between maternal diagnoses. Despite the methodological rigor, we emphasize that the findings cannot be interpreted as reflective of causal relationships. In the Discussion section, we elaborate on potential confounding effects and the further work required to understand the etiological relevance of our findings to ASD.

## Methods

### Sample

Our sample is population-based, derived through a case-cohort ascertainment design, as described in detail previously (Janecka et al., 2018; Levine et al., 2018). Briefly, the data source was Meuhedet, a large health maintenance organization (HMO) in Israel. Legislation requires all Israeli citizens to purchase medical insurance from an HMO (each offering equivalent medical provision and fees) and prohibits HMOs refusing a citizen membership, thus limiting the risk of ascertainment bias in the sample.

The cohort birth years were defined as 1 January 1997 through 31 December 2008, and children were followed up until January 26 2015. The study sample comprised of 19.5% of 270 799 children born within the Meuhedet HMO within those years, sampled without stratification, as well as all ASD cases born within those years. Additionally, all siblings of those individuals, born within the cohort birth years, were also included in the sample.

This ascertainment design produced a subcohort including random 19.5% of Meuhedet children born between 1997 and 2008, together with all siblings of ASD cases and controls. Due

to random sampling, the subcohort included some ASD cases, and thus overlapped with the individuals selected independently as ASD cases. Individuals sampled both into the subcohort, and as ASD cases or their siblings, contributed to all analyses only once. Family relations (siblings, parents) in the dataset were identified through the Meuhedet Family Relations Register.

In order to ensure complete coverage of maternal diagnoses during, and prior to pregnancy (see exposure definition in Maternal diagnosis classification) for all births, we used the subset of children born on, or after 1 January 1999 through 31 December 2008, as maternal medical records in the first year of the follow-up (1997) may be incomplete. Due to the low likelihood of reproduction beyond ages 13–55 in females, and below 13 in males, we excluded all children born to mothers younger than 13 or older than 55, or fathers older than 55, as records of those children were likely to include administrative errors.

This study was approved by the institutional review board of the University of Haifa and the Helsinki Ethics Committee of the Meuhedet. Those bodies waived the need for informed consent because the study data were fully de-identified.

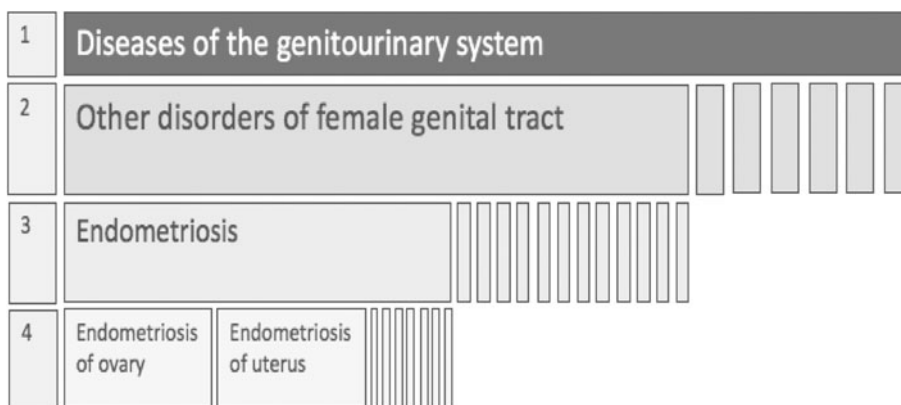
### Exposure

The exposures were maternal diagnoses classified according to the International Classification of Diseases, Ninth Revision (ICD-9). Although the ICD-10 taxonomy was comprehensively adopted in Israel in 2015, i.e. considerably later than the end of the exposure period in our study, few providers began the transition already in 2008, resulting in a small fraction of the diagnoses in our dataset coded using the ICD-10 system. In order to ensure completeness of the data, these diagnoses were harmonized with the remainder of the data and re-coded to ICD-9, using publicly available resources (ICD10Data, 2021; online Supplementary Table S1). All diagnostic codes were identified through the Meuhedet Diagnostic Classification Register. The ICD-9 diagnoses are organized hierarchically, with four levels of information going from least to most specific to the given diagnosis (see Fig. 1 for an example). For the current analyses, we used the information at level 3 as it provides both a useful summary and sufficient detail regarding the underlying health condition. In the current analyses, we included all recorded maternal diagnoses, irrespective of the internal system they affect, their chronicity or severity. The exposure period was defined as the pregnancy period (here defined as 271 days prior to the child's date of birth) and the preceding year (i.e. a total of 636 days prior to the child's date of birth). Widening of the exposure window beyond pregnancy allowed us to ascertain temporally proximal diagnoses which could still exert effects on the fetus.

Prior to the analyses, we filtered out the diagnoses not recorded in any women during the exposure period, and those where the available information did not allow assignment to any ICD-9 code. The exposures were coded as binary variables (yes/no) indicating the presence/absence of any given level 3, ICD-9 diagnosis within the exposure period. Earlier analyses have demonstrated that the diagnostic rates of all interrogated conditions approximate those recorded in Sweden (Janecka et al., 2018).

### Outcome

ASD cases were ascertained using the ICD-9 and ICD-10 criteria for ASD from the ICD (ICD-9 codes 299 and ICD-10 codes F84,



**Fig. 1.** Example of the hierarchical organization of the ICD-9 taxonomy. ICD-9 categories are organized from the most general (level 1, top row), through most specific diagnostic codes (level 4, bottom row). Level 3 diagnoses were used in the current study.

including all subcodes within those categories). In the Meuhedet system, all children with suspected ASD underwent evaluation by a panel of social workers, a psychologist, and either a trained psychiatrist, a developmental-behavioral pediatrician, or a child neurologist. The final diagnosis was made by a board-certified developmental-behavioral pediatrician. All children were followed up until 26 January 2015, first ASD diagnosis, or death, whichever occurred first. Earlier analyses have demonstrated that age-specific ASD prevalence in this sample matches that reported previously in Israel (Janecka et al., 2018).

### Covariates

In all adjusted models, the covariates included child's year of birth, maternal age at child's birth, number of all maternal diagnoses during the exposure period (636 days prior to child's date of birth), and residential socioeconomic status (SES). Child's year of birth was included in the models in order to account for the temporal changes in ASD diagnosis (Davidovitch, Hemo, Manning-Courtney, & Fombonne, 2013), as well as other possible temporal effects influencing the patterns of maternal diagnoses. Maternal age at child's birth – to account for differential ASD risk observed across different maternal age categories (Janecka et al., 2019; Sandin et al., 2012), and the age-dependent nature of many maternal medical diagnoses. SES – to account for factors that could contribute to differential healthcare utilization, impacting the probabilities of the diagnosis of both any maternal condition and ASD (Davidovitch et al., 2013). Finally, the number of maternal diagnoses in the exposure period – to account for differential utilization of healthcare around pregnancy, which influences the likelihood of maternal receipt of any diagnosis (we have also shown that this number and is also associated with ASD risk (Janecka et al., 2018; Rahman et al., 2020)). Information about all covariates was obtained from the Meuhedet records, except for SES, which was obtained from the Central Bureau of Statistics Registry (Demographic Characteristics of the Population in Localities and Statistical Areas, 1995). Residential SES was derived from household census data and represented an index of the number of electrical appliances per person and per capita income. In unadjusted (crude) models, the only covariate was child's year of birth.

### Statistical analysis

All analyses were done using R software, version 3.6.3 (R Core Team, 2014). The relative risk of ASD and the associated two-

sided 95% confidence intervals were estimated by the hazard ratios (HRs) from Cox proportional hazard regression models using *survival* package (Therneau & Li, 1999). Proportional hazard assumption was examined using standardized Schoenfeld residuals (Grambsch & Therneau, 1994). In all models, we adjusted for within-family correlation of data, arising due to the presence of siblings in the dataset, by calculating robust standard error estimates (Barlow, 1994).

We applied inverse probability weighting to account for the sample ascertainment procedures, which were based on both the ASD status, and family size (i.e. due to inclusion of siblings of the randomly selected 19.5% individuals, larger families were more likely to be ascertained). Each individual was assigned a weight. All ASD cases and their siblings received a weight of 1, even when, due to the random subcohort sampling, they were also sampled into the subcohort. For other control individuals in the subcohort that weight was always higher, reflecting a lower probability of being included in the sample for those individuals; the exact weight given to those individuals was dependent on the family size, with individuals from smaller families receiving higher regression weights (see online Supplementary Table S2).

**Filtering of potential false-positive findings.** Statistical analyses of the associations between maternal diagnoses and ASD risk in offspring were conducted in four steps in order to remove the associations with a high likelihood of representing Type I errors.

First, we filtered out maternal diagnoses recorded in fewer than 10 cases and 10 controls. This is due to the instability of the maximum likelihood regression coefficients when the number of events per variable is small – a problem known as sparse data bias (Greenland, Mansournia, & Altman, 2016). Imposing the cut-off of 10 events per variable has been shown as suitable to eliminate such bias (Peduzzi, Concato, Kemper, Halford, & Feinstein, 1996).

Threshold for nominal statistical significance for analytical  $p$  values was set at 0.05. For each of the nominally significant diagnoses in Cox proportional hazards model, we performed permutation testing. Such tests allow the estimation of the probability of observing any given association under the null, i.e. in the absence of a real association between exposure and outcome (Ludbrook & Dudley, 1998). The rate of ASD, relationship between the follow-up time and ASD status, and correlations between the exposure and covariates in each permuted dataset remained the same as in the original dataset; however, the original associations between ASD and maternal exposure were broken in the permutations – allowing estimation of regression coefficients

expected under the null. We performed 1000 permutations of each nominally significant association, in order to determine empirical  $p$  values [defined as the proportion of tests producing a  $p$  value equal to, or smaller than the analytical  $p$  value (i.e. in the non-permuted dataset)]. To validate the analytical  $p$  value derived from the analyses in the original dataset, we required that at least 95% of the  $p$  values observed after permutations are higher than that analytical  $p$  value, i.e. producing an empirical  $p$  value  $<0.05$ .

Due to the multitude of tests being performed (equal to the number of distinct maternal diagnoses), we then used false discovery rate (FDR) correction for multiple testing (Benjamini & Hochberg, 1995) on empirical  $p$  values. The permissible FDR ( $Q$ -value) was set at 0.05.

Finally, all diagnoses that remained significant after the FDR correction were entered into a joint multiple regression model to account for possible correlation between those diagnoses. The model specifications were analogous to those used in initial adjusted univariate regressions, as described above. An outline of the analytical framework is presented in online Supplementary Fig. S1. Throughout the Results section, we refer to single-diagnosis models adjusted only for the child's year of birth as 'crude models'; to single-diagnosis models, adjusted for the full set of covariates as 'adjusted univariate'; and to multiple-diagnoses models, adjusted for the full set of covariates as 'multiple regression'.

**Missing data and sensitivity analyses.** In order to evaluate the effects of missing covariate data on the results, we verified if the covariate missingness was related to the study variables, as well as the consistency of the results in the data with and without missing data imputed using multivariate imputation by chained equation, implemented in the *mice* package (Zhang, 2016). We used full outcome (ASD diagnosis, and time to diagnosis) and covariate information [maternal age and number of diagnoses in pregnancy; child's date of birth; SES; number of siblings (used for weights' calculation)], as well as auxiliary variables (child's sex and paternal age) to perform five imputations of the dataset. None of the variables was transformed prior to the imputation process.

Earlier studies have suggested high levels of comorbidity between ASD and other psychiatric and physical conditions (Mannion & Leader, 2013). In order to ensure that the associations observed in our study do not reflect comorbidities in mothers with ASD, we re-analyzed the data after exclusion of all families where the mother herself had a recorded ASD diagnosis.

## Results

There were 88 713 children in the Meuhedet dataset born 1999–2008. Of those, 23 individuals were removed from the sample due to being born to mothers younger than 13 or older than 55, or fathers older than 55. Additional 8503 individuals had missing SES data, and no individuals were removed due to missing information on any other covariates - producing a final analytical sample of 80 187 children (90% of the 1999–2008 birth cohort; see the population flowchart in online Supplementary Fig. S2), born to 30 864 mothers. There were 1132 children diagnosed with ASD, and 79 055 controls, with the median age at the end of follow-up 11.2 (IQR: 8.8–13.6). We identified five mothers of children in the cohort who themselves received an ASD diagnosis during the follow-up period. Sample characteristics are presented in Table 1 (see online Supplementary Table S3 for the characteristics of the sample before exclusions, as well as of the excluded subset of children).

**Table 1.** Analytical sample demographic characteristics ( $N = 80\,193$ )

	Controls ( $N = 79\,055$ )	ASD ( $N = 1132$ )
% Female	49.2	20.1
Mean SES (s.d.)	7.6 (4.3)	10.3 (4.2)
Mean maternal age (s.d.)	29.8 (5.4)	30.0 (5.4)
Mean paternal age (s.d.)	32.4 (6.1)	32.9 (6.2)
N children in the dataset born each year (% total sample)		
1999	7991 (10.1%)	88 (7.8%)
2000	8068 (10.2%)	91 (8.0%)
2001	8161 (10.3%)	111 (9.8%)
2002	8431 (10.7%)	114 (10.1%)
2003	8555 (10.8%)	145 (12.8%)
2004	8451 (10.7%)	153 (13.5%)
2005	8162 (10.3%)	135 (11.9%)
2006	7824 (9.9%)	141 (12.5%)
2007	8057 (10.2%)	126 (11.1%)
2008	5335 (6.8%)	28 (2.5%)
% Mothers with ASD	<0.1	0.3

## Associations between maternal diagnoses and offspring ASD

There were 822 distinct level 3 ICD-9 diagnoses recorded in women during pregnancy and the preceding year in the Meuhedet records (online Supplementary Fig. S3). Of those, 453 maternal diagnoses were nominally statistically significantly associated with offspring ASD risk (online Supplementary Fig. S4). Most of these associations were between ASD and very rare maternal diagnoses, which in our sample were recorded in mothers of very few controls, and none of the cases; consequently, majority of these associations indicated a negative association between maternal diagnosis and ASD (point estimate  $HR < 0.01$ ; online Supplementary Fig. S5), likely reflecting high rates of Type I error when testing such rare exposures. Only 52 (11%) of those 453 diagnoses were associated with an increased risk of ASD (point estimate  $HR > 1$ ; online Supplementary Fig. S6).

After filtering out diagnoses recorded in mothers of less than 10 cases or 10 controls, due to unreliability of the regression coefficients for sparse predictors, we retained 148 maternal diagnoses in the dataset. Among those, 22 were nominally significantly associated with offspring ASD (Table 2 and Fig. 2; see online Supplementary Table S4 for all results), distributed across a broad range of diagnostic categories (Fig. 3a). Fourteen of those diagnoses (64%) were associated with an increased risk of ASD [ $HR > 1$ ; cf. 42% in unadjusted models (online Supplementary Table S5)]. Frequencies of those diagnoses in cases and controls are presented in online Supplementary Table S6.

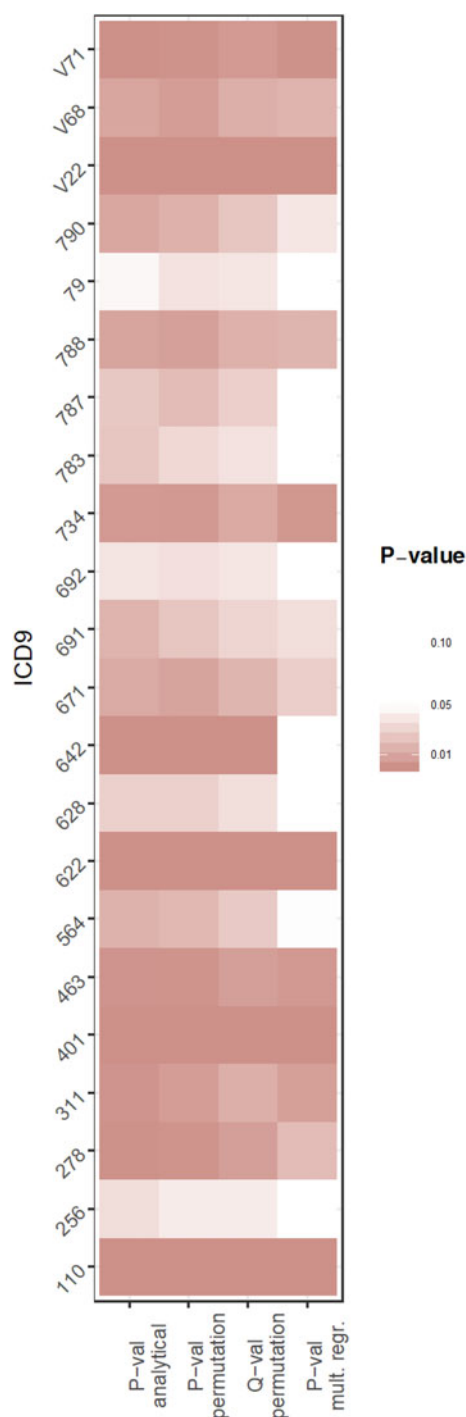
Permutation testing revealed that none of those 22 associations could arise under the null (empirical  $p$  value  $<0.05$ ), and all of them remained significant after controlling for multiple testing. In the final multiple regression step, 16 of those diagnoses remained significantly associated with ASD (Table 2 and



**Table 2.** Top associations between maternal diagnoses and offspring ASD, ordered by the *p* values from adjusted univariate models

ICD-9	Diagnosis	Crude HR (CI)	Adjusted HR (CI) <sup>a</sup>	Analytical <i>p</i> value <sup>a</sup>	Empirical <i>p</i> value <sup>a</sup>	Multiple regression <i>p</i> value	Exposed(n)	
							ASD	Control
<b>V22</b>	Supervision of normal pregnancy	0.62 (0.54–0.72)	0.62 (0.54–0.71)	$1.80 \times 10^{-11}$	0	$4.71 \times 10^{-10}$	827	62483
<b>401</b>	Essential hypertension	2.17 (1.54–3.07)	2.74 (1.92–3.90)	$2.43 \times 10^{-08}$	0	$3.62 \times 10^{-05}$	36	958
<b>110</b>	Dermatophytosis	0.55 (0.42–0.72)	0.57 (0.43–0.74)	$3.13 \times 10^{-05}$	0	$9.35 \times 10^{-05}$	59	6904
<b>622</b>	Non-inflammatory disorders of cervix	1.99 (1.46–2.71)	1.88 (1.38–2.57)	$7.06 \times 10^{-05}$	0	$1.82 \times 10^{-04}$	44	1208
<b>V71</b>	Observation and evaluation for suspected conditions not found	1.54 (1.24–1.92)	1.53 (1.23–1.91)	$1.66 \times 10^{-04}$	$1.00 \times 10^{-3}$	$5.37 \times 10^{-04}$	98	4231
<b>642</b>	Hypertension complicating pregnancy childbirth and the puerperium	1.64 (1.21–2.22)	1.77 (1.30–2.42)	$2.91 \times 10^{-04}$	0	$5.00 \times 10^{-02}$	44	1549
<b>278</b>	Overweight, obesity and other hyperalimentation	1.49 (1.11–2.00)	1.69 (1.26–2.27)	$5.49 \times 10^{-04}$	$2.00 \times 10^{-3}$	$2.00 \times 10^{-02}$	53	2264
<b>463</b>	Acute tonsillitis	0.75 (0.64–0.88)	0.78 (0.67–0.91)	$1.58 \times 10^{-03}$	$2.00 \times 10^{-3}$	$3.90 \times 10^{-03}$	196	16962
<b>311</b>	Depressive disorder, not elsewhere classified	1.81 (1.14–2.86)	2.11 (1.32–3.35)	$1.70 \times 10^{-03}$	$6.00 \times 10^{-3}$	$6.92 \times 10^{-03}$	19	652
<b>734</b>	Flat foot	1.73 (1.04–2.88)	2.10 (1.26–3.52)	$4.53 \times 10^{-03}$	$4.00 \times 10^{-3}$	$3.28 \times 10^{-03}$	15	710
<b>788</b>	Symptoms involving urinary system	1.24 (0.96–1.59)	1.40 (1.09–1.80)	$9.47 \times 10^{-03}$	$7.00 \times 10^{-3}$	$1.67 \times 10^{-02}$	68	3504
<b>V68</b>	Encounters for administrative purposes	0.72 (0.58–0.89)	0.76 (0.61–0.93)	$9.65 \times 10^{-03}$	$6.00 \times 10^{-3}$	$1.63 \times 10^{-02}$	100	10611
<b>790</b>	Non-specific findings on examination of blood	1.48 (1.03–2.13)	1.60 (1.12–2.29)	$1.04 \times 10^{-02}$	$1.50 \times 10^{-2}$	$3.89 \times 10^{-02}$	35	1654
<b>671</b>	Venous complications in pregnancy and the puerperium	0.43 (0.27–0.67)	0.56 (0.36–0.88)	$1.19 \times 10^{-02}$	$9.00 \times 10^{-3}$	$2.82 \times 10^{-02}$	21	4153
<b>564</b>	Functional digestive disorders, not elsewhere classified	1.31 (1.00–1.73)	1.41 (1.07–1.85)	$1.60 \times 10^{-02}$	$1.80 \times 10^{-2}$	$4.91 \times 10^{-02}$	57	2688
<b>691</b>	Atopic dermatitis and related conditions	0.46 (0.26–0.82)	0.50 (0.28–0.88)	$1.62 \times 10^{-02}$	$2.40 \times 10^{-2}$	$3.54 \times 10^{-02}$	12	1823
<b>783</b>	Symptoms concerning nutrition metabolism and development	1.56 (1.04–2.35)	1.60 (1.06–2.41)	$2.46 \times 10^{-02}$	$3.30 \times 10^{-2}$	$6.71 \times 10^{-02}$	24	1010
<b>787</b>	Symptoms involving digestive system	1.23 (1.00–1.51)	1.27 (1.03–1.56)	$2.58 \times 10^{-02}$	$2.00 \times 10^{-2}$	$7.32 \times 10^{-02}$	106	5171
<b>628</b>	Female infertility	1.27 (1.07–1.51)	1.22 (1.02–1.45)	$3.07 \times 10^{-02}$	$2.90 \times 10^{-2}$	$1.35 \times 10^{-01}$	161	7084
<b>256</b>	Ovarian dysfunction	1.76 (1.16–2.68)	1.59 (1.03–2.44)	$3.58 \times 10^{-02}$	$4.10 \times 10^{-2}$	$2.15 \times 10^{-01}$	23	668
<b>692</b>	Contact dermatitis and other eczema	0.75 (0.61–0.94)	0.79 (0.64–0.99)	$3.77 \times 10^{-02}$	$3.60 \times 10^{-2}$	$1.46 \times 10^{-01}$	95	8120
<b>79</b>	Viral and chlamydial infection in conditions classified elsewhere and of unspecified site	0.92 (0.77–1.10)	0.84 (0.70–1.00)	$4.62 \times 10^{-02}$	$3.70 \times 10^{-2}$	$6.08 \times 10^{-02}$	156	10386

<sup>a</sup>Univariate models, adjusted for maternal age at child's birth, child's year of birth, SES, and total number of diagnoses in pregnancy.



**Fig. 2.** Diagnoses (ICD-9) nominally associated with ASD through the consecutive stages of filtering.

Fig. 3b; online Supplementary S6), including e.g. essential hypertension ( $p = 3.62 \times 10^{-05}$ ), non-inflammatory disorders of the cervix ( $p = 1.82 \times 10^{-04}$ ), and depressive disorder ( $p = 6.92 \times 10^{-03}$ ). The Schoenfeld residuals did not indicate a violation of the proportional hazards assumption (online Supplementary Table S7 and Fig. S8).

Online Supplementary Table S8 shows the counts of all diagnoses recorded in at least 10 cases and 10 controls, as well as their counts significant in the consecutive filtering stages, categorized by broad (level 1) ICD-9 categories.

### Sensitivity analyses

All results remained virtually unchanged after exclusion of women with recorded ASD diagnosis (online Supplementary Table S9;  $n_{\text{mothers}} = 5$  and  $n_{\text{children}} = 13$  were excluded).

Missing information in our study was limited to the SES variable, and was related to the ASD diagnosis (10% missing among the controls, 15% among ASD cases), validating our approach to re-analyze the data after the SES imputation. We observed a very close alignment between the effect sizes and levels of significance observed when using models with and without data imputation (respectively,  $N = 88\,690$  and  $N = 80\,187$ ), suggesting data missingness had no substantial effects on the study results (online Supplementary Table S10).

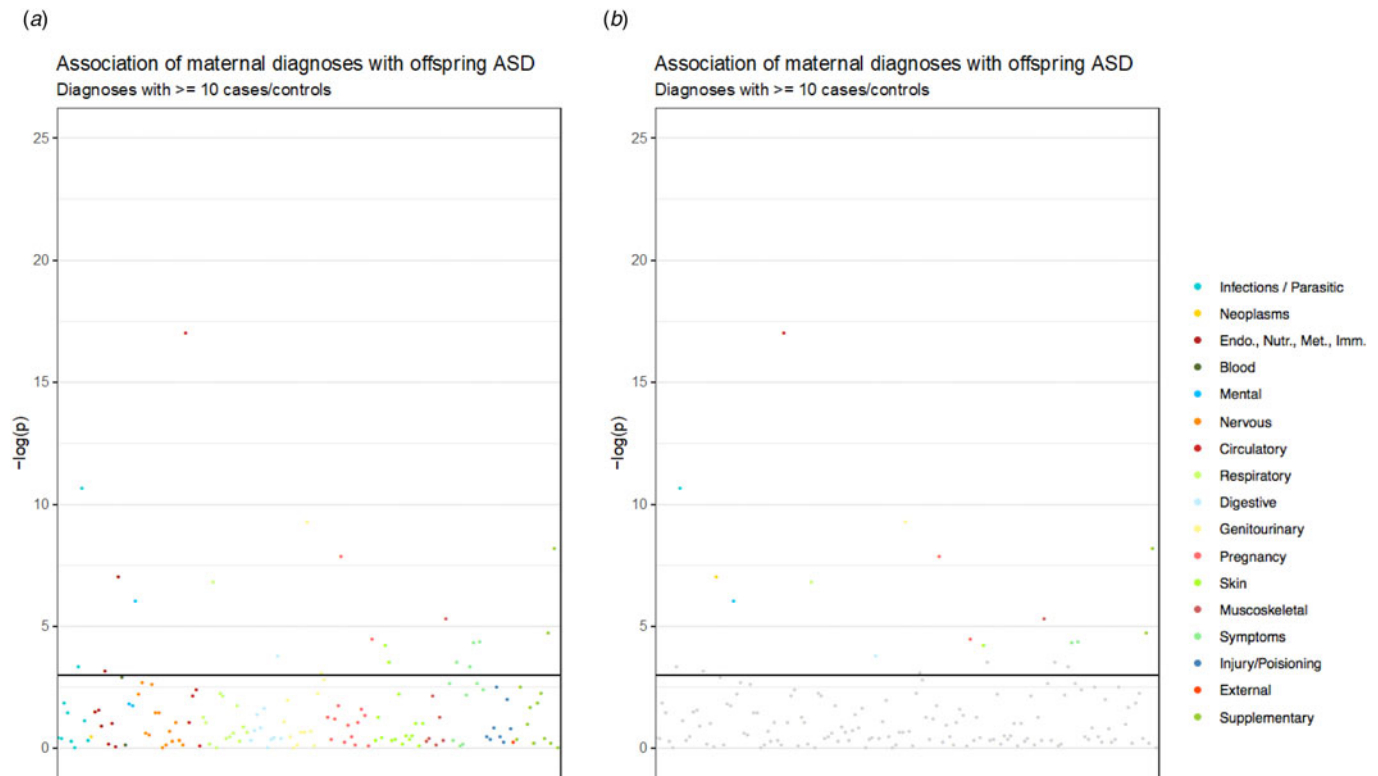
### Discussion

Using our approach, we screened over 820 maternal ICD-9 diagnoses around pregnancy for an association with ASD, and through an iterative process of identifying likely false-positive associations, we identified 16 maternal diagnoses with robust evidence for an association with offspring ASD. Those 16 diagnoses that passed through all filtering stages included previously reported ASD risk factors, e.g. depression (Rai et al., 2013) and hypertension (Gardener, Spiegelman, & Buka, 2009). In addition, our systematic approach allowed us to identify also novel associations that warrant further attention in ASD research, e.g. non-inflammatory disorders of the cervix, and urinary symptoms.

The mechanisms underlying these associations remain to be elucidated. Advancing our knowledge about the etiology of ASD will require careful consideration of potential causal factors underlying these associations, including e.g. shared genetic effects (whereby maternal genetic variation transmitted to offspring contributes to the risk of both maternal diagnosis and offspring ASD), maternal use of medication, or socioeconomic factors associated with receiving specific diagnoses. For example, apparent 'protective' effects ( $HRs < 1$ ) observed in our study likely reflect differential utilization of healthcare – whereby women with more medical supervision during pregnancy were less likely to have a child with ASD, but through their frequent medical contacts, were also more likely to receive relatively minor diagnoses (e.g. acute tonsillitis or venous complications of pregnancy). In support of this, (i) we observed that mothers of children with ASD in this sample were less likely to attend a prenatal care appointment or see a healthcare professional for administrative purposes (ICD-9 V22, V68), and (ii) adjusting for the total number of diagnoses received during the exposure period as well as SES – both indexing healthcare utilization – removed mostly the associations with  $HRs < 1$ .

Therefore, interpreting our results – and other findings on this topic that rely on registry data – it is important to bear in mind that they do not necessarily reflect causal relationships. In order to better understand the etiology of ASD, identifying maternal diagnoses associated with an increased risk of the disorder needs to be followed by careful examination of the causal factors driving the association, taking into account genetic and social determinants of health and disease, and other factors associated with receiving a medical diagnosis (e.g. medication).

From the methodological standpoint, the systematic nature of our approach allowed comparisons of risk estimates (HRs) and the strength of the evidence for an association ( $p$  value) across a wide range of diagnoses, using a unified analytical process.



**Fig. 3.**  $p$  Values of the associations between maternal diagnoses and ASD, for diagnoses recorded in at least 10 cases and 10 controls. Each dot represents a  $-\log(p)$  value of an association between a distinct maternal diagnosis (level 3 of the ICD-9 codes) and offspring ASD. The results are color-coded by broad ICD-9 categories (level 1), as shown in the legend. Figure 3a represents  $p$  values for all diagnoses recorded in at least 10 cases and 10 controls; Fig. 3b shows the same results, but the diagnoses that were filtered out in permutation testing or subsequent control for multiple testing are shown in grey (N.B. the diagnoses that were non-significant in the multiple regression step are not shown in grey).

The key analytical advantages of such an approach are (i) facilitated synthesis of information regarding ASD risk factors pertaining to maternal diagnoses, compared to pooling of many individual studies, each investigating single/few diagnoses, and (ii) limiting false-positive findings through explicit acknowledgement of, and addressing the issue of multiple testing.

While our study represents a novel approach for analyzing associations between maternal diagnoses around pregnancy and ASD, its limitations need to be acknowledged. Importantly, the definition of the exposure window in our study (pregnancy and one year preceding it) likely did not allow us to ascertain the associations between ASD and maternal chronic diagnoses, particularly those that are well-managed and do not necessitate frequent contacts with healthcare professionals (e.g. diabetes). Therefore, we do not propose that the list of associations detected by us is exhaustive. Likewise, the results need to be interpreted as specific to the sample at hand, and further validation should be sought in other cohorts. Rates of ASD differ by country (Delobel-Ayoub et al., 2020) – with prevalence in Israel [0.5% (Davidovitch et al., 2013)] representing a relatively low estimate compared to the USA [1.7% (Baio et al., 2018)] – as likely do patterns of maternal morbidity in pregnancy. Replication of the observed associations in other cohorts will be thus crucial for the determination of the robustness of the associations reported here, an essential step prior to causal analysis. Owing to the left-censoring of the data, as well as the evidence of under-diagnosis of ASD in the past (Rutter, 2005), likely we could not identify all mothers with ASD in the sample. As a result, the sensitivity analyses could fail to fully account for the effects of comorbidities

in ASD females. Similarly, lack of emigration data for the HMO members precluded accurate right-censoring of the data for the individuals who became unavailable for follow-up prior to 26 January 2015. Furthermore, exposure in our study was defined as the presence of at least one record of a given diagnosis during the exposure period, rather than two, as reported in some other studies (e.g. Razaz, Tomson, Wikström, & Cnattingius, 2017). While this could introduce some fraction of false positives (non-exposed children classified as exposed, e.g. due to administrative errors), our study focused on relatively common diagnoses where those would have limited influence; additionally, false negatives (exposed children classified as not exposed) would be more likely in a study with a relatively narrow exposure period, compromising statistical power. Finally, we did not have access to information allowing to estimate family-level SES, and thus could only control for residential measures of SES. While those two are expected to strongly correlate, this correlation will not be perfect, and thus the SES adjustment in our study should be considered incomplete.

In conclusion, our work applied a novel approach for identifying associations between maternal diagnoses around pregnancy and offspring ASD, consisting of systematic testing of a wide range of maternal diagnoses, followed by a series of validation steps to filter out the likely false-positive associations. We showed that such an approach replicates many of the previously reported ASD maternal clinical risk factors, and identifies novel associations, warranting replication and follow-up in future studies designed to elucidate the underlying causal mechanisms.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0033291721001021>

**Data.** The data underlying this article cannot be shared publicly due to concerns for the privacy of individuals that participated in the study. Interested researchers should discuss access options with Arad Kodesh and Stephen Z. Levine.

**Code.** All code is available upon request from the corresponding author.

**Acknowledgements.** We would like to acknowledge the generous support of the Seaver Foundation.

**Authors contributions.** MJ designed the study, planned the statistical analyses, assisted in carrying out the analyses, was involved in results interpretation, manuscript write-up, and revisions. AK and SZL provided the data, assisted in carrying out the analyses, were involved in results interpretation, manuscript write-up, and revisions. VK, RR, AS, PFO, JG, DS, LC, and AR contributed to the study design, were involved in results interpretation, manuscript write-up, and revisions. SS contributed to the study design, data analysis, was involved in results interpretation, manuscript write-up, and revisions. All authors approved the manuscript version to be published.

**Financial support.** This work was supported in part by a grant from the Eunice Kennedy Shriver National Institute of Child Health and Human Development, the National Institute of Environmental Health Sciences, and the National Institute of Neurological Disorders and Stroke (Dr Reichenberg, Dr Kodesh, Dr Levine [grant number HD 073978]); by a grant from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (Dr Reichenberg, Dr Sandin, Dr Schendel, Dr Buxbaum [grant number HD098883]); by a grant from the National Institute of Mental Health (Dr Janecka, Dr Reichenberg, Dr Sandin, Dr Schendel, Dr Grove, Dr Croen [grant number MH124817]); and by T32 award from the National Institute of Mental Health to Dr Khachadourian [grant number MH122394]. The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**Conflict of interest.** None.

## References

- Acosta, O., Ramirez, V., Powell, L., Jansson, T., Lager, S., Gaccioli, F., ... Jansson, T. (2015). Increased glucose and placental GLUT-1 in large infants of obese nondiabetic mothers. *American Journal of Obstetrics and Gynecology*, *212*(2), 227e1–227e7.
- Atladóttir, H. Ó., Pedersen, M. G., Thorsen, P., Mortensen, P. B., Deleuran, B., Eaton, W. W., & Parner, E. T. (2009). Association of family history of autoimmune diseases and autism spectrum disorders. *Pediatrics*, *124*(2), 687–694.
- Atladóttir, H. Ó., Thorsen, P., Østergaard, L., Schendel, D. E., Lemcke, S., Abdallah, M., & Parner, E. T. (2010). Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. *Journal of Autism and Developmental Disorders*, *40*(12), 1423–1430. <https://doi.org/10.1007/s10803-010-1006-y>.
- Bai, D., Hon, B., Yip, K., Windham, G. C., Sourander, A., Francis, R., ... Sandin, S. (2019). Association of genetic and environmental factors with autism in a 5-country cohort. *JAMA Psychiatry*, *76*(10), 1035–1043. <https://doi.org/10.1001/jamapsychiatry.2019.1411>.
- Baio, J., Wiggins, L., Christensen, D., Maenner, M., Daniels, J., Warren, Z., ... Dowling, N. (2018). Prevalence of autism spectrum disorder among children aged 8 years – autism and developmental disabilities monitoring network, 11 sites, United States, 2014. *MMWR Surveillance Summaries*, *67*(6), 1–23.
- Barlow, W. (1994). Robust variance estimation for the case-cohort design. *Biometrics*, *50*(4), 1064–1072.
- Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society*, *157*(1), 289–300.
- Castanha, P. M. S., Braga, C., Cordeiro, M. T., Souza, A. I., Jr, C. D. S., Martelli, C. M. T., ... Marques, E. T. A. (2016). Placental transfer of dengue virus (DENV) – specific antibodies and kinetics of DENV infection – enhancing activity in Brazilian infants. *The Journal of Infectious Diseases*, *214*, 265–272. <https://doi.org/10.1093/infdis/jiw143>.
- Chan, S. Y., Vasilopoulou, E., & Kilby, M. D. (2009). The role of the placenta in thyroid hormone delivery to the fetus. *Nature Endocrinology*, *5*, 45–54.
- Croen, L., Grether, J., Yoshida, C., Odouli, R., & Van de Water, J. (2005). Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders. *Archives of Pediatrics & Adolescent Medicine*, *159*, 151–157.
- Dancis, J., Jansen, V., & Levitz, M. (1980). Placental transfer of steroids: Effect of binding to serum albumin and to placenta. *American Physiological Society*, *7*, 208–213.
- Davidovitch, M., Hemo, B., Manning-Courtney, P., & Fombonne, E. (2013). Prevalence and incidence of autism spectrum disorder in an Israeli population. *Journal of Autism and Developmental Disorders*, *43*(4), 785–793. <https://doi.org/10.1007/s10803-012-1611-z>.
- Delobel-Ayoub, M., Saemundsen, E., Ego, A., Moilanen, I., Ebeling, H., Rafnsson, V., ... Schendel, D. (2020). Prevalence of autism spectrum disorder in 7–9-year-old children in Denmark, Finland, France and Iceland: A population-based registries approach within the ASDEU project. *Journal of Autism and Developmental Disorders*, *50*, 949–959.
- Demographic Characteristics of the Population in Localities and Statistical Areas. (1995) Jerusalem: State of Israel, Central Bureau of Statistics.
- Gardener, H., Spiegelman, D., & Buka, S. L. (2009). Prenatal risk factors for autism: Comprehensive meta-analysis. *The British Journal of Psychiatry: The Journal of Mental Science*, *195*(1), 7–14. <https://doi.org/10.1192/bjp.bp.108.051672>.
- Grambsch, P., & Therneau, T. (1994). Proportional hazard tests and diagnostics based on weight residuals. *Biometrika*, *81*(3), 515–526.
- Greenland, S., Mansournia, M. A., & Altman, D. G. (2016). Sparse data bias: A problem hiding in plain sight. *BMJ (Online)*, *353*, 1–6. <https://doi.org/10.1136/bmj.i1981>.
- ICD10Data. (2021). Convert ICD-9-CM codes to ICD-10-CM/PCS, or convert ICD-10-CM/PCS codes to ICD-9-CM.
- Janecka, M., Hansen, S. N., Modabbernia, A., Browne, H. A., Buxbaum, J. D., Schendel, D. E., ... Grice, D. E. (2019). Parental age and differential estimates of risk for neuropsychiatric disorders: Findings from the Danish birth cohort. *Journal of American Academy of Child Psychiatry*. <https://doi.org/https://doi.org/10.1016/j.jaac.2018.09.447>.
- Janecka, M., Kodesh, A., Levine, S. Z., Lusskin, S. I., Viktorin, A., Rahman, R., ... Reichenberg, A. (2018). Association of autism spectrum disorder with prenatal exposure to medication affecting neurotransmitter systems. *JAMA Psychiatry*, *10029*(12), 1217–1224. <https://doi.org/10.1001/jamapsychiatry.2018.2728>.
- Kohler, P., & Farr, R. (1966). Placental IgG transport. *Nature*, *210*(5040), 1070–1071.
- Levine, S. Z., Kodesh, A., Viktorin, A., Smith, L., Uher, R., Reichenberg, A., & Sandin, S. (2018). Association of maternal use of folic acid and multivitamin supplements in the periods before and during pregnancy with the risk of autism spectrum disorder in offspring. *JAMA Psychiatry*, *75*(2), 176–184. <https://doi.org/10.1001/jamapsychiatry.2017.4050>.
- Ludbrook, J., & Dudley, H. (1998). Why permutation tests are superior to t and F tests in biomedical research. *American Statistician*, *52*(2), 127–132. <https://doi.org/10.1080/00031305.1998.10480551>.
- Mahadevan, U., Wolf, D. C., Dubinsky, M., Cortot, A., Lee, S., Siegel, C. A., ... Abreu, M. (2013). Placental transfer of anti-tumor necrosis factor agents in pregnant patients with inflammatory bowel disease. *Clinical Gastroenterology and Hepatology*, *11*(3), 286–292.
- Mannion, A., & Leader, G. (2013). Comorbidity in autism spectrum disorder: A literature review. *Research in Autism Spectrum Disorders*, *7*(12), 1595–1616. <https://doi.org/10.1016/j.rasd.2013.09.006>.
- Palmeira, P., Quinello, C., Ana, L., Zago, A., & Carneiro-sampaio, M. (2012). IgG placental transfer in healthy and pathological pregnancies. *Clinical and Developmental Immunology*, *2012*, 1–13. <https://doi.org/10.1155/2012/985646>.
- Peduzzi, P., Concato, J., Kemper, E., Halford, T. R., & Feinstein, A. R. (1996). A simulation study of the number of events per variable recommended in



- multivariable regression analyses. *Journal of Clinical Epidemiology*, 49(12), 1373–1379. [https://doi.org/10.1016/0197-2456\(93\)90084-q](https://doi.org/10.1016/0197-2456(93)90084-q).
- Rahman, R., Kodesh, A., Levine, S. Z., Sandin, S., Reichenberg, A., & Schlessinger, A. (2020). Identification of newborns at risk for autism using electronic medical records and machine learning. *European Psychiatry*, 63(1), e22. <https://doi.org/10.1192/j.eurpsy.2020.17>.
- Rai, D., Lee, B. K., Dalman, C., Golding, J., Lewis, G., & Magnusson, C. (2013). Parental depression, maternal antidepressant use during pregnancy, and risk of autism spectrum disorders: Population based case-control study. *BMJ (Clinical Research Ed.)*, 346(April), f2059. <https://doi.org/10.1136/bmj.f2059>.
- Razaz, N., Tomson, T., Wikström, A. K., & Cnattingius, S. (2017). Association between pregnancy and perinatal outcomes among women with epilepsy. *JAMA Neurology*, 74(8), 983–991. <https://doi.org/10.1001/jamaneurol.2017.1310>.
- R Core Team. (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing. <http://www.r-project.org/>.
- Rutter, M. (2005). Incidence of autism spectrum disorders: Changes over time and their meaning. *Acta Paediatrica, International Journal of Paediatrics*, 94(1), 2–15. <https://doi.org/10.1080/08035250410023124>.
- Sandin, S., Hultman, C. M., Kolevzon, A., Gross, R., MacCabe, J. H., & Reichenberg, A. (2012). Advancing maternal age is associated with increasing risk for autism: A review and meta-analysis. *Journal of the American Academy of Child and Adolescent Psychiatry*, 51(5), 477–486.e1. <https://doi.org/10.1016/j.jaac.2012.02.018>.
- Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Larsson, H., Hultman, C. M., & Reichenberg, A. (2014). The familial risk of autism. *JAMA : The Journal of the American Medical Association*, 311(17), 1770–1777. <https://doi.org/10.1001/jama.2014.4144>.
- Therneau, T. M., & Li, H. (1999). Computing the Cox model for case cohort designs. *Lifetime Data Analysis*, 5(2), 99–112. <https://doi.org/10.1023/A:1009691327335>.
- Viktorin, A., Uher, R., Reichenberg, A., Levine, S. Z., & Sandin, S. (2017). Autism risk following antidepressant medication during pregnancy. *Psychological Medicine*, 47(16), 2787–2796. <https://doi.org/10.1017/S0033291717001301>.
- Walker, N., Filis, P., Shaughnessy, P. J. O., Bellingham, M., & Fowler, P. A. (2019). Nutrient transporter expression in both the placenta and fetal liver are affected by maternal smoking. *Placenta*, 78(September 2018), 10–17. <https://doi.org/10.1016/j.placenta.2019.02.010>.
- Wiggins, L. D., Baio, J., & Rice, C. (2006). Examination of the time between first evaluation and first autism spectrum diagnosis in a population-based sample. *Journal of Developmental and Behavioral Pediatrics*, 27(2), 79–87. <https://doi.org/10.1097/00004703-200604002-00005>.
- Xiang, A. H., Wang, X., Martinez, M. P., Walthall, J. C., Curry, E. S., Page, K., ... Getahun, D. (2019). Association of maternal diabetes with autism in offspring. *JAMA*, 91101(14), 1425–1434. <https://doi.org/10.1001/jama.2015.2707>.
- Zhang, Z. (2016). Multiple imputation with multivariate imputation by chained equation (MICE) package. *Annals of Translational Medicine*, 4(2), 30.