Article

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

Sarah E. Canetta, Ph.D.

Yuanyuan Bao, M.S.

Mary Dawn T. Co, M.D.

Francis A. Ennis, M.D.

John Cruz, B.S.

Masanori Terajima, M.D., Ph.D.

Ling Shen, Ph.D.

Christoph Kellendonk, Ph.D.

Catherine A. Schaefer, Ph.D.

Alan S. Brown, M.D., M.P.H.

Objective: The authors examined whether serologically confirmed maternal exposure to influenza was 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 used a nested case-control design in the Child Health and Development Study birth cohort. In all, 85 individuals with bipolar disorder were identified following extensive ascertainment and diagnostic assessment and matched to 170 comparison subjects in the analysis. Serological documentation of maternal exposure to influenza was determined using the hemagglutination inhibition assay.

Results: No association was observed between serologically documented maternal exposure to influenza and bipolar disorder in offspring. However, maternal serological influenza exposure was related to a significant fivefold greater risk of bipolar disorder with psychotic features.

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

(Am J Psychiatry 2014; 171:557–563)

While substantial research has supported prenatal exposure to infection as a risk factor for schizophrenia, few studies have examined whether this environmental insult increases the risk for other psychiatric syndromes such as bipolar disorder (1). Although previous studies relying on ecological data on influenza have 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 greater risk of bipolar disorder among offspring (4).

In the present study, we examined the relationship between maternal influenza and bipolar disorder by quantifying influenza antibodies 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 that produced offspring with and without bipolar disorder, similar to the methods of a previous study (5) that found an association between serological evidence of influenza exposure during pregnancy and schizophrenia in offspring. In the study cited above on maternal influenza and bipolar disorder (4), we reported a nearly sixfold greater risk of bipolar disorder with psychotic features in offspring, but a much weaker twofold greater 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 psychosis in offspring apart from a traditional psychiatric diagnosis. Consequently, we examined separately the relationships between serologically documented maternal influenza exposure and bipolar disorder with and without psychotic features in offspring, applying the Bonferroni correction to adjust for multiple comparisons.

Method

Description of Cohort

The study is based on a nested case-control design that has been previously described in detail (4). Case and comparison subjects were identified after longitudinal follow-up from the Child Health and Development Study birth cohort (5, 6). Briefly, the cohort is a representative sample that consists of all offspring of pregnant women receiving obstetric care from the Kaiser Permanente Medical Care Plan, Northern California Region, in

This article is the subject of a CME course (p. 593) and is discussed in an Editorial by Dr. Meyer (p. 485)

Alameda County, Calif., born from 1959 to 1966. Maternal serum samples from virtually all gravidae were prospectively collected, frozen, and archived in a single biorepository. Although all pregnant women 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 Table S1 in the data supplement that accompanies the online edition of this article. The blood drawing was not deliberately timed with influenza symptoms, as serological methods are not used to diagnose influenza.

Definition of Exposure

Serological evidence of influenza exposure was defined as the first occurrence during pregnancy of an influenza antibody titer ≥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). The viruses were selected because 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 and Scientific Corporation, Westbury, N.Y.) 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 individual 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, N.Y.) were added and incubated for 45 minutes at room temperature. The assay titer was determined as the reciprocal of the highest dilution of serum that 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

Case subjects with potential DSM-IV bipolar disorder were ascertained via screening procedures from 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 case subjects is provided in Figure S1 in the online data supplement.

Ascertainment through Kaiser. Potential case subjects 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 to 2010 (maximum duration of follow-up was 29 years). Individuals from the inpatient and outpatient databases screened positive for potential bipolar disorder based on discharge diagnoses of ICD-9 codes 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. Individuals screened positive from this source if they had prescriptions for mood-stabilizing medications (lithium, carbamazepine, and valproic acid).

Ascertainment through Alameda County. Individuals 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 to 2009, and the maximum duration of follow-up from this source was 16 years. These individuals screened positive based on ICD-9 outpatient diagnosis codes 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 to 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 section) who administered the Family Interview for Genetic Studies (8) to screen for possible bipolar disorder or psychotic illness in the cohort member. If the interview indicated at least one bipolar or psychotic symptom, then the cohort member was considered to have screened positive.

Individuals identified by any of these methods were invited to participate in the study. Repeat appointments were scheduled for individuals who failed to attend their scheduled interviews. Extensive efforts were made to locate all individuals, including searches of Department of Motor Vehicles records, telephone directories, and 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 case subjects from the above ascertainment procedures to schedule a diagnostic interview using the Structured Clinical Interview for DSM-IV-TR (SCID) (see Figure S1 in the online data supplement). 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 case subjects.

Ascertainment from the Prenatal Determinants of Schizophrenia Study. Additional case subjects ascertained through Kaiser records by an earlier study (Prenatal Determinants of Schizophrenia; PDS) (6) were included. Although the purpose of the PDS study was to identify schizophrenia spectrum disorder case subjects, bipolar disorder case subjects were also diagnosed by interview. The protocol for the PDS study included the same electronic linkages with the Kaiser inpatient, outpatient, and pharmacy databases, and it used the same ICD-9 diagnostic codes as in the present study. Ascertainment covered the period from 1981 to 1998. The Diagnostic Interview for Genetic Studies (9), rather than the SCID, was used to diagnose bipolar disorder case subjects in the PDS study; these two interviews are very similar with regard to assessment of psychotic and major affective disorders. The PDS study yielded 23 case subjects. Combined, these two protocols yielded a total of 95 case subjects.

TABLE 1. Characteristics of Analytic Sample in a Study of Maternal Influenza and Bipolar Disorder in Offspring

Characteristic	Case Subjects (N=85)		Comparison Subjects (N=170)		Analysis
	Mean	SD	Mean	SD	p
Maternal age (years)[a]	27.5	6.6	28.2	6.0	0.37
Gestational age (days)[a]	281.4	16.3	280.5	16.5	0.68
	N	%	N	%	p
Maternal race[a]					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[b]					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[c]					0.33
Yes	22	26.2	35	20.7	
No	62	73.8	134	79.3	

[a] Data missing for one case subject.
[b] Data missing for six case subjects and nine comparison subjects.
[c] Maternal psychiatric disorder was defined as psychoses, schizophrenia, affective disorder, anxiety, alcohol/substance abuse, mental deficiency, or other mental disorders. Data missing for one case subject and one comparison subject.

Participants provided written informed consent after receiving a complete description of the study. The study protocol was approved by the institutional review boards of the New York State Psychiatric Institute and Kaiser.

Selection of Matched Comparison Subjects

We first excluded the birth cohort members who screened positive for potential bipolar disorder or schizophrenia spectrum disorders (N=448). All siblings of case subjects were excluded from potential comparison subjects. Comparison subjects were matched to case subjects 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 patient was first treated as reported in the SCID/Diagnostic Interview for Genetic Studies. Siblings of selected comparison subjects were excluded from further control selection, so that all comparison subjects 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 or availability of maternal archived sera. Initially, an 8:1 ratio of comparison subjects to case subjects was used, as it represented the maximum number of comparison subjects that could be successfully matched to case subjects on all criteria and maximized statistical power. This protocol yielded 754 matched comparison subjects; the case and comparison subjects formed 95 matched sets.

Description of the Analytic Sample

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

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, the 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 0.0167.

Covariates

Potential confounders were identified in the literature (10), 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, Cary, N.C.).

Results

Sample Characteristics

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

TABLE 2. Characteristics and Serological Evidence of Influenza Exposure in a Study of Maternal Influenza and Bipolar Disorder in Offspring

Characteristic	Exposed to Influenza (N=63)		Unexposed to Influenza (N=192)		Analysis
	Mean	SD	Mean	SD	p
Maternal age (years)[a]	26.7	6.0	28.4	6.2	0.06
Gestational age (days)[b]	278.6	15.4	281.5	16.7	0.23
	N	%	N	%	p
Maternal race[a]					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[c]					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[d]					0.95
Yes	14	22.2	43	22.6	
No	49	77.8	147	77.4	

[a] Data missing for one unexposed individual.
[b] Data missing for one exposed individual.
[c] Data missing for seven exposed and eight unexposed individuals.
[d] Maternal psychiatric disorder was defined as psychoses, schizophrenia, affective disorder, anxiety, alcohol/substance abuse, mental deficiency, and other mental disorders. Data missing for two unexposed individuals.

TABLE 3. Serological Evidence of Maternal Exposure to Influenza and Risk of Bipolar Disorder in Offspring

	Case Subjects			Comparison Subjects					
	Total	Exposed		Total	Exposed		Analysis		
Disorder	N	N	%	N	N	%	Odds Ratio	95% CI	p
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

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. Exposed mothers were slightly younger, but this relationship did not reach significance.

Serological Influenza Exposure and Bipolar Disorder in Offspring

Maternal influenza exposure and bipolar disorder. In the analysis of all bipolar disorder case subjects, no increase in risk was found among offspring of mothers with serological documentation of influenza exposure at any time during pregnancy (odds ratio=1.26, 95% confidence interval [CI]=0.65–2.44, p=0.49; Table 3). Additionally, no trimester-specific associations of influenza and bipolar disorder were found (see Table S2a in the online data supplement).

Maternal influenza exposure and bipolar disorder with and without psychotic features. The offspring of mothers with serological documentation of influenza exposure at any time during pregnancy had a fivefold greater risk of bipolar disorder with psychotic features (odds ratio=5.03, 95% CI=1.38–18.38, p=0.015; Table 3). This association was significant after applying the Bonferroni correction to adjust for multiple comparisons. Although no covariate was related to both influenza and bipolar disorder, for further assurance we adjusted for maternal race and maternal psychiatric history, and the association persisted (odds ratio=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 as a result of small sample sizes. No significant associations were observed between maternal influenza exposure and bipolar disorder without psychotic features (Table 3 and see Table S2b in the online data supplement).

Discussion

The major finding of this study is that serologically documented maternal influenza exposure is related to a fivefold greater risk of bipolar disorder with, but not without, psychotic features. In our previous study in this same birth cohort (4), a clinical diagnosis of maternal influenza was significantly associated with a nearly sixfold

TABLE 4. Serological Evidence of Maternal Exposure to Influenza by Trimester and Risk of Bipolar Disorder With Psychotic Features in Offspring

	Case Subjects			Comparison Subjects			Analysis		
	Total	Exposed		Total	Exposed				
Gestational Timing of Influenza Exposure	N	N	%	N	N	%	Odds Ratio	95% CI	p
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

TABLE 5. Composite Measure of Exposure to Maternal Influenza During Pregnancy and Risk of Bipolar Disorder in Offspring[a]

	Case Subjects			Comparison Subjects			Analysis		
	Total	Exposed		Total	Exposed				
Disorder	N	N	%	N	N	%	Odds Ratio	95% CI	p
Bipolar disorder	85	27	31.8	168	42	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

[a] Defined as serological evidence of maternal exposure to influenza or a clinical diagnosis of influenza during pregnancy.

greater risk of bipolar disorder with psychotic features (odds ratio=5.74, 95% CI=1.52–21.72, $p<0.01$). In contrast, the risk for bipolar disorder without psychotic features in that study was substantially lower and did not reach significance (odds ratio=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 compared with antibody) yielded convergent and significant results with similar odds ratios for bipolar disorder with psychotic features. Since prenatal influenza has been previously associated with schizophrenia (1), 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 the risk for psychosis apart from traditional diagnostic categories. Although the association between maternal influenza exposure and bipolar disorder without psychotic features did not reach statistical significance per se, such early life infectious insults may 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 (11, 12).

As noted above, our previous study demonstrated a significant association between clinical diagnosis of maternal influenza and all types of bipolar disorder (with or without psychotic features combined) (4), while serological evidence of influenza was not related to this outcome in our present study. This discrepancy may have been a result of the difference in methods of ascertaining influenza. However, the results are largely concordant between the two methods of assessing exposure status (10 of 13 women [76.9%] who had a clinical diagnosis of influenza were seropositive). Nonetheless, clinical assessment and serological measures each have their own relative strengths with regard to diagnosis of influenza, and thus in surveillance studies they are used in combination (13). 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 and nonpsychotic subtypes in all 85 case subjects and 168 comparison subjects with both clinical and serological data (two comparison subjects without clinical data were not included in this analysis). In this analysis, a pregnant woman was considered to have been exposed to influenza based on the presence of either a maternal influenza antibody titer $\geq$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 observed 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 result adds to a growing literature documenting neurobiological differences between these two subtypes. For example, kynurenic acid levels were significantly increased in the CSF of bipolar disorder patients with psychotic episodes, while no differences were found for bipolar disorder without psychosis (14). As kynurenic acid augments the dopaminergic system, this finding is consistent with previous work suggesting a hyperactive dopaminergic system specifically in bipolar disorder patients with psychotic features. For example, a positron emission tomography imaging study (15) demonstrated greater dopamine D_2 striatal receptor binding in bipolar disorder patients with versus without psychotic

features. Furthermore, animal models suggest that prenatal maternal influenza infection and immune activation cause dopaminergic hyperactivity (16–20).

Our study had several strengths, including a prospectively obtained maternal serological biomarker for influenza from archived specimens drawn during pregnancy, case subjects from a population-based birth cohort, directly administered research assessments using standard interviews, and comparison subjects who were representative of the source population from which the case subjects were drawn. In addition to greater diagnostic validity, the detailed diagnostic assessments permitted differentiation of psychotic from nonpsychotic 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 antibodies can remain elevated for several months following infection, and prepregnancy serum samples were not available. As a result, some portion of pregnancies may have been misclassified as exposed during pregnancy. However, we consider it most likely that maternal influenza exposure during pregnancy was primarily responsible for the greater 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 antibodies from preconceptional exposure (1, 21). The presence of these "false positives" most probably decreased the magnitude of an association between maternal influenza exposure and bipolar disorder in the offspring because classification of the timing of exposure is likely to have been nondifferential 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 case subjects whose status changed from seronegative to seropositive between successive blood draws among mothers of case subjects and matched comparison subjects with at least two prenatal maternal serum samples. Three of 26 case subjects with bipolar disorder with psychotic features (11.54%) compared with three of 52 matched comparison subjects (5.77%) had mothers who evidenced these changes in antibody status (odds ratio=2.38, 95% CI=0.38–14.97, p=0.36). Although this result is not statistically significant, possibly because of a loss of power from the considerably decreased sample size, the finding supports the hypothesis.

A second limitation is the potential for bias as a result of 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 case subjects who both remained in Kaiser and 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 case and comparison subjects 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 may decrease the incidence of bipolar disorder with psychotic features in the population. While bipolar disorder and schizophrenia are considered separate disorders in DSM and ICD diagnostic classification systems, our results suggest that psychotic symptoms in both syndromes may share a common etiology. These results provide further evidence that parsing bipolar disorder case subjects into those with and those 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 (22), to improve the classification and understanding of psychiatric disorders.

Presented in part at the 14th International Congress of Schizophrenia Research, Orlando, Fla., April 21–25, 2013. Received July 17, 2013; revision received Sept. 30, 2013; accepted Oct. 25, 2013 (doi: 10.1176/appi.ajp.2013.13070943). From the Department of Psychiatry, Columbia University College of Physicians and Surgeons, N.Y. State Psychiatric Institute, N.Y.; the Division of Infectious Diseases and Immunology, Department of Medicine, University of Massachusetts Medical School, Worcester, Mass., the Division of Research, Kaiser Permanente, Oakland, Calif.; and the Department of Epidemiology, Mailman School of Public Health, Columbia University, N.Y. Address correspondence to Dr. Brown (asb11@columbia.edu).

Supported by NIMH grants 5R01 MH073080, 5K02 MH065422, 5R01 MH069819, and T32 MH16434-31; National Institute on Child Health and Development grants N01-HD-1-3334 and NO1-HD-6-325; and National Institute of Allergy and Infectious Diseases grant U19 AI-057319.

Dr. Co has received funding from GlaxoSmithKline, the National Institute of Allergy and Infectious Diseases, Sanofi-Pasteur Biologics, and Walter Reed Army Institute of Research. Drs. Ennis, Cruz, and Terajima have received funding from GlaxoSmithKline, the National Institute of Allergy and Infectious Diseases, and Walter Reed Army Institute of Research. The other authors report no financial relationships with commercial interests.

The authors thank Jacky Chow for technical support and Daniel Pilowsky, M.D., Mark McCormick, M.D., and Lauren Ellman, Ph.D., for contributions to the psychiatric diagnoses.

References

1. Brown AS, Derkits EJ: Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. Am J Psychiatry 2010; 167:261–280
2. Boyd JH, Pulver AE, Stewart W: Season of birth: schizophrenia and bipolar disorder. Schizophr Bull 1986; 12: 173–186

3. Machón RA, Mednick SA, Huttunen MO: Adult major affective disorder after prenatal exposure to an influenza epidemic. Arch Gen Psychiatry 1997; 54:322–328
4. Parboosing R, Bao Y, Shen L, Schaefer CA, Brown AS: Gestational influenza and bipolar disorder in adult offspring. JAMA Psychiatry 2013; 70:677–685
5. Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Bresnahan M, Babulas VP, Susser ES: Serologic evidence of prenatal influenza in the etiology of schizophrenia. Arch Gen Psychiatry 2004; 61:774–780
6. Susser ES, Schaefer CA, Brown AS, Begg MD, Wyatt RJ: The design of the Prenatal Determinants of Schizophrenia study. Schizophr Bull 2000; 26:257–273
7. Szretter KJ, Balish AL, Katz JM: Influenza: propagation, quantification, and storage. Curr Protoc Microbiol 2006; Chapter 15:1
8. Maxwell E: The Family Interview for Genetic Studies Manual. Washington, DC, National Institute of Mental Health, Intramural Research Program, Clinical Neurogenetics Branch, 1992
9. Nurnberger JI Jr, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J, Severe JB, Malaspina D, Reich T (NIMH Genetics Initiative): Diagnostic Interview for Genetic Studies: rationale, unique features, and training. Arch Gen Psychiatry 1994; 51: 849–859
10. Tsuchiya KJ, Byrne M, Mortensen PB: Risk factors in relation to an emergence of bipolar disorder: a systematic review. Bipolar Disord 2003; 5:231–242
11. Giovanoli S, Engler H, Engler A, Richetto J, Voget M, Willi R, Winter C, Riva MA, Mortensen PB, Schedlowski M, Meyer U: Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice. Science 2013; 339:1095–1099
12. Lipina TV, Zai C, Hlousek D, Roder JC, Wong AH: Maternal immune activation during gestation interacts with DISC1 point mutation to exacerbate schizophrenia-related behaviors in mice. J Neurosci 2013; 33:7654–7666
13. Killbourne ED: Influenza. New York, Plenum Medical Book Co, 1987
14. Olsson SK, Sellgren C, Engberg G, Landén M, Erhardt S: Cerebrospinal fluid kynurenic acid is associated with manic and psychotic features in patients with bipolar I disorder. Bipolar Disord 2012; 14:719–726
15. Pearlson GD, Wong DF, Tune LE, Ross CA, Chase GA, Links JM, Dannals RF, Wilson AA, Ravert HT, Wagner HN Jr, DePaulo JR: In vivo D2 dopamine receptor density in psychotic and nonpsychotic patients with bipolar disorder. Arch Gen Psychiatry 1995; 52:471–477
16. Vuillermot S, Weber L, Feldon J, Meyer U: A longitudinal examination of the neurodevelopmental impact of prenatal immune activation in mice reveals primary defects in dopaminergic development relevant to schizophrenia. J Neurosci 2010; 30:1270–1287
17. Aguilar-Valles A, Flores C, Luheshi GN: Prenatal inflammation-induced hypoferremia alters dopamine function in the adult offspring in rat: relevance for schizophrenia. PLoS ONE 2010; 5:e10967
18. Meyer U, Feldon J: Prenatal exposure to infection: a primary mechanism for abnormal dopaminergic development in schizophrenia. Psychopharmacology (Berl) 2009; 206:587–602
19. Zuckerman L, Rehavi M, Nachman R, Weiner I: Immune activation during pregnancy in rats leads to a postpubertal emergence of disrupted latent inhibition, dopaminergic hyperfunction, and altered limbic morphology in the offspring: a novel neurodevelopmental model of schizophrenia. Neuropsychopharmacology 2003; 28:1778–1789
20. Ozawa K, Hashimoto K, Kishimoto T, Shimizu E, Ishikura H, Iyo M: Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: a neurodevelopmental animal model of schizophrenia. Biol Psychiatry 2006; 59:546–554
21. Boksa P: Effects of prenatal infection on brain development and behavior: a review of findings from animal models. Brain Behav Immun 2010; 24:881–897
22. Cuthbert BN, Insel TR: Toward the future of psychiatric diagnosis: the seven pillars of RDoC. BMC Med 2013; 11:126