Infections During Pregnancy and After Birth, and the Risk of Autism Spectrum Disorders: A Register-based Study Utilizing a Danish Historic Birth Cohort

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Summary

Objective: Mounting evidence suggests that immune dysfunction may play a crucial role in the pathophysiology of autism spectrum disorders (ASD). In addition, several studies have reported that congenital and postnatal infections may contribute to the neurobiological basis of ASD. This study aimed to investigate the relationship between infections during pregnancy and after birth, and ASD.

Methods: A case-control study design was adopted. Both cases and controls were retrieved from a historic birth cohort (HBC) maintained at Statens Serum Institute in Copenhagen/Denmark and were followed up retrospectively during pregnancy and after birth over four pre-defined periods. Study subjects were followed-up utilizing Danish nation-wide health registers for outpatient and hospital admissions due to infections. Associations between infections and ASD were analyzed using Mantel-Haenszel estimate of the odds ratio (OR) and logistic regression models.

Results: In total, 414 ASD cases and 820 controls were followed-up during pregnancy and a mean 16.3 years after birth. Crude, but not adjusted estimates showed that ASD cases had an increased risk of hospital admission due to infection at the end of the first year of life (OR = 1.48 [range: 1.07-2.05], P = 0.02) and at the end of the follow-up period (OR = 1.30 [range: 1.02-1.64], P = 0.03).

Conclusion: The present findings indicate that infections have a potential role in the pathophysiology of ASD; however, further studies are necessary to determine if infections etiologically contribute to ASD or if they act as an epiphenomenon due to distorted immunity in children with ASD.

Keywords: Autistic disorder, infections, population registers, birth cohort

INTRODUCTION

Autism spectrum disorders (ASD), or pervasive developmental disorders (PDD), according to the International Classification of Diseases (ICD) (WHO 2010), are a group of complex disorders characterized by a triad of pervasive qualitative abnormalities in social interactions and communication, and restricted, stereotyped behavior (APA 2000). The neurobiological basis of ASD is complex and several lines of research suggest that both genetic and environmental factors contribute etiologically to ASD (Hertz-Picciotto et al. 2006; Newschaffer et al. 2007). Mounting evidence suggests that immune processes play a key role in the pathophysiology of ASD (Onore et al. 2011); however, although inflammatory changes in the central nervous system (CNS) (Pardo-Villamizar 2008), and peripheral dysfunctional responses of humoral and cellular immunity (Ashwood and Van De Water 2004) have been repeatedly reported, convergence toward a single immunopathology is still lacking.

Congenital infections have been linked to behavioral abnormalities and several psychiatric disorders, including ASD (Patterson 2011). This was proposed as early as 1963, when a Dutch physician (Dr. Van Krevelen) reported congenital rubella infection in a patient with infantile autism (Rimland 1964). Several subsequent studies reported associations between ASD and other prenatal infections, such as cytomegalovirus (CMV) (Yamashita et al. 2003; Libbey et al. 2005).

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Postnatal childhood infections, however, have been less extensively investigated. Whilst Rosen et al. (2007) reported a slightly lower frequency of infections in children with subsequent diagnoses of ASD than in frequency-matched controls during the first 2 years of life, a large population-based study by Atladottir et al. (2010a) reported a high rate of ASD diagnoses in children admitted to hospital due to infectious diseases.

For a subset of the study population investigated by Atladottir et al. (2010a) amniotic fluid (AF) samples are available, which has made it possible to examine the association between maternal/fetal immune activation and ASD (Abdallah et al. 2011b, 2012). Consequently, it is important to analyze the association between registered infections and ASD in this particular sample. As such, the present study aimed to investigate the association between infection and ASD during 4 pre-defined time periods utilizing a Danish historic birth cohort (HBC).

MATERIAL and METHODS

Study design and the Danish HBC

To examine the association between ASD and infection diagnoses during pregnancy and after birth, a case-control study design was adopted. Cases and controls were retrieved from an HBC maintained at Statens Serum Institute (SSI) in Copenhagen/Denmark. The HBC is based on a collection of antenatal biologic samples obtained during screening/diagnostic procedures and kept later at SSI. The collection of samples is from the late 1970s-2004, and includes more than 100,000 samples of AF, and first and second trimester maternal serum samples (Abdallah et al. 2011b). Conventionally, after a screening or diagnostic test was performed (for example, measuring α-fetoprotein levels), residual biologic material was centrifuged and the supernatant was collected and stored at –20 °C, according to the routine SSI procedures for storage and handling of biologic materials (Nørgaard-Pedersen and Hougaard 2007).

Study population and selection of cases and controls

The ASD case population comprised all singleton offsprings with a registered AF sample in the HBC who were born between 1982 and 2000, and had ≥1 ASD diagnosis. The controls were also retrieved from the HBC and included non-ASD children with a corresponding AF sample and were frequency-matched to cases by gender and year of birth. Cases and controls were followed-up for somatic and psychiatric disorders through nation-wide health registers until 01 September 2009. Retrieval of data from different Danish nation-wide health registers (see below) was made possible using a unique personal identification number (CPR number), which is assigned to every person living in Denmark; the CPR number is used in all national registers, enabling accurate linkage (Pedersen et al. 2006).

All somatic and psychiatric diagnoses from the various registers were retrieved using their corresponding International Classification of Diseases (ICD) codes. In Denmark, ICD-8 codes (Sundhedsstyrelsen 1986) were used until 1993, followed by ICD-10 codes (Sundhedsstyrelsen 2008). The diagnostic codes used to identify diagnoses of ASD and infectious diseases are available elsewhere (Atladottir et. al. 2010a, 2010b; Abdallah et al. 2011b). All register-retrieved diagnoses of infections during pregnancy for the mothers of ASD children and in those children until the end of the follow-up were included. Four follow-up time periods were introduced: 1. Infections during pregnancy; 2. Infections during the first month after birth; 3. Infections during the first year after birth. 4. Infections until the end of follow-up (01 September 2009). Furthermore, infections were examined in 2 subcategories: viral and bacterial.

Danish nation-wide health registers

Psychiatric, somatic, and obstetric data for the cases and controls were retrieved from Danish nation-wide health registers. ASD and other psychiatric diagnoses were retrieved from the Danish Psychiatric Central Register (DPCR). The DPCR includes data on psychiatric hospital admissions in Denmark since 1969 and data regarding outpatient status since 1995, with a coverage rate of 95%-100% (Munk-Jorgensen and Mortensen 1997). For ASD diagnoses the DPCR has relatively high validity. A recent validation study based on abstraction of medical records of all infantile autism diagnoses in the DPCR between 1990 and 1999 confirmed the diagnoses in 94% of the cases (Lauritsen et al. 2010).

Somatic diagnoses (including admissions due to infectious diseases) were retrieved from the Danish National Hospital Register (DNHR), which includes somatic hospital admissions in Denmark since 1977 and outpatient contacts since 1995 (Andersen et al. 1999). Only primary diagnoses were included in the present study's analysis due to their higher validity in the DNHR (Andersen et al. 1999). In general, the diagnostic validity of the DNHR ranges from 74% to 87% for admissions registered in pediatric and internal medicine departments (Andersen et al. 1999). For infectious disease admissions, however, the validity ranges around 70.4% utilizing ICD-8 codes and expected to be higher when ICD-10 codes are implemented (Mosbech et al. 1995). A recent small-scale pilot validation study that included 60 patients confirmed the diagnoses via positive culture results in hospital records for about 88% of the cases (Atladottir et al. 2010a).

Obstetric history and birth records for the study population were retrieved from the Danish Medical Birth Registry (MBR). The MBR comprises data on all live births and stillbirths by women in Denmark since 1973, and has good quality administrative and obstetric data (Knudsen and Olsen 1998).

Statistical analysis

Background characteristics of the study population were analyzed using the Mantel-Haenszel (MH) chi-square test and MH estimates of the odds ratio (OR), controlling for the variables used for frequency matching (gender and year of birth). Logistic regression models were used to analyze the association between infection diagnoses during the 4 pre-defined follow-up periods, and ASD diagnoses. Furthermore, infection diagnoses were analyzed in 3 categories (any infection, bacterial infections, and viral infections) and associations were reported using ORs with a 95% confidence interval (CI).

Both crude and adjusted ORs were calculated. Gender and year of birth were included as covariates in both crude and adjusted estimates. Additionally, in the adjusted analyses we controlled for parity, gestational age, Apgar score, maternal and paternal age at delivery, and birth weight, as well as diagnoses of any congenital malformation or other childhood psychiatric co-morbidity. Furthermore, we analyzed the data stratified by gender, and only including individuals born after 1994, when both inpatient and outpatient data became available and ICD-10 was introduced in Denmark.

IBM PASW Statistics v.18.0 (SPSS Inc. 2009) was used for data management and statistical analyses were performed using STATA Statistical Software v.11.2 (StataCorp LP 2009). This study was approved by the Danish Data Protection Agency (record no. 2009-41-3173) and The Danish Ethics Committee of Midtjylland Region (record no. M-20090066).

RESULTS

The study population included 414 ASD cases (infantile autism: n = 94; Asperger's syndrome: n = 126; atypical autism: n = 16; other ASD: n = 178) and 820 controls frequency-matched for gender and year of birth. The background characteristics of the study population are presented in Table 1. Mean age at time of ASD diagnosis (based on the DPCR registration) ranged from 7.8 ± 4.3 years for infantile autism to 11.1 ± 4.0 years for Asperger's syndrome. As compared to the controls, ASD cases were more likely to have an older mother (> 35 years old) (OR = 1.28 [range: 1.01-1.63], P = 0.04), be the first child (OR = 1.32 [range: 1.03-1.69], P = 0.03), have a low Apgar score (< 7) (OR = 2.47 [range: 1.01-6.01], P = 0.04), and be co-diagnosed with a congenital malformation (OR = 2.78 [range: 1.96-3.96], P < 0.001), mental retardation (OR = 20.52 [range: 10.31-40.86], P < 0.001), or psychiatric comorbidity (OR = 17.89 [range: 17.34-25.92], P < 0.001).

Table 1. Characteristics of the study population.

Characteristic	ASD Cases, n = 414 n (%)	Controls, n = 820 n (%)
Gender		
Female	79 (19.1)	160 (19.5)
Male	335 (80.9)	660 (80.5)
Age (years)[1]		
Mean (SD)	16.28 (4.55)	16.26 (4.58)
Mothers' age (years)		
<30	109 (26.3)	226 (27.6)
30-35	120 (29.0)	283 (34.5)
>35*	185 (44.7)	311 (37.9)
Fathers' age (years)[2]		
<30	73 (17.6)	181 (22.1)
30-35	130 (31.4)	261 (31.8)
36-40	112 (27.1)	220 (26.8)
>40	92 (22.2)	154 (18.8)
Gestational age (d)		
Preterm (<260)	43 (10.4)	87 (10.6)
Birth weight (BW) (g)		
Low BW (<2500)	25 (6.0)	47 (5.7)
Parity[2]		
1st Child*	156 (37.7)	258 (31.5)
≥2nd Child	258 (62.3)	561 (68.4)
APGAR Score (recorded 5 min after birth)		
<7*	11 (2.7)	9 (1.1)
Congenital Malformation[3]*	83 (20.1)	68 (8.3)
Mental Retardation[4]*	88 (21.3)	10 (1.2)
Psychiatric Comorbidity[5]*	300 (72.5)	106 (12.9)
History of Infection Admissions		
Maternal infection during pregnancy	7 (1.7)	13 (1.6)
Childhood infection during first month of life	11 (2.7)	18 (2.2)
Childhood infection during first year of life*	74 (17.9)	105 (12.8)
Childhood infection until end of follow-up[6]*	202 (48.8)	347 (42.3)
Any bacterial infection until end of follow-up[6]	57 (13.8)	131 (16.0)
Any viral infection until end of follow-up[6]	45 (10.9)	73 (8.9)

*Chi square P value <0.05.
[1]Age at the end of register-based follow-up (01 September 2009).
[2]Percentages do not equal 100% due to missing data.
[3]ICD-8 codes 740.x-759.x and ICD-10 codes DQ00.x-DQ99.x were used to diagnose congenital malformations.
[4]ICD-8 codes 310.x-315.x and ICD-10 codes DF70.x-DF79.x were used to diagnose mental retardation.
[5]Any psychiatric diagnosis in the Danish Central Psychiatric Register other than ASD.
[6]Register-based follow-up ended 01 September 2009.

Table 2. Overall diagnoses of infection in the children with ASD and controls during 4 follow-up periods.

Childhood/Maternal Infection	Crude OR (95% CI)				Adjusted OR (95% CI)
	Overall*	Male*	Female*	Post -1994*[1]	Overall*
Maternal infection during pregnancy	1.06 (0.42-2.70)	1.38 (0.52-3.66)	NE	2.52 (0.66-9.60)	1.08 (0.27-4.39)
Childhood infection during first month of life	1.22 (0.57-2.63)	1.45 (0.66-3.21)	NE	0.54 (0.15-1.98)	1.25 (0.45-3.42)
Childhood infection during first year of life	1.48 (1.07-2.05)	1.62 (1.14-2.32)	0.91 (0.39-2.10)	0.98 (0.56-1.70)	1.14 (0.72-1.81)
Childhood infection from age 1 year until the end of follow-up[2]	1.30 (1.02-1.64)	1.37 (1.05-1.78)	1.03 (0.59-1.77)	1.19 (0.78-1.81)	1.0 (0.77-1.50)

*Number of overall cases with ASD: n = 414; male ASD cases: n = 335; female ASD cases: n = 79; post-1994 ASD cases: n = 133.
[1] Individuals born after 1994, when ICD-10 was the only diagnostic tool used and the Danish National Hospital Register included out-patient/emergency ward diagnoses along with inpatient diagnoses.
[2] Register-based follow-up ended 01 September 2009.

Mean follow-up period in the ASD cases was 16.28 ± 4.55 years, versus 16.26 ± 4.58 years in the controls. The prevalence of diagnosed infections was higher in the ASD cases during all 4 follow-up periods, but was significantly higher during the last 2 follow-up periods (Table 2). ASD was associated with a higher risk of hospital admittance due to infection during the first year of life (OR = 1.48 [range: 1.07-2.05], P = 0.02), and at the end of the follow-up period (OR = 1.30 [rage: 1.02-1.64], P = 0.03). Interestingly, the ORs for hospital admittance due to infections were higher in the male ASD cases than in the female ASD cases (Table 2), but the difference was not statistically significant (P value of the homogeneity test of the OR based on gender = 0.35). Additionally, analysis of cases and controls born after 1994 did not yield significant differences in terms of diagnosed infections between the cases compared to the frequency-matched controls (Table 2).

Finally, analysis of diagnosed infections according viral and bacterial infection subgroups is presented in Table 3. Although significant associations were not observed between ASD, and viral and bacterial infections, the observed associations were in the same direction as the overall infection estimates, except for bacterial infections at the end of the follow-up period, which were slightly less prevalent in the ASD cases than in the controls, (OR = 0.84 [range: 0.60-1.18], P = 0.31) (Table 3).

DISCUSSION

In the present study children diagnosed with ASD later in life were more likely to have been admitted to hospital due to infections than the controls. This association was observed before the diagnosis of ASD was established (at the end of 1 year post birth) as well as after ASD was diagnosed (at the end of the follow-up period). These findings are similar to those reported previously by Atladottir et al. (2010a), who studied a larger Danish population of almost 1.5 million children and reported that admissions to hospital for various childhood infections were associated with the diagnosis of ASD.

The potential role of maternal infections during pregnancy in the pathophysiology of autism has been reported in several animal and epidemiological studies (Atladottir et al. 2010b;

Table 3. Viral and bacterial infections in the children with ASD and controls during 2 follow-up periods.

Childhood/Maternal Infection	Crude OR (95% CI)				Adjusted OR (95% CI)
Viral Infections[2]	Overall*	Male*	Female*	Post -1994*1	Overall*
Childhood viral infection during first year of life	1.20 (0.58-2.50)	1.36 (0.62-2.98)	0.54 (0.06-4.88)	1.19 (0.42-3.36)	1.16 (0.42-3.19)
Childhood viral infection until end of follow-up[3]	1.25 (0.85-1.84)	1.39 (0.91-2.11)	0.63 (0.20-1.95)	1.00 (0.47-2.13)	1.12 (0.64-1.95)
Bacterial Infections[2]					
Childhood bacterial infection during first year of life	1.41 (0.75-2.67)	1.46 (0.72-2.96)	1.23 (0.28-5.33)	1.13 (0.37-3.43)	1.21 (0.52-2.85)
Childhood bacterial infection until end of follow-up[3]	0.84 (0.60-1.18)	0.85 (0.58-1.25)	0.80 (0.39-1.63)	0.75 (0.36-1.54)	0.70 (0.43-1.12)

* Number of overall cases with ASD: n = 414; male ASD cases: n = 335; female ASD cases: n = 79; post-1994 ASD cases: n = 133.
[1] Individuals born after 1994, when ICD-10 was the only diagnostic tool used and the Danish National Hospital Register included both out-patient/emergency ward diagnoses and inpatient diagnoses.
[2] Bacterial and viral infection estimates during pregnancy and the first month of life are not presented due to the small number of admissions.
[3] Register-based follow-up ended 01 September 2009.

Table 4. Application of Hill's criteria for causation to the relationship between infection and ASD.

Hill's Criteria for causation	Application of Hill's criteria to the relationship between infections and ASD
Strength of the Association	While a significant association between ASD and infection was observed based on our crude estimates, the adjusted estimates did not suggest a strong association, which may indicate that the association reported is distorted due to confounding. Furthermore, the population-based study by Atladottir et. al. (2010a) reported an overall hazard ratio of 1.76 (did not reach 2.0), which means that the probability of causation is <50%, suggesting that it is more likely that the infection-causal relationship is not true (Freeman and Kohles 2011).
Consistency of the Evidence	This criterion is not completely fulfilled. A large body of research indicates that congenital infection may lead to ASD (Yamashita et al. 2003; Libbey et al. 2005; Patterson 2011); however, research regarding childhood infections is relatively limited and inconsistent (Rosen et al. 2007; Atladottir et al. 2010a).
Specificity	Although the fulfillment of this criterion indicates a causal relationship, monocausality seldom exists in biologic psychiatry, which limits the usefulness of applying this criterion (van Reekum et al. 2001).
Temporal sequence	This criterion is not completely fulfilled. Evidence shows that congenital infections are associated with ASD later in life (Patterson 2011); however, childhood infections are also seen more frequently in children with ASD after being diagnosed (Atladottir et al. 2010a).
Biological Gradient	It is difficult to apply this criterion to psychiatric research (van Reekum et al. 2001). Additionally, we did not perform dose-response analysis in the present study to examine this criterion. Nonetheless, research suggests that a dose-response relationship could be present (children diagnosed later with ASD may suffer more frequently from infections than healthy children) (Atladottir et al. 2010a).
Biologic Rationale	This criterion is fulfilled when the causal relationship between infections and ASD is examined. Exposure to infections is biologically plausible as an etiologic factor in ASD later in life via different pathways (maternal/fetal immune activation, neurotropic viruses, direct brain damage, etc)(Patterson et al. 2008).
Coherence	This criterion is partially fulfilled. Consensus has not been achieved regarding the etiologic role of infections in ASD (Atladottir et al. 2010a); however, research in this field is moving in that direction. Yet, many questions from other perspectives need to be answered. For example, if infections play an etiologic role in the development of ASD, we will expect more children with ASD in developing countries than in developed countries, and this has not yet been documented.
Experimental Evidence	This criterion is partially fulfilled when the relationship between infection and ASD is examined. Whereas compelling evidence is available from animal studies, human studies in this area are limited in number (Patterson et al. 2008).
Analogous Evidence	This criterion can be fulfilled when considering the etiologic factors for schizophrenia. The linkage between schizophrenia and infections has been repeatedly suggested and the numerous similarities between ASD and schizophrenia yield analogous evidence when analyzing the relationship between ASD and infections (Meyer et al. 2011; Meyer et al. 2007).

Patterson 2011). Interestingly, such a role is proposed to function indirectly via triggering a maternal immune activation (MIA) state along with elevation of different cytokines and chemokines, which eventually leads to the clinical phenotype (Parker-Athill and Tan 2010). A major advantage of the present study was the availability of corresponding intrauterine biologic materials (AF samples) for the ASD cases and controls, which facilitated examination of the association between maternal/fetal immune activation and ASD in the same study population. AF samples for the ASD cases had higher levels of interleukin-4 (IL-4), IL-10, tumor necrosis factor-α (TNF-α), TNF-β (Abdallah et al. 2011b), and monocyte chemotactic protein-1 (MCP-1) (Abdallah et al. 2012) than those for the controls. Given the ability of different pathogens during pregnancy to trigger similar clinical psychopathologic outcomes in offspring later in life and the fact that most infectious microorganisms are limited to specific maternal areas with minimal direct invasion to fetal tissue (Patterson et al. 2008), it is possible that the contribution of prenatal infections (along with other inflammatory triggers, such as allergens, toxins, and autoimmunity) to the pathophysiology of ASD might be rather indirect via induction of a "cytokine-storm" in the fetus (Buehler 2011).

The increased risk of hospital admission due to infections after birth observed in the present study, however, could be attributed to a number of biological and psychological factors. Children that develop ASD may be genetically more susceptible to infections than healthy controls. For example, Geier et al. (2009) reported reduced levels of glutathione due to the lack of precursor availability in ASD, which diminishes a child's ability to resist infections, resolve inflammation, and detoxify environmental contaminants. Additionally, children

with ASD may have a higher incidence of physical anomalies that increase their risk of infections. Konstantareas et al. (1987) reported that low-set ears were more prevalent in children with ASD than in controls, which made the ASD children more susceptible to ear infections. Furthermore, feeding problems and childhood failure to thrive (FTT) may be more common in children with ASD, leading to greater susceptibility to infections (Keen 2008).

Psychological factors might also have contributed to the higher rate of hospital admissions due to infections in the present study. Such factors may be related to parents, children with autism, or healthcare providers. For example, parents of children with ASD report significantly higher levels of stress than parents of normally developing children, and they also report reductions in stress when their affected children receive professional care (Baker-Ericzén et al. 2005). On the other hand, children with ASD also report increased levels of stress (demonstrated by elevated levels of cortisol) due to non-social stressors (Levine et al. 2005). As such, it is plausible that a stressful home environment increases biological susceptibility to infections via the neurohormonal outflow that occurs during stress (Freestone et al. 2008), or parental stress may be associated with increased alertness toward clinical symptoms—i.e. parents become more aware and react more frequently to their children's symptomatic complaints due to infections. Increased alertness may also be observed in healthcare providers due to the special needs of children with ASD and their comorbid medical (Newschaffer et al. 2007) and psychiatric (Abdallah et al. 2011a) disorders. As in the present study, earlier research has shown that children with ASD (on an aggregate level and not only due to infections) had significantly more annual outpatient visits and physicians spent significantly more time with ASD patients during their visits compared to non-ASD children (Liptak et al. 2006).

The present study has a number of limitations. Due to the reliance on register-based data, no data on autistic or infection symptomatology was present to validate the diagnoses. While several validation studies on DPCR and DNHR diagnoses were performed (Mosbech et al. 1995; Atladottir et al. 2010a; Lauritsen et al. 2010) that suggest both registers are satisfactory sources of clinical diagnoses, it was not possible to use standard diagnostic tools, such as parental reports (e.g. Autism Diagnostic Interview-Revised [ADI-R]) or direct observation (e.g. Autism Diagnostic Observation Schedule-Generic [ADOS-G]) to validate the ASD diagnoses. Furthermore, the present study did not examine the severity of infections that led to hospital admissions. Due to the relatively small study population, numerous infection categories were collapsed to dichotomized variables; therefore, it was not possible to examine a dose-response effect. Additionally, the relatively small study population limited our ability to analyze infections in viral and bacterial subcategories. The power was 50% to detect an OR of 1.8 at a significance level of P = 0.05. With the present study's sample size, 80% power would require an OR ≥2.5. In addition, diagnoses of infection used in the present study were based on admissions to secondary and tertiary healthcare facilities in Denmark, not infections reported by general practitioners or those not reported to a healthcare provider. As a result, the diagnoses of infections may reflect only severe infections that actually required hospitalization of the mother or child.

Despite the observed association between ASD and infections, no causal relationship can be inferred based on the present study's findings. Application of Hill's criteria for causation (Hill 1965; van Reekum et al. 2001) shows that current evidence (including the present findings) is not sufficient to indicate a causal relationship (Table 4). Whereas a number of criteria, such as biologic rationale, and experimental and analogous evidence, can be fulfilled to some extent, other criteria, including temporal sequence, biological gradient, and specificity of the evidence, have not been sufficiently met.

In conclusion, the present study shows that there was a higher risk of admission due to postnatal infections in children with ASD, as compared to the controls. However, the study did not confirm this association for admissions due to infections during pregnancy and this was mainly because of limited statistical power. A noteworthy strong point of the present study is that all covariates were obtained independent of exposure, which minimized differential misclassification and selection bias. In addition, as the study was based on an HBC with corresponding biologic samples, it remains possible that additional examination of the biologic material can be performed in the search for a more definitive understanding of the relationship between ASD, and infections (during pregnancy and after birth) and maternal/fetal immune activation.

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