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Serological Documentation of Maternal Influenza Exposure and Bipolar Disorder in Adult Offspring

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Abstract

Objective—The goal of the present study was to evaluate whether serologically confirmed maternal exposure to influenza is associated with an increased risk of bipolar disorder in the offspring and with subtypes of bipolar disorder, with and without psychotic features.

Method—The study utilized a nested case-control design in the Child Health and Development Study birth cohort. Eighty-five cases of bipolar disorder were identified following extensive ascertainment and diagnostic assessment and matched to 170 controls in the analysis. Serological documentation of maternal exposure to influenza was determined using the hemagglutination inhibition assay.

Results—There was no association between serologically documented maternal exposure to influenza and bipolar disorder in offspring. However, maternal serologic influenza exposure was related to a significant, fivefold increased risk of bipolar disorder with psychotic features.

Conclusions—These results suggest that maternal influenza exposure may increase the risk for the offspring developing bipolar disorder with psychotic features. Taken together with earlier associations between prenatal influenza exposure and schizophrenia, this may suggest that prenatal influenza is a risk factor for psychosis, rather than for a specific psychotic disorder diagnosis.

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Dr. Brown takes responsibility for the integrity of the data and the accuracy of the data analysis, and all authors had full access to all the data in the study. Ms. Yuanyuan Bao performed the statistical analysis for this study.

Introduction

While substantial prior work has supported prenatal exposure to infection as a risk factor for schizophrenia, few studies have examined whether this environmental insult increases risk for other psychiatric syndromes, such as bipolar disorder (1). Although previous studies relying on ecologic data on influenza suggested an association between prenatal infection and bipolar disorder, these findings were limited by exposure misclassification (2, 3), as reviewed in a previous publication (4). Recently, these limitations were circumvented by a nested case-control study which demonstrated that clinical diagnosis with influenza during gestation was associated with a significant, fourfold increased risk of bipolar disorder among offspring (4).

In the present study, we examined the relationship between maternal influenza and bipolar disorder by quantifying influenza antibody in maternal serum specimens from these pregnancies. This method offers certain advantages to clinical diagnoses of influenza, which may be missed if mothers chose not to seek treatment or were asymptomatic. Consequently, we examined influenza antibody titers in prospectively drawn archived maternal serum samples from pregnancies of offspring with and without bipolar disorder, similar to the methods of a previous study that found an association between serologic evidence of influenza exposure during pregnancy and schizophrenia in offspring (5). In the study cited above on maternal influenza and bipolar disorder (4), we reported a nearly sixfold increase in risk of bipolar disorder with psychotic features in offspring, but a much weaker, twofold elevated risk of bipolar disorder without psychotic features, which fell short of statistical significance (unpublished data). These findings suggested that maternal influenza may be a risk factor for offspring psychosis apart from a traditional psychiatric diagnosis. Consequently, we separately examined the relationship between serologically documented maternal influenza exposure and bipolar disorder with, and without, psychotic features, applying Bonferroni correction to adjust for multiple comparisons.

Methods

Description of Cohort

The study is based on a nested case-control design, described in detail in a previous publication (4). Cases and controls were identified following longitudinal follow-up from the Child Health and Development Study birth cohort (5, 6). Briefly, the cohort is a representative sample which consists of all offspring of pregnant women receiving obstetric care from the Kaiser Permanente Medical Care Plan, Northern California Region, in Alameda County, California, born from 1959–1966. Maternal serum samples from virtually all gravidae were prospectively collected, frozen, and archived in a single biorepository. Although all gravidae in the cohort were instructed to obtain blood draws at least once during each trimester, there was variability in the sampling periods. The distribution of serum samples for each pregnancy is provided in Supplementary Table 2. The blood drawing was not deliberately timed with influenza symptoms as serologic methods are not used to diagnose influenza.

Definition of Exposure

Serologic evidence of influenza exposure was defined as the first occurrence during pregnancy of an influenza antibody titer of 1:20 using the hemagglutination inhibition assay, based on a previous validation study of influenza and schizophrenia using archived maternal serum specimens in this birth cohort (5).

Hemagglutination Inhibition Assay

Influenza strains A/Japan/170/62(H2N2) and A/Taiwan/1/64(H2N2) were obtained from the Biodefense and Emerging Infections Research Resources Repository to assay serum influenza titers using a standard protocol (7). These viruses were selected as they were prevalent during the years of the pregnancies (5). These viruses were propagated in Madin-Darby Canine Kidney cells (American Type Culture Collection number CCL-34) and verified by sequencing (GENEWIZ DNA sequencing services) to ensure that no mutations conferring resistance to neuraminidase inhibitors were introduced during propagation. Sera were thawed and an aliquot was heat-inactivated at 56°C for 30 minutes and then stored at -20°C until testing. Sera were treated with Receptor Destroying Enzyme II (Accurate Chemical & Scientific Corporation, Westbury, NY) by incubating in a 37°C water bath for 16–20 hours and again heat-inactivated at 56°C for 30 minutes. All available serum samples from each subject were assayed in duplicate in a 96-well plate format. Serial twofold dilutions of serum from 1:5–1:2560 were prepared, and an equal volume of standardized antigens (4 HA units) was added and incubated for 20 minutes at room temperature, after which 0.05 ml 0.5% turkey red blood cells (Bio Link Inc., Liverpool, NY) were added and incubated for 45 minutes at room temperature. The assay titer was determined as the reciprocal of the highest dilution of serum which completely inhibits hemagglutination. If duplicate samples differed by a factor of >2, those samples were retested. As another quality assurance measure, serial twofold dilutions (1:40–1:640) of pooled sera from donors who showed >1:80 titer against both influenza strains A/Japan/170/62(H2N2) and A/Taiwan/1/64(H2N2) were used as a positive control in later assays.

Case Ascertainment and Diagnosis

Cases with potential DSM-IV bipolar disorder were ascertained via screening procedures in three sources: the Kaiser electronic database, the Alameda County Behavioral Health Care Services database, and a mailed survey to all living mothers and children in the birth cohort. The flow chart of the ascertainment and diagnosis of cases is provided in Supplemental Figure 1.

Ascertainment through Kaiser

Potential cases were identified by screening the inpatient and outpatient databases of Kaiser. The inpatient database included all psychiatric hospitalizations of Kaiser members, whether in Kaiser or non-Kaiser hospitals, and covered the period from 1981–2010 (maximum duration of follow-up was 29 years). Subjects from the inpatient and outpatient databases screened positive for potential bipolar disorder based on discharge diagnoses of ICD-9 295–298 excluding unipolar major depressive disorder. A comprehensive electronic database of outpatient treatment was introduced in 1995. Case ascertainment was complemented by the

Kaiser outpatient pharmacy database, which began in 1992. Cases screened positive from this source based on prescriptions for mood stabilizing medications (lithium, carbamazepine, valproic acid).

Ascertainment through Alameda County

Subjects with potential bipolar disorder treated as outpatients were also ascertained by electronic record linkage between the cohort and Alameda County identifiers; the database included treatment from 1993–2009, and thus the maximum duration of follow-up from this source was 16 years. These subjects screened positive based on ICD-9 outpatient diagnoses of 295–298, excluding unipolar major depressive disorder.

Ascertainment through the Birth Cohort by Mailed Questionnaire and Follow-Up

The third method of ascertainment was initiated by letters mailed to all living mothers (N=6,971) and cohort members (N=13,009) with known addresses in the entire cohort along with a mental and physical health questionnaire. This protocol was conducted from 2009–2011. Questionnaire respondents who reported “mental health problems” in an eligible cohort member (including the respondent him or herself and family members) were contacted by a trained Kaiser study interviewer (see “Diagnostic Protocol”), who administered the Family Interview for Genetic Studies to screen for possible bipolar disorder or psychotic illness in the cohort member. If the interview indicated at least one bipolar and/or psychotic symptom, then the cohort member was considered to have screened positive.

Subjects identified by any of these methods were invited to participate in the study. Repeat appointments were scheduled for subjects who failed to attend their scheduled interviews. Extensive efforts were made to locate all individuals; searches utilized Department of Motor Vehicles records, telephone directories, and the subjects’ parents or siblings.

The total number of potential cases of major psychiatric disorder ascertained from these three sources was 448.

Diagnostic Protocol

We sought all potential cases from the above ascertainment procedures to schedule a diagnostic interview using the Structured Clinical Interview for DSM-IV-TR (SCID) (Supplementary Figure 1). Study interviewers had a minimum of a master’s degree in a mental health field and were trained to reliability. DSM-IV-TR diagnoses, including diagnostic qualifiers representing subtypes of bipolar disorder, were systematically assigned by consensus of three experienced doctoral-level clinicians based on review of the SCID and medical records. This protocol yielded 72 total cases.

Ascertainment from PDS I Study

Additional cases ascertained through Kaiser records by an earlier study (Prenatal Determinants of Schizophrenia I, PDS I) (6) were included. Although the purpose of PDS I was to identify schizophrenia spectrum disorder cases, bipolar disorder cases were also diagnosed by interview. The protocol for the PDS I study included the same electronic

linkages with the Kaiser inpatient, outpatient, and pharmacy databases, and utilized the same ICD-9 diagnostic codes as in the present study. Ascertainment covered the period from 1981–1998. The Diagnostic Interview for Genetic Studies, rather than the SCID, was used to diagnose bipolar disorder cases in the PDS I; these two interviews are very similar with regard to assessment of psychotic and major affective disorders. The PDS I study yielded 23 cases. Combined, these two protocols yielded a total of 95 cases.

After complete description of the study to the subjects, written informed consent was obtained. The study protocol was approved by the Institutional Review Boards of the New York State Psychiatric Institute and Kaiser.

Selection of Matched Controls

We first excluded the birth cohort members who screened positive for potential bipolar disorder or schizophrenia spectrum disorders (N=448). All siblings of cases were excluded from potential controls. Controls were matched to cases on membership in Kaiser (for cases ascertained through Kaiser records) or residence in Alameda County (for cases ascertained through Alameda records or the birth cohort mailing) in the year the case was first treated as reported in the SCID/Diagnostic Interview for Genetic Studies. Siblings of selected controls were excluded from further control selection, so that all controls were independent observations, each representing a single family or pregnant woman.

The other matching criteria were date of birth (± 30 days), sex, and gestational timing/availability of maternal archived sera. Initially, an 8:1 ratio of controls to cases was used, as it represented the maximum number of controls that could be successfully matched to cases on all criteria and maximized statistical power. This protocol yielded 754 matched controls; the cases and controls formed 95 matched sets.

Description of the Analytic Sample

Of the initial 95 bipolar disorder cases, two were siblings; one of these was excluded at random, since these two cases represented non-independent observations, resulting in 94 cases. Eight matched controls corresponding to the excluded case were also excluded, resulting in 746 matched controls. Of the 94 cases, 85 had maternal archived sera available for the present study. The two controls from each matched set that most closely matched the case with regard to trimester of each serum draw were selected. Thus, 85 cases and 170 controls comprised the analytic sample for this study and all were assayed for influenza antibody. These 85 cases included 36 with psychotic features and 49 without psychotic features. Although we did not analyze the data by other bipolar disorder subtypes, 71 had bipolar disorder I, 10 had bipolar disorder II and 4 had bipolar disorder NOS.

Statistical Analysis

Point and interval estimates of odds ratios were obtained by fitting conditional logistic regression models for matched sets. Statistical significance was judged at $\alpha = 0.05$. For analyses of bipolar disorder with and without psychotic features, Bonferroni correction was applied; given that there were three primary analyses—bipolar disorder and bipolar disorder

with and without psychotic features—the Bonferroni-corrected p-value for significance was set at $p=0.0167$.

Covariates

Potential confounders were identified in the literature (8), including maternal age, race, education, and psychiatric history. Each of these covariates, with the exception of maternal psychiatric history, was obtained from a maternal interview administered by the Child Health and Development Study during pregnancy; this last covariate was obtained from Kaiser maternal medical records. Categories and definitions of each covariate are provided in Tables 1 and 2; we also included gestational age in the tables for descriptive purposes. Bivariate analyses were conducted to determine the association between each of these covariates and the outcome, as well as serological influenza exposure; the criteria for adjustment in the models were associations between the covariate and both the outcome and the exposure ($p < 0.1$). Statistical analyses were performed using SAS 9.2© (SAS Institute Inc., Cary, NC, USA).

Results

Sample Characteristics

Covariates related to case or control status—No covariates tested were related to case or control status (Table 1).

Covariates related to serological influenza exposure—Serological evidence of maternal influenza exposure was significantly associated with maternal race ($p=0.01$, Table 2) with exposed mothers more likely to be black and unexposed mothers more likely to be white. There was also a trend for exposed mothers to be slightly younger ($p=0.06$).

Serological Influenza Exposure and Bipolar Disorder in Offspring

Maternal influenza exposure and bipolar disorder—In the analysis of all bipolar disorder cases, there was no increased risk among offspring of mothers with serological documentation of influenza exposure at any time during pregnancy [Odds Ratio (OR)=1.26 (95% CI, 0.65–2.44), $p=0.49$, Table 3]. There were also no trimester-specific associations of influenza and bipolar disorder (Supplementary Table 1a).

Maternal influenza exposure and bipolar disorder with and without psychotic features—Offspring of mothers with serological documentation of influenza exposure at any time during pregnancy had a fivefold increased risk of bipolar disorder with psychotic features [OR=5.03 (95% CI, 1.38–18.38), $p=0.015$, Table 3]. This association was statistically significant after applying Bonferroni correction to adjust for multiple comparisons (see “Statistical Analysis”). Although no covariate was related to both influenza and bipolar disorder, for further assurance, we adjusted for maternal race and maternal psychiatric history in the analysis of maternal influenza at any time during pregnancy and bipolar disorder with psychotic features. The association persisted [OR=4.87 (95% CI, 1.18–20.06), $p=0.028$]. Increases in risk were observed for the first and second trimesters, although each fell short of statistical significance (Table 4), possibly due to small

sample sizes. There were no significant associations between maternal influenza exposure and bipolar disorder without psychotic features (Table 3 and Supplementary Table 1b).

Discussion

The major finding of this study is that serologically documented maternal influenza exposure is related to a fivefold increased risk of bipolar disorder with, but not without, psychotic features. In our previous study in this same birth cohort, a clinical diagnosis of maternal infection was associated with a similar, nearly sixfold and statistically significant association with bipolar disorder with psychotic features [OR=5.74 (95% CI, 1.52 – 21.72), $p<0.01$] (4). In contrast, the increased risk for bipolar disorder without psychotic features in that study was less than threefold and non-significant [OR=2.81 (95% CI, 0.84–9.35), $p=0.092$] (unpublished results). It is intriguing that the use of two independent methods of prospective assessment of maternal influenza exposure (clinical versus antibody) yielded convergent, significant results with similar odds ratios for bipolar disorder with psychotic features. Since prenatal influenza has been previously associated with schizophrenia, a disorder characterized in large part by psychotic episodes such as hallucinations and delusions, our results support the hypothesis that maternal influenza exposure may preferentially increase risk for psychosis apart from traditional diagnostic categories (1). Even though the association between maternal influenza exposure and bipolar disorder without psychotic features does not attain statistical significance *per se*, such early life infectious insults might nevertheless ‘prime’ latent pathologies (or risks) that could be unmasked by other adverse factors. Hence, prenatal influenza infection could be viewed as a ‘general disease primer’. The adverse effects of infection may reflect an early entry into a neuropathological process, but the specificity of subsequent disease could be strongly influenced by the genetic or environmental context (9, 10).

As noted above, our previous study demonstrated a significant association between clinical diagnosis of maternal influenza and all cases of bipolar disorder (4) (with/without psychotic features combined) while serologic evidence of influenza was not related to this outcome in the present study. This discrepancy may have been due to the difference in methods of ascertaining influenza. However, the results are largely concordant between the two methods of assessing exposure status [ten of the thirteen (76.9%) of women who had a clinical diagnosis of influenza were seropositive]. Nonetheless, clinical assessment and serologic measures each have their own relative strengths with regard to diagnosis of influenza and thus in surveillance studies they are used in combination (11). Consequently, in order to more completely ascertain influenza exposure, we conducted an exploratory analysis, which combined results from these two diagnostic approaches into a single composite measure and examined its association with risk for bipolar disorder and the psychotic/non-psychotic subtypes in all 85 cases and 168 controls with both clinical and serological data. In this analysis, a gravida was considered to have been exposed to influenza based on the presence of either a maternal influenza antibody titer ≥ 20 or a clinical diagnosis of maternal influenza. This analysis confirmed the significant association between maternal influenza exposure and bipolar disorder with psychotic features, but no association was found for all cases or cases without psychotic features (Table 5).

If the association between maternal exposure to influenza and bipolar disorder is stronger for bipolar disorder with, versus without, psychotic features, this adds to a growing literature documenting neurobiological differences between these two subtypes. For example, kynurenic acid levels were significantly increased in cerebrospinal fluid of bipolar disorder patients with psychotic episodes, while no differences were found for cases without psychosis (12). As kynurenic acid augments the dopaminergic system, this finding is consistent with prior work suggesting a hyperactive dopaminergic system specifically in bipolar disorder patients with psychotic features. For example, a previous positron emission tomography imaging study demonstrated greater dopamine D2 striatal receptor binding in bipolar disorder patients with versus without psychotic features (13). Furthermore, animal models suggest that prenatal maternal influenza infection and immune activation cause dopaminergic hyperactivity (14–18).

The study had several strengths, including a prospectively obtained maternal serologic biomarker for influenza from archived specimens drawn during pregnancy, cases from a population-based birth cohort, directly administered research assessments using standard interviews, and controls who were representative of the source population from which the cases were drawn. In addition to greater diagnostic validity, the detailed diagnostic assessments permitted differentiation of psychotic from non-psychotic features in this sample, which is not possible in studies of psychiatric registries.

One limitation is that we cannot conclusively distinguish influenza infection prior to pregnancy from infection during pregnancy, given that antibody can remain elevated for several months following infection, and pre-pregnancy serum samples were not available. This may result in some portion of pregnancies being misclassified as having been exposed during pregnancy. However, we consider it most likely that maternal influenza exposure during pregnancy was primarily responsible for the increased risk of bipolar disorder with psychotic features. First, in this same birth cohort we observed a similar magnitude of association for clinically diagnosed maternal influenza, which occurred exclusively during pregnancy, and bipolar disorder with psychotic features. Second, influenza infection during pregnancy is a more biologically plausible disruptor of fetal nervous system development than residual elevated antibody from preconceptional exposure (1, 19). The presence of these ‘false positives’ most probably decreased the magnitude of an association between maternal influenza exposure and offspring bipolar disorder because classification of the timing of exposure is likely to have been non-differential with regard to outcome status, which occurred many years after the pregnancies. To more rigorously examine whether pregnancy is the critical period for influenza infection exposure, we compared the proportions of subjects whose status changed from seronegative to seropositive between successive blood draws among mothers of cases and matched controls with at least two prenatal maternal serum samples. Three of 26 (11.54%) cases of bipolar disorder with psychotic features versus 3 of 52 (5.77%) matched controls had mothers who evidenced these changes in antibody status [OR=2.38 (95% CI, 0.38 – 14.97), $p=0.36$]. Although this result is not statistically significant, possibly due to a loss of power from the considerably decreased sample size, the finding supports the hypothesis.

A second limitation is the potential for bias due to loss to follow-up. For bias to have occurred, however, loss to follow-up would need to be related both to maternal influenza exposure and to bipolar disorder with psychotic features, which does not seem plausible. Moreover, our ascertainment method captured both cases who remained in Kaiser, and those who left Kaiser before they could be ascertained, representing an improvement on our previous follow-up study of schizophrenia in this cohort (5). Finally, the serum samples were frozen for more than 30 years, which could compromise protein stability. However, the samples were uniformly stored and handled, and cases and controls were matched on date of birth and trimester of serum draws.

Although replication in independent samples is essential, these findings imply that prevention of influenza exposure during pregnancy might decrease the incidence of bipolar disorder with psychotic features in the population. While bipolar disorder and schizophrenia are considered separate disorders in the DSM and ICD diagnostic classification systems, our results suggest that psychotic symptoms in both syndromes may share a common etiology. This provides further evidence that parsing bipolar disorder cases into those with and without psychotic symptoms may be another meaningful way to reduce the heterogeneity of this disorder in order to provide insight into particular neurobiological systems that may be disturbed. Moreover, studies of maternal infection and other environmental factors may provide a new strategy to shed light on the Kraepelinian dichotomy of psychosis, and provide further rationale for new biologically-based approaches, such as the Research Domain Criteria (20), to improve the classification and understanding of psychiatric disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographic characteristics and bipolar disorder

	Cases (N=85)			Controls (N=170)		
	Mean	SD	Mean	SD	P	
Maternal Age (yrs) ¹	27.5	6.6	28.2	6.0	0.37	
Gestational Age (days) ¹	281.4	16.3	280.5	16.5	0.68	
	N	%	N	%	P	
Maternal Race ¹					0.20	
White	58	69.0	109	64.1		
Black	22	26.2	41	24.1		
Other	4	4.8	20	11.8		
Maternal Education ²					0.64	
Less than high school	16	20.2	26	16.1		
High school graduate	30	38.0	59	36.7		
Some college/college graduate	33	41.8	76	47.2		
Maternal Psychiatric History ^{4, 3}					0.33	
Yes	22	26.2	35	20.7		
No	62	73.8	134	79.3		

^aMaternal psychiatric disorder was defined as psychoses, schizophrenia, affective disorder, anxiety, alcohol/substance abuse, mental deficiency, and other mental disorders

¹Missing for 1 case

²Missing for 6 cases, 9 controls

³Missing for 1 case, 1 control

Table 2

Demographic characteristics and serologic evidence of influenza exposure

	Exposed to Influenza (N=63)			Unexposed to Influenza (N=192)		
	Mean	SD		Mean	SD	P
Maternal Age (yrs) ¹	26.7	6.0		28.4	6.2	0.06
Gestational age (days) ²	278.6	15.4		281.5	16.7	0.23
	N	%		N	%	P
Maternal Race ¹						0.01
White	32	50.8		135	70.7	
Black	24	38.1		39	20.4	
Other	7	11.1		17	8.9	
Maternal Education ³						0.97
Less than high school	10	17.9		32	17.4	
High school graduate	20	35.7		69	37.5	
Some college/college graduate	26	46.4		83	45.1	
Maternal Psychiatric History ^{4, 4}						0.95
Yes	14	22.2		43	22.6	
No	49	77.8		147	77.4	

^aMaternal psychiatric disorder was defined as psychoses, schizophrenia, affective disorder, anxiety, alcohol/substance abuse, mental deficiency, and other mental disorders¹Missing 1 unexposed²Missing 1 exposed³Missing 7 for exposed, 8 unexposed⁴Missing 2 unexposed

Table 3
Serologic evidence of maternal exposure to influenza and risk of bipolar disorder in offspring

	Cases				Controls				Odds Ratio	95% CI	P
	Total		Exposed		Total		Exposed				
	N	%	N	%	N	%	N	%			
Bipolar Disorder	85	23	27.1		170	40	23.5	1.26	0.65–2.44	0.49	
Bipolar Disorder with Psychotic Features	36	14	38.9		72	13	18.1	5.03	1.38–18.38	0.015	
Bipolar Disorder without Psychotic Features	49	9	18.4		98	27	27.6	0.54	0.21–1.36	0.19	

Serologic evidence of maternal exposure to influenza by trimester and risk of bipolar disorder with psychotic features in offspring

Table 4

	Cases				Controls				Odds Ratio	95% CI	P
	Total		Exposed		Total		Exposed				
	N	%	N	%	N	%	N	%			
Gestational Timing of Influenza Exposure											
First Trimester	21	7	33.3	42	6	14.3	3.36	0.83–13.55	0.09		
Second Trimester	30	6	20.0	60	5	8.3	4.00	0.77–20.87	0.10		
Third Trimester	24	1	4.2	48	2	4.2	1.00	0.05–18.92	1.00		

Composite measure of exposure to maternal influenza during pregnancy and risk of bipolar disorder in offspring^a.

Table 5

	Cases				Controls				Odds Ratio	95% CI	P
	Total		Exposed		Total		Exposed				
	N	%	N	%	N	%	N	%			
Bipolar Disorder	85	27	31.8	42	168	25.0	1.52	0.79–2.91	0.21		
Bipolar Disorder with Psychotic Features	36	15	41.7	71	13	18.3	5.39	1.49–19.46	0.01		
Bipolar Disorder without Psychotic Features	49	12	24.5	97	29	29.9	0.73	0.30–1.74	0.47		

^aDefined as serologic evidence of maternal exposure to influenza or a clinical diagnosis of influenza during pregnancy