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Prenatal toxoplasmosis antibody and childhood autism

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Scientific Abstract

There is evidence that some maternal infections during the prenatal period are associated with neurodevelopmental disorders, such as childhood autism. However, the association between autism and *Toxoplasma gondii* (*T. gondii*), an intracellular parasite, remains unclear. The authors examined whether serologically confirmed maternal antibodies to *T. gondii* are associated with odds of childhood autism in offspring. The study is based on a nested case-control design of a large national birth cohort (N=1.2 million) and the national psychiatric registries in Finland. There were 874 cases of childhood autism and controls matched 1:1 on date of birth, sex, birthplace and residence in Finland. Maternal sera were prospectively assayed from a national biobank for *T. gondii* IgM and IgG antibodies; IgG avidity analyses were also performed. High maternal *T. gondii* IgM antibody was associated with a significantly decreased odds of childhood autism. Low maternal *T. gondii* IgG antibody was associated with increased offspring odds of autism. In women with high *T. gondii* IgM antibodies, the IgG avidity was high for both cases and controls, with the exception of three controls. The findings suggest that the relationship between maternal *T. gondii* antibodies and odds of childhood autism may be related to the immune response to this pathogen or the overall activation of the immune system.

Lay Abstract

Some maternal infections during the prenatal period have been associated with childhood autism. However, the association between autism and *Toxoplasma gondii* (*T. gondii*), an intracellular parasite, remains unclear. The authors examined whether maternal antibodies to *T. gondii* are associated with odds of childhood autism in offspring. The study, which is based on a large national birth cohort and the psychiatric registries in Finland, included 874 cases of childhood

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autism and matched controls. Maternal sera were prospectively assayed from a national biobank for *T. gondii* specific IgM and IgG antibodies. In order to determine the timing of *T. gondii* infection, IgG avidity analyses were performed on all IgM seropositive samples. The findings suggest that high *T. gondii* specific IgM levels in pregnant women may be associated with a decrease in offspring odds of autism, while low IgG levels may be related to increased odds of this disorder. In women with high *T. gondii* IgM antibodies, the IgG avidity was high for both cases and controls, with the exception of three controls, indicating past infection. While further studies are necessary to replicate this finding in independent birth cohorts these studies have the potential for improving our understanding of developmental pathways to childhood autism.

Keywords

Toxoplasmosis; Autism; antibody; childhood

Introduction

Autism is a neurodevelopmental disorder that has been associated with several genetic variants and with environmental factors originating with the mother and the child. Several studies suggest that maternal infections or disruptions in the immune system during the pre-/perinatal period may increase risk of autism (Lee et al., 2014; Brown et al., 2014; Meldrum et al., 2013; Zerbo et al., 2014). Given the effects of congenital *Toxoplasma gondii* (*T. gondii*), an intracellular parasite, on early brain development (Berrebi et al., 2010; Roizen et al., 2006; Swisher et al., 1994), the infection is a viable prenatal exposure to study in relation to risk of autism. However, there is a paucity of research on the association between maternal *T. gondii* and autism to date.

Approximately one third of the world population is infected with *T. gondii* (CDC, 2013). The parasite is primarily transmitted via food (undercooked meat), contaminated water, and cat waste (Abdoli et al., 2014; CDC, 2013). Direct transmission of the parasitic infection in an adult or child most often causes no symptoms. However congenital transmission via the placenta to offspring of pregnant women has detrimental effects on the fetus affecting primarily the central nervous and muscular systems (CDC, 2013; Prigione et al., 1995). Congenital symptoms include chorioretinitis, intracranial calcifications, hydrocephalus, and intellectual dysfunction (Berrebi et al., 2010; Roizen et al., 2006; Swisher et al., 1994). Some children exposed to *T. gondii* in utero remain asymptomatic until later school age (Berrebi et al., 2010). Common manifestations at that period of development include delayed motor development (Kankova et al., 2012) and intellectual dysfunction (Berrebi et al., 2010).

Only one study has investigated associations between maternal *T. gondii* antibodies and risk for autism in offspring. In that study, the authors assayed for IgG antibody to select pathogens, including *T. gondii*, from newborn blood samples among subjects later diagnosed with autism and controls (Grether et al., 2010). IgG antibody in the neonate derives mainly from the mother and therefore represents a marker of maternal IgG. *T. gondii* IgG levels in the 2nd and 4th quartiles were associated with significantly lower odds of autism compared

to the 1st quartile (reference group), suggesting a protective effect of adequate maternal IgG. The study was limited by an insufficient sample size to measure IgM antibody, a marker of *T. gondii* infection, and the authors did not provide antibody ranges for each quartile. Moreover, because newborn blood rather than maternal sera during pregnancy were assayed, maternal immune measures during pregnancy could not be examined.

In the present study, we assayed maternal serum specimens for *T. gondii* specific antibodies during pregnancy (first/second trimesters) in relation to childhood autism in offspring. During this period of gestation, the fetus is dependent on the maternal immune response (Prigione et al., 1995). Specifically, we quantified maternal *T. gondii* specific antibodies, IgM and IgG, from archived maternal sera. The IgG antibodies are considered an indicator of a past infection and IgM antibodies of an acute primary or ongoing infection. However, we note that the presence of IgM antibodies may sometimes remain detectable for months or years after a primary infection and thereby the validity of IgM as a single marker of recent infection may be questionable (Wong and Remington, 1994). To further evaluate the role of *T.gondii* infection and the time of infection in association with childhood autism, we determined IgG avidity for all IgM seropositive subjects (Jones et al., 2001; Hedman et al., 1989).

We first tested the relationship between maternal *T. gondii* IgM and odds of childhood autism in offspring (Grether et al., 2010). We then evaluated whether lower levels of maternal *T. gondii* IgG antibody are related to an increased odds of childhood autism, consistent with the Grether study (Grether et al., 2010) described above. The study was conducted in the Finnish Prenatal Study of Autism (FiPS-A), which capitalizes on a large and representative sample of pregnancies from a national birth cohort with prospectively collected and archived maternal serum specimens from an extensive biobank and well-validated offspring diagnoses of virtually all childhood autism cases in Finland.

Methods

Description of the cohort and biobank

All offspring in the FiPS-A were derived from the Finnish Maternity Cohort, which consists of over 1 million pregnancies with archived prenatal serum specimens that were drawn beginning in 1983. Sera were obtained primarily during the first and early second trimesters (mean = 10.93 weeks, SD = 3.49) from over 98% of pregnant women in Finland, following informed consent, for screening of HIV, syphilis and hepatitis. One maternal serum sample was obtained for each pregnancy. After the screening, serum samples were stored as one aliquot at minus 25°C in a single, centralized biorepository at the National Institute of Health and Welfare in Oulu, Finland. All of the serum samples in the Finnish Maternity Cohort were linked with offspring by a unique personal identification number (PIN), which has been assigned to all residents of Finland since 1971. The PINs were also used to link these samples with the other registries, described below.

Finnish Medical Birth Registry

The Finnish Medical Birth Registry includes comprehensive and standardized data on the demographic and prenatal/neonatal periods up to age 7 days on all births in Finland. The registry included the PIN of mothers and live born children that were used to link the subjects with the other registries.

Case and control identification

The Finnish Hospital and Outpatient Discharge Registry was used to identify all recorded diagnoses of childhood autism for psychiatric hospital admissions and outpatient visits. Computerized data were available from 1987 to the present. The registry contains the personal and hospital identification code and primary/secondary psychiatric diagnoses. In order to identify the autism cases for the present study, we conducted a record linkage between the Finnish Medical Birth Registry and the Finnish Hospital Discharge and Outpatient Registry, using the mother and offspring PINs. Cases with childhood autism diagnosis (ICD-10 F84.0) in the sampling frame consisted of all offspring born in Finland from 1987 to 2005 (Lampi et al., 2011). Over this time period, there were 1.2 million births. The total number of childhood autism cases in the entire sample was 1,132 (Lampi et al., 2011). In order to validate the registry diagnoses, 80 cases of infantile autism from the Finnish Hospital and Outpatient Discharge Registry were assessed in a previous study using the Autism Diagnostic Interview-Revised (ADI-R). Among these cases, 77 (96%) met the criteria from the ADI-R for childhood autism. The childhood autism cases were matched 1:1 to controls drawn from the birth cohort who were without diagnosis of childhood autism or severe/profound intellectual disability on date of birth, sex, birthplace and residence in Finland. A total of 874 cases and 874 matched controls had maternal sera available for *T. gondii* testing and were included in all analyses.

The study was approved by the ethical committees of the hospital district of Southwest Finland, the National Institute of Health and Welfare, and the Institutional Review Board of the New York State Psychiatric Institute. Informed consent was obtained before acquisition of all maternal serum specimens after the nature and possible consequences of the procedure and data derived from serum analyses were explained.

Laboratory Assay

The *T. gondii* assays were carried out blind to case/control status. The chemiluminescent microparticle immunoassay (CMIA) using an Architect i200 automatic analyzer (Abbott Diagnostics, Abbott Park, IL) was used in the assessment of *T. gondii* IgG and IgM antibody levels. To verify the quality of the test results, each set of 100 specimens was included with negative and positive control samples and the test values were obtained according to the manufacturer's instruction. The results are expressed in IU/mL for IgG and in S/CO for IgM. According to the manufacturer, the reactive cut-off of IgG is >=1.6 IU/mL. The corresponding reactive cut-off value for IgM is >=0.50 S/CO for IgM (Gay-Andrieu et al., 2009). The relative sensitivity is 99.7% (functional sensitivity = 0.03–0.25 S/CO) for IgG and 89.9% (functional sensitivity = 0.03–0.25 IU/mL) for IgM and the relative specificity for IgG is 99.1 % and for IgM is 99.8% (Gay-Andrieu et al., 2009). In order to examine the timing of *T. gondii* infection, we tested for IgG antibody avidity to *T. gondii* among all IgM

seropositive subjects using the IgG avidity (AVIcomp) test (Abbott Diagnostics, USA). IgG avidity indicates the strength of the binding of IgG antibody to antigen (Rahbari et al., 2012; Delforge et al., 2015). The percent binding increases with the length of time following *T. gondii* infection. Low avidity (<50%) is indicative of a recent infection and high avidity (>60%) of an infection at least four months earlier (Hedman et al., 1989). No clinical interpretation is drawn from intermediate results (50–60%).

Statistical Analysis

Maternal IgG and IgM were examined as categorical variables to provide clinically interpretable results and to account for the non-normal distribution of the data (Streiner, 2002). This follows on previous precedents in the literature (Markovitz et al., 2015; Brown et al., 2005; Coccaro et al., 2016; Groër et al., 2011). Values within the reactive cut-off are categorized as seropositive; they include maternal IgM levels >=0.5 S/CO and IgG levels >=1.6 IU/ml. All other values, including those below the level of detection (<0.25) were classified as seronegative. Secondary analyses were performed with maternal IgM and IgG antibodies as continuous variables in relation to offspring autism case-control status. The *T. gondii* IgG levels among seropositive subjects (>=1.6 IU/ml) in the case group, and control group, respectively, were classified into quartiles (defined based on the distribution of IgG levels in seropositive controls), and compared to the numbers of seronegative cases and controls. Odds ratios representing the association between each quartile of *T. gondii* IgG antibody levels and autism were calculated. We wished to keep the seropositive and seronegative groups separate, given prior precedents in the literature (Markovitz et al., 2015; Brown et al., 2005; Coccaro et al., 2016; Groër et al., 2011). Secondary analyses were performed with quartiles derived from the entire distribution of controls to determine offspring odds of autism across all levels of *T. gondii* IgG antibody. In all analyses, point and interval estimates of odds ratios and 95% confidence intervals (95% CIs) were obtained by fitting conditional logistic regression models for matched sets. Statistical significance was judged at $P < 0.05$ for the primary hypothesis and Bonferroni corrected $P < 0.016$ for the secondary hypothesis and post-hoc analysis. Statistical analyses were performed using SAS 9.2 (SAS Institute Cary, N.C.).

Covariates

Maternal and neonatal demographic data were analyzed using frequency statistics. In order to address the potential for confounding, we examined relationships between covariates and *T. gondii* IgG antibody among controls and between these variables and case-control status. The covariates included offspring sex, maternal age (< median, ≥ median) paternal age (< median, ≥ median), number of previous births (≥1), socioeconomic status (upper white collar, lower white collar, blue collar, other), gestational age (<37 weeks), birth weight (<2500 g), and gestational week of the blood draw (< median, ≥ median). Data on offspring sex, maternal age, paternal age, and socioeconomic status were acquired from the Finnish Hospital and Outpatient Discharge Registry and the Finnish Population Registry. Data on previous births, gestational age at birth, and birth weight were acquired from the Finnish Medical Birth Registry. Data on gestational week of the blood draw were obtained from the FMC. In accord with extant epidemiologic literature, covariates were included in the

adjusted model based on associations between the covariate and both *T. gondii* seropositivity and autism ($P < 0.1$).

Results

Sample Characteristics

Demographic information for the study sample is provided in STable 1. Relationships between the covariates and maternal IgM and IgG seropositivity and childhood autism are provided in Table 1. No covariate was associated with seroprevalence of maternal *T. gondii* IgM antibodies. Greater maternal age, paternal age, and previous childbirths were associated with a higher seroprevalence of maternal *T. gondii* IgG antibodies and an increased odds of childhood autism. Low birth weight was associated with an increased odds of childhood autism. Hence, maternal age, paternal age, and previous childbirths met the a priori criteria for inclusion in the statistical models, as they were associated with both seropositive maternal *T. gondii* IgG and childhood autism. Information about the distribution of IgM and IgG antibody data are provided in Stables 2 and 3.

Maternal T. gondii Seroprevalance and Childhood Autism in Offspring

The results of the analysis of *T. gondii* IgM by category and childhood autism are presented in Table 2. A significant decrease in odds of childhood autism was found in offspring of mothers who were seropositive for *T. gondii* IgM antibodies (odds ratio=0.40, 95% CI=0.18–0.91, p=0.03).

The results of the analysis of *T. gondii* IgG by category and childhood autism are presented in Table 3. No significant increase in odds of autism was found among offspring whose mothers were seropositive for *T. gondii* IgG antibodies (odds ratio=1.09, 95% CI=0.86–1.38, p=0.47). The relationship was similar after adjusting for maternal age, paternal age, and previous childbirths (odds ratio=1.02, 95% CI=0.80–1.29, p=0.90).

Post-hoc Analysis

The results for maternal *T. gondii* IgG antibody by quartile, for the seropositive group, in comparison to the seronegative (reference) group, and childhood autism are presented in Table 4. Offspring of mothers with low seropositive levels of *T. gondii* IgG antibodies (1^{st} quartile) relative to the seronegative group had an increase in odds of childhood autism (odds ratio [OR] =1.65, 95% CI=1.10–2.49, p=0.018) that fell slightly short of the Bonferroni corrected value of 0.016. There was no consistent relationship between *T. gondii* IgG level for the 2^{nd} to 4^{th} quartiles and childhood autism. Additional analysis of maternal *T. gondii* IgG antibody by quartile, including the entire range of values regardless of seronegative and seropositive status, and childhood autism are presented in STable 4. Offspring of mothers with higher levels of *T. gondii* IgG antibodies (2^{nd} to 4^{th} quartiles) relative to the reference group (1^{st} quartile) had mild increases in odds of childhood autism, though the findings fell well short of statistical significance.

In women with seropositive *T. gondii* IgM antibodies, the IgG avidity was high for cases and controls, indicating past infection, with the exception of three controls (Table 5). Similarly,

in these subjects, we found that the 8 cases and 20 controls were positive for IgG antibodies, with antibody levels ranging from 3.20 – 141.30 IU/mL.

For IgM, the continuous analysis was consistent with our findings in Table 2 of a decrease in odds of autism among offspring of mothers with higher levels of *T. gondii* IgM antibodies, though the finding was only a statistical trend (STable 5). For IgG, the continuous analysis was consistent with our findings in Table 3 of no significant increase in odds of autism among offspring with higher maternal *T. gondii* IgG antibodies (STable 5).

The analysis of *T. gondii* IgM by category and childhood autism presented in Table 2 was re-run removing the control with an outlying value (15.94 S/CO) and their matched case. The analysis of *T. gondii* IgG by category and quartile and childhood autism presented in Tables 2–3 were also re-run removing the case with an outlying value (325.30 IU/mL) and their matched control. The point estimates for all analyses did not change. For the analysis of *T. gondii* IgG by quartile, the p-values for analysis comparing the seronegative group to the 1st quartile of the seropositive group survived the Bonferroni correction, and to the 2nd quartile of the seropositive group fell slightly below the Bonferroni corrected p-value (STable 6).

Discussion

In the present study, we investigated the relationship between maternal *T. gondii* and childhood autism by measuring maternal IgM and IgG antibodies to this pathogen in archived prenatal serum specimens in a Finnish national birth cohort. There were three main findings. First, we observed that seropositive maternal IgM antibody was associated with a 60% decreased odds of childhood autism. Second, low seropositive *T. gondii* IgG antibodies (1st quartile) were associated with increased odds of autism, a finding that just fell short of significance following Bonferroni correction. Third, in women with seropositive *T. gondii* IgM antibodies, the IgG avidity was high for both cases and controls, indicating past infection. This is the first study to examine maternal *T. gondii* antibody in relation to childhood autism.

As IgM secretion is one of the first responses of the immune system to protect against a pathogen (Remington et al., 2010), one interpretation of the IgM findings is that a sufficient immune response to acute maternal *T. gondii* infection during pregnancy is protective against offspring odds of autism. However, considering the timing of exposure to the pathogen, the IgG avidity testing suggests that most subjects with seropositive *T. gondii* IgM antibody did not experience very recent infection. The likelihood of active infection is also low given that only two cases and two controls were given a diagnosis of congenital toxoplasmosis in childhood (see S.I.). It is therefore more probable that these women had a past or latent infection at the time of gestational blood draw, as IgM antibodies can remain detectable for years after an acute infection. This leads to a second possible explanation for the IgM findings: that an acute infection in the maternal circulation was more likely to have been eliminated prior to pregnancy among subjects without autism. Serial blood draws or at least a pre-pregnancy blood draw, which was not available in the current study, would be necessary to confirm this.

A third explanation is that the women who are seropositive for IgM may have more active immune systems during pregnancy, independent of exposure to *T. gondii.* To determine whether the maternal immune system was more active in subjects with higher IgM antibody levels, we assessed the relationship between IgM levels and C-reactive protein, an acute-phase reactant that is part of the innate immune response (CRP; see S.I.). While we did not find a significant association, previous studies suggest that CRP levels may not correlate with all antibodies (Nguyen et al., 2016; Sanders et al., 1987) and can vary across different types of infections or autoimmune disorders (Durán et al., 2016). Fourth, unknown genetic or environmental factors not measured in our study may contribute to the *T. gondii* IgM association with childhood autism. Lastly, given the small number of seropositive IgM cases and controls, it is possible that the findings represent a statistical artifact. While it is reassuring that all women with seropositive *T. gondii* IgM antibodies also had seropositive IgG antibodies, the results should be interpreted with caution until replicated.

With regard to our second finding, of an association between maternal *T. gondii* IgG levels and autism that fell slightly short of significance following Bonferroni correction, we note that secretion of IgG antibody generally represents a later immune response to an infection and for *T. gondii*, these antibodies are believed to prevent reactivation of latent infection (Remington et al., 2010). Hence, the association between seropositivity to *T. gondii* IgG antibody in the lowest quartile and childhood autism could be related to a protective effect of IgG antibody against past or latent *T. gondii* infection. While we did not observe a significantly lower odds of autism with increasing *T. gondii* IgG antibodies, it is conceivable that a protective effect is present for IgG levels above a certain antibody threshold.

As noted above, the single previous study that utilized *T. gondii* IgG antibodies to investigate the association of infection with odds of childhood autism was based on analyses of neonatal blood samples (Grether et al., 2010). While in both studies the lowest quartile for IgG was associated with the greatest odds of autism, the prior study did not provide antibody ranges for each quartile; thus, it is not possible to directly compare IgG levels and their respective associations with autism across the two studies. Though IgG antibody in the neonate derives mainly from the mother, an advantage of the present study is the analysis of *maternal* sera which allowed for a more direct assessment of maternal humoral immune status in response to *T. gondii* infection.

Other infections have been associated with odds of autism. In a study that included many infections based on clinical diagnoses, mothers of ASD children were more likely to have two or more such infections during pregnancy (Zerbo et al., 2013). Additional studies showed that other types of maternal infections, such as viral infection in the first trimester and bacterial infection in the second trimester were associated with autism (Atladottir et al., 2010). Alterations of the immune response, including elevated mid-gestational levels of interleukin-4 and −5 (Goines et al., 2011) and elevated first to second trimester levels of C-reactive protein (Brown et al., 2014) during pregnancy were also associated with autism.

The study has several strengths. First, exposure was prospectively documented with maternal biomarkers of *T. gondii* exposure. Second, the cases and controls were derived from a large, population-based national birth cohort including all childhood autism cases diagnosed in

Finland through psychiatric registries which cover the entire population. The cases with maternal sera represented over 85% of all childhood autism cases in the population and their selection was not predicated on the exposure, or related factors. This indicates that the potential for selection bias was small. Third, the sample size was large, with over 700 matched case-control pairs. Fourth, while registry data were used for diagnosis, the diagnoses were confirmed via parent interview with a subsample of cases (Lampi et al., 2011).

The study has several limitations. First, we have no information on the biological effect of low *T. gondii* IgG. This could reflect an increased likelihood of reactivation of infection or a more subtle effect of immune system control of the organism. Unfortunately, this has not been adequately studied in relation to maternal infection and fetal outcomes. Second, although there was no evidence of confounding following testing of many covariates, residual factors related to maternal lifestyle or health may be associated with *T. gondii* exposure and odds of autism. Third, the timing of the infection in relation to when the serum sample was drawn is unclear. Our data from avidity testing suggested that most subjects exposed to *T. gondii* IgM antibody did not experience very recent infection, and we cannot rule out the possibility that for some subjects this may have occurred prior to pregnancy. Fourth, IgM antibodies to other infections, such as cytomegalovirus were not measured in the current study. Therefore we cannot rule out the presence of other maternal infections. Fifth, although the total sample size was large, the number of subjects who tested seropositive for *T. gondii* IgM was small.

Conclusion

Our findings suggest that maternal *T. gondii* IgM antibody, measured during pregnancy, is related to a decrease in offspring odds of childhood autism. Moreover, low seropositive levels of *T. gondii* IgG antibody are related to an increase in offspring odds of this disorder. Fetal *T. gondii* infection significantly alters development of the brain, later becoming symptomatic during postnatal life, from infancy through school age (Berrebi et al., 2010). This infection is preventable through public health measures (Brown and Derkits, 2010). Hence, further studies aimed at replicating this association in independent birth cohorts and further exploring the relationship between maternal *T. gondii*, the immune response to this parasite, and autism have the potential for improving our understanding of developmental pathways to this disorder and providing new strategies for prevention. Future studies should also examine the relationship between maternal immune activation, other infections, and *T. gondi*i in the observed associations with childhood autism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Covariates in relation to maternal *T. gondii* IgM and IgG seropositivity and diagnostic classification (childhood autism, controls)

Covariates	IgM for Controls (n=874)				IgG for Controls (n=874)				Diagnostic Classification			
	Seronegative n (%)	Seropositive n (%)	X^2/t	P	Seronegative n (%)	Seropositive n (%)	X^2/t	P	Cases (n=874) n (%)	Controls (n=874) n (%)	X^2/t	P
Maternal age			0.02	0.89			12.29	0.0005			8.72	0.003
< Median (29.00)	391 (45.78)	9 (45.00)			331 (48.96)	69 (34.85)			339 (38.79)	400 (45.77)		
≥Median	463 (54.22)	11 (55.00)			345 (51.04)	129 (65.15)			535 (61.21)	474 (54.23)		
Paternal age			0.12	0.73			8.07	0.005			6.55	0.01
< Median (31.00)	394 (46.14)	10 (50.00)			330 (48.82)	74 (37.37)			351 (40.16)	404 (46.22)		
≥Median	460 (53.86)	10 (50.00)			346 (51.18)	124 (62.63)			523 (59.84)	470 (53.78)		
Previous births (≥ 1)	494 (57.85)	12 (60.00)	0.04	0.84	371 (54.88)	135 (68.18)	11.11	0.0009	581 (66.48)	506 (57.89)	13.68	0.0002
Gestational Age (< 37 weeks)	42 (4.92)	2 (10.00)	1.06	0.30	33 (4.88)	11 (5.56)	0.15	0.70	54 (6.18)	44 (5.03)	1.08	0.30
Birth weight (< 2500 grams)	22 (2.58)	1 (5.00)	0.45	0.50	18 (2.66)	5 (2.53)	0.01	0.92	39 (4.46)	23 (2.63)	4.28	0.04
Maternal SES[a]			2.00	0.57			0.76	0.86			1.43	0.70
Upper white collar	109 (15.87)	2 (12.50)			92 (16.25)	19 (13.87)			98 (13.59)	111 (15.79)		
Lower white collar	311 (45.27)	5 (31.25)			254 (44.88)	62 (45.26			331 (45.91)	316 (44.95)		
Blue collar	146 (21.25)	5 (31.25)			122 (21.55)	29 (21.17)			157 (21.78)	151 (21.48)		
Others	121 (17.61)	4 (25.00)			98 (17.31)	27 (19.71)			135 (18.72)	125 (17.78)		
Sex			0.03	0.86			0.39	0.53			---	---
Male	669 (78.34)	16 (80.00)			533 (78.85)	152 (76.77)			685 (78.38)	685 (78.38)		
Female	185 (21.66)	4 (20.00)			143 (21.15)	46 (23.23)			189 (21.62)	189 (21.62)		
Gestational week of blood draw[b]			1.72	0.19			0.05	0.82			0.63	0.43
< Median (10.00)	321 (39.48)	5 (25.00)			253 (39.35)	73 (38.42)			308 (37.24)	326 (39.14)		
≥Median	492 (60.52)	15 (75.00)			390 (60.65)	117 (61.58)			519 (62.76)	507 (60.86)		

Abbreviations: IgM, immunoglobulin M; SES, Socioeconomic Status;

[a] frequency missing = 153 cases, 171 controls;

[b] frequency missing = 47 cases, 41 controls.

Table 2.

Maternal *T. gondii* IgM seropositivity in childhood autism cases and matched controls

IgM by category [Range (S/CO)]	Cases (n, %) (n=874)	Controls (n, %) (n=874)	OR (95% CI)	P
Seronegative, < 0.5	866 (99.08)	854 (97.71)	1	NA
Seropositive, ≥ 0.5	8 (0.92)	20 (2.29)	0.40 (0.18 – 0.91)	0.03

Abbreviations: IgM, immunoglobulin M; CI, confidence interval; OR, odds ratio

Table 3.

Maternal *T. gondii* IgG seropositivity in childhood autism cases and matched controls

			Unadjusted		Adjusted*	
IgG by category [Range (IU/mL)]	**Cases (n, %) (n=874)**	**Controls (n, %) (n=874)**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**
Seronegative, < 1.6	664 (75.97)	676 (77.35)	1	NA	1	NA
Seropositive, ≥ 1.6	210 (24.03)	198 (22.65)	1.09 (0.86 – 1.38)	0.47	1.02 (0.80 – 1.29)	0.90

Abbreviations: IgG, immunoglobulin G; CI, confidence interval; OR, odds ratio.

*Model adjusted for maternal age, paternal age, and previous births

Table 4.

Maternal *T. gondii* IgG level by quartile in childhood autism cases and matched controls

IgG by quartile [Range (IU/mL)]	Cases (n, %) (n=874)	Controls (n, %) (n=874)	OR (95% CI)	P
Seronegative, < 1.6	664 (75.97)	676 (77.35)	1	NA
Seropositive by Quartile				
1st, ≤ 25 (1.6–3.39)	73 (8.35)	48 (5.49)	1.65 (1.09 – 2.49)	0.018
2nd, 26–50 (3.4–8.14)	33 (3.78)	51 (5.84)	0.65 (0.41 – 1.04)	0.074
3rd, 51–75 (8.15–18.39)	60 (6.86)	48 (5.49)	1.27 (0.85 – 1.89)	0.249
4th, ≥ 76 (18.4–325.30)	44 (5.03)	51 (5.84)	0.91 (0.60 – 1.39)	0.655

Abbreviations: IgG, immunoglobulin G; CI, confidence interval; OR, odds ratio

Table 5.

Maternal IgG avidity by category in childhood autism cases and matched controls among seropositive IgM subjects

IgG Avidity by category [Range (% binding)]	Cases (n, %) (n=7)	Controls (n, %) (n=16)
Low, < 50%	0 (0.0)	3 (18.75)
Intermediate, 50 – 60%	1 (14.29)	2 (12.50)
High, ≥ 60%	6 (85.71)	11 (68.75)

Abbreviations: IgG, immunoglobulin G; IgG avidity data was missing for 1 case and 4 controls that were positive to *T. gondii* IgM antibody.