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Maternal serum cytokine levels and risk of bipolar disorder

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Abstract

Prenatal exposure to influenza has previously been associated with increased risk of bipolar disorder (BD), an association that may be mediated by maternal cytokines. The objective of this study was to determine the association between maternal levels of cytokines measured during each trimester of pregnancy and the risk of BD in offspring. We conducted a case-control study nested in the Child Health and Development Study, a birth cohort that enrolled pregnant women in 1959–1966. Potential cases with DSM-IV-TR bipolar I disorder, bipolar II disorder, BD not otherwise specified, and BD with psychotic features were ascertained through electronic medical records, a public agency database, and a mailing to the cohort. Diagnoses were confirmed by clinical interview. Nine cytokines (IL-1 β , IL-4, IL-5, IL-6, IL-8, IL-10, IFN- γ , TNF- α and GM-CSF) were measured simultaneously by Luminex assays in archived prenatal maternal serum samples from 85 cases and 170 matched controls. Data were analyzed using conditional logistic regression. In the overall study sample, there were no significant associations between prenatal maternal cytokine levels and BD after adjustment for confounders. The risk of BD without psychotic features was decreased among subjects with higher maternal levels of first trimester log-transformed IL-4 (OR (95% CI)=0.76 (0.58, 0.98); $p=0.04$) and third trimester log-transformed IL-6 (OR (95% CI)=0.64 (0.42, 0.98); $p=0.04$). In conclusion, higher levels of prenatal maternal cytokines were not associated with increased risk for BD. Further studies with larger samples are necessary to confirm the finding.

Keywords

bipolar disorder; cytokines; immune; prenatal; maternal; IL-4; IL-6

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1. Introduction

Bipolar disorder (BD) is generally thought to result from a combination of genetic and non-genetic factors (1). The potential relevance of exposures occurring during the prenatal period is suggested by structural brain abnormalities associated with BD (2) and by evidence that the risk of BD is increased among offspring of pregnancies affected by obstetric complications (3–5), pre-term birth (6), exposure to famine (7) and maternal smoking (8). Moreover, the overlap in clinical features and genetic risk factors between BD and schizophrenia (9–11) suggests that non-genetic risk factors may be shared as well. Prenatal exposure to maternal infection has been repeatedly identified as a risk factor for schizophrenia (reviewed in (12)). The findings that IL-8 was elevated during the second trimester among mothers of persons with schizophrenia (13) and that TNF- α and IL-8 were elevated in late pregnancy among mothers of those with psychosis (14) indicate that this association may be mediated through immune activation, including altered cytokine levels. Prenatal maternal immune activation has been demonstrated to disrupt neurodevelopment in animal models (reviewed in (15)).

There have been relatively few studies on prenatal exposure to infection and BD (16). Recently, a clinical diagnosis of maternal influenza was associated with a nearly four-fold increased risk of BD in the Child Health and Development Study (CHDS) cohort (17). Serologically documented maternal influenza was later found to confer a fivefold increased risk of BD with psychotic features, but not without psychotic features, in the same cohort (18). Findings from other studies of influenza exposure that were not based on antibody measures in maternal sera have been mixed (19–21). To our knowledge, no previous studies have examined relationships between levels of maternal cytokines and BD.

To determine whether prospectively assayed prenatal maternal cytokine levels are associated with BD in the offspring, we measured 9 cytokines in archived maternal serum samples from different periods during pregnancy in BD cases and matched controls drawn from the CHDS cohort. We hypothesized that the pro-inflammatory cytokines IL-1 β , IL-6, TNF- α , and in particular IL-8, would be elevated in mothers of cases relative to controls. We further hypothesized that this association would be strongest in the second trimester and in BD cases with psychotic features.

2. Methods

2.1. Description of the cohort

Cases and controls were drawn from the Child Health and Development Study (CHDS) cohort (22). Nearly all women receiving prenatal care from the Kaiser Permanente Medical Care Plan, Northern California Region (KPNC) in Alameda County, California from 1959–1966 ($n=19,044$ live births) were recruited to the study. Extensive data were collected prospectively from sources including medical records and maternal interviews. Additionally, biospecimens were obtained and archived, including maternal serum samples from each trimester of pregnancy.

2.2. Case ascertainment and diagnosis

Ascertainment of BD cases has been described in depth elsewhere (18), and is summarized here. Cases with DSM-IV-TR BD, including bipolar I disorder, bipolar II disorder, BD not otherwise specified, and BD with psychotic features, were identified through the following 3 sources: the KPNC electronic medical records database, the Alameda County Behavioral Health Care Services (ACBHCS) database, and a mailing to the entire living CHDS birth cohort (mothers and children).

KPNC inpatient (covering 1981–2010), outpatient (beginning in 1995), and outpatient pharmacy (beginning in 1992) databases were used to screen for CHDS subjects with potential BD and other psychotic disorders through computerized record linkages. Subjects were considered to have potential BD if they met either of the following criteria: inpatient discharge diagnoses of ICD-9 codes 295 through 298; outpatient diagnoses of ICD-9 codes 295 through 298, excluding unipolar major depressive disorder. Potential BD cases also included subjects treated with prescriptions for mood-stabilizing medications used in the treatment of BD (lithium carbonate, carbamazepine, and valproic acid). An additional means of identifying patients was by a mailing to all living cohort members (n=13,009) and mothers (n=6971) with known addresses who had not already been identified in one of the database sources; this was conducted in 2009–2011. The mailing included a questionnaire on mental and physical health, and those reporting “mental health problems” in a cohort member were administered the Family Interview for Genetic Studies. Subjects were considered to have potential BD if the interview indicated the presence of at least one bipolar or psychotic symptom.

Subjects with potential BD as identified above were invited to participate in a clinical interview. Of the 448 subjects with potential BD, 214 were interviewed by qualified, trained study interviewers using the Structured Clinical Interview for DSM-IV-TR (SCID-I/P). The subjects who did not complete an interview consisted of those who could not be contacted, refused to participate, did not keep an interview, or could not be interviewed. Diagnoses were assigned by a consensus of three experienced doctoral-level psychiatric clinicians. This resulted in a total of 72 diagnosed BD cases.

An additional 23 cases with BD identified through KPNC records by an earlier study (Prenatal Determinants of Schizophrenia Study (23)) were also included. These cases were diagnosed using the Diagnostic Interview for Genetic Studies (DIGS) (24). This resulted in a total of 95 BD cases identified in the CHDS birth cohort. One of a pair of siblings with BD was excluded at random. For 85 of these 94 cases there was at least one maternal serum sample from pregnancy available with a sufficient quantity of serum for the analysis. These 85 cases were included in the present study. A schematic diagram of this ascertainment process can be found in (18).

2.3. Selection of matched controls

Controls were initially matched to all 95 confirmed BD cases in an 8:1 ratio. All CHDS cohort members who screened positive for potential BD or schizophrenia spectrum disorders (n=448) were excluded as potential controls. Siblings of selected controls were also

excluded, in order to maintain independence of observations. Matching criteria included: date of birth (+/- 30 days), sex, gestational timing, the availability of archived maternal sera, and membership in KPNC (for cases ascertained through KPNC records) or residence in Alameda County (for cases ascertained through ACBHCS or CHDS mailing) in the year the case was first treated as reported in the SCID/DIGS. Siblings of selected controls were excluded from further control selection to ensure independence of observations. For each of the 85 cases with maternal serum samples available, the two matched controls most closely resembling the case with regard to timing of each serum draw were selected, for a total of 170 controls.

2.4. Measurement of cytokines in maternal serum samples

Serum samples were spun for 5 minutes at 10,000 rpm in a minicentrifuge. The supernatant was assayed for 9 cytokines (IL-1 β , IL-4, IL-5, IL-6, IL-8, IL-10, IFN- γ , TNF- α and GM-CSF) in duplicate. All analytes were assayed simultaneously in a multiplex format using human high sensitivity cytokine/chemokine kits (Milliplex Map Kits, EMD Millipore Corporation, Billerica, MA) according to the manufacturer's instructions. In brief, samples were incubated overnight at 4 C with a mixture of microspheres; each set of spheres is supplied with a single capture antibody specific for one of the analytes. Each microsphere is also internally coded with a unique mixture of fluorescent dyes. The 96 well filter plate was then washed twice by vacuum filtration, incubated for 1 hour at room temperature with biotinylated detection antibodies, washed, and then incubated for 30 minutes at room temperature with streptavidin-phycoerythrin. Plates were read in a Luminex 100 Analyzer (Luminex, Austin, TX) controlled by STarStation 2.0 software (Applied Cytometry, Sheffield, UK). Two fluorescent signals were measured from individual beads; one identified the bead type and the other measured the fluorescent intensity of the bound phycoerythrin. Values for each analyte were determined from a standard curve of log dose versus median fluorescent intensity using a 5 parameter logistic fit.

2.5. Quality control

All samples were analyzed in duplicate, resulting in two measures per subject for each trimester-cytokine combination. Five samples were excluded for all cytokines because data from the duplicate was not available. In order to assess the reliability of the cytokine measurements, we calculated the coefficient of variation (CV; defined as standard deviation/mean, expressed as a percentage) for each pair of replicate measures. Cytokine measures were excluded as not sufficiently reliable for observations where the CV exceeded 50%. This criterion resulted in within-assay CVs that were comparable to previously reported ranges (see below). Observations were additionally excluded from analysis for a given trimester-cytokine combination if there was not at least one case and one control from the matched set remaining following the exclusions listed above. Because controls were matched 2:1 to cases, this resulted in higher percentages of controls than of cases being excluded; nonetheless, the percentages of case and control observations that were excluded for each trimester-cytokine combination were highly correlated ($r^2 = 0.95$). The percentage of observations excluded ranged from 5.0–55.3% depending on the trimester-cytokine combination (details shown in Supplementary Table 1).

The average within-assay CVs for included samples ranged from 8.3% to 21.2%, comparable to previously reported ranges (25, 26). Between-assay CVs calculated for control samples with known cytokine concentrations ranged from 8.6% to 36.9%. This is comparable to ranges of analytic variation previously reported using this assay (26–28).

Following the exclusions listed above, the duplicate measures were averaged, resulting in a single value per subject for each trimester-analyte combination; these averaged values were used for the statistical analyses described below.

2.6. Statistical analyses

Covariates (listed in Table 1) were compared between cases and controls using chi-square tests or Fisher's exact test for categorical covariates, and t-tests for continuous covariates, for the full sample (N=255; 85 cases and 170 controls) and for each trimester. Descriptive statistics (median, mean, standard deviation, range) for the levels of each cytokine in each trimester were calculated separately for cases and controls. Appropriate to the nested case-control study design, crude and adjusted conditional logistic regression models for matched sets were fit for each trimester-cytokine combination. All cytokine levels were log-transformed prior to inclusion in models due to skewed distributions. Where cytokine values were below the limit of detection (LOD), a value of 0.5*LOD was used for analyses. To address potential confounding, adjusted models included covariates associated with case status with $p \leq 0.10$ (maternal smoking and maternal age). To address possible differences in findings between BD with and without psychotic features, analyses were also stratified by the presence of psychotic features in cases. To further test for effect modification by maternal psychiatric history, given prior evidence for interaction between genetic liability and prenatal exposure to infection in the risk of schizophrenia (29), we conducted analyses including terms for maternal psychiatric history (broad) and the product of maternal psychiatric history and cytokine level in each model.

The primary analysis focused on levels of individual cytokines. However, some previous work has suggested the balance (i.e. between pro- and anti-inflammatory) may be more important than the absolute levels of cytokines (30, 31). Therefore, we conducted a secondary analysis by fitting adjusted models as described above, using the ratios of pro-inflammatory (IL-1B, IL-6, IL-8, TNF- α) to anti-inflammatory (IL-10, IL-5) cytokines, log-transformed due to skewness. IL-4, IFN- γ , and GM-CSF were omitted from these analyses due to high numbers of missing values.

All tests were two sided. Statistical significance was judged at $\alpha=0.05$.

3. Results

3.1. Demographic characteristics of cases and controls

Characteristics of the study sample are shown in Table 1. The study sample was 32% male and 68% female. In the total sample (N=255), there were no significant ($p < 0.05$) differences between cases and controls with regard to maternal age, race, education, family income, or maternal psychiatric history (Table 1). Maternal smoking was more prevalent among cases (53%) than controls (39%), as shown previously (8). There were no differences in mean

gestational age at maternal blood draw for cases versus controls in any of the three trimesters. Comparing case and control characteristics for those subjects with serum samples available for each trimester, maternal smoking was more prevalent in first trimester cases versus controls ($p=0.06$); second trimester case mothers were younger than those of controls ($p=0.10$); and maternal smoking was more prevalent in third trimester cases than controls ($p=0.01$) (data not shown).

3.2. Association of trimester-specific maternal cytokine levels with BD

Results are presented here for IL-1 β , IL-5, IL-6, IL-8, IL-10 and TNF- α . The results for the 3 of 9 cytokines for which >30% of observations were excluded (IL-4, IFN- γ and GM-CSF) are available in Supplementary Table 2.

Table 2 shows the median levels of prenatal maternal cytokines for cases and controls and the results of conditional logistic regression analyses of log-transformed cytokine levels (referred to hereafter as “cytokine levels”), by trimester. Additional summary statistics are available in Supplementary Table 3.

Only 3rd trimester IL-6 showed a significant association between maternal cytokine level and BD in the unadjusted conditional logistic regression analysis, with higher IL-6 levels being associated with a decreased risk of BD (OR (95% CI) = 0.72 (0.51, 1.00); $p=0.05$). However, this association did not remain significant after adjustment for potential confounders (OR (95% CI) = 0.74 (0.52, 1.07); $p=0.11$). None of the other cytokines tested showed a significant association between cytokine values and BD in unadjusted or adjusted analyses (Table 2).

IL-4, IFN- γ and GM-CSF were also measured, but are not shown in Table 2 due to the high proportions of observations that did not meet inclusion criteria. Following adjustment, no significant associations were found between levels of these cytokines and BD in any trimester. A marginally significant association between BD and third trimester IFN- γ was observed (OR (95% CI) = 0.70 (0.49, 1.02); $p=0.06$) in the adjusted model, however this was based on only 36 cases and 55 controls.

3.3. Association of maternal cytokine levels with BD by presence of psychotic features

Psychotic features were present in 42% of cases. No significant associations were observed between any trimester-cytokine combination and BD with psychotic features (Table 3). The positive association (increased risk associated with higher cytokine level) of greatest magnitude was for second trimester IL-5 (OR (95% CI)=1.45 (0.92, 2.28); $p=0.11$) and the negative association (decreased risk associated with higher cytokine level) of greatest magnitude was for third trimester TNF- α (OR (95% CI)=0.34 (0.09, 1.23); $p=0.10$).

Fifty-eight percent of cases did not have psychotic features. As shown in Table 3, statistically significant negative associations were observed between BD without psychotic features and first trimester IL-4 (OR (95% CI)=0.76 (0.58, 0.98); $p=0.04$) and third trimester IL-6 (OR (95% CI)=0.64 (0.42, 0.98); $p=0.04$).

3.4. Association of maternal cytokine levels with BD by maternal psychiatric history

The only trimester-cytokine combination for which there was marginally significant evidence for effect modification by maternal psychiatric history was first trimester IL-10 (p-value for interaction=0.06). However, neither of the stratum-specific odds ratios indicated significant association between first trimester IL-10 and risk for BD. Among children of mothers with a history of psychiatric disorders, the OR (95% CI) was 0.13 (0.01, 1.15) and among children of mothers with no history of psychiatric disorders the OR (95% CI) was 1.10 (0.75, 1.61).

3.5. Association of BD with ratios of pro- to anti-inflammatory cytokines

The ratio of IL-6/IL-5 during the first trimester showed a significant negative association with the risk of BD (OR (95% CI)= 0.73 (0.55, 0.98)), signifying that increases in the levels of IL-6 relative to those of IL-5 were associated with decreases in the risk of BD. None of the other ratio measures examined were significantly associated with BD.

4. Discussion

We conducted the first study, to our knowledge, to examine the association between prenatal maternal cytokine levels and the development of BD in the offspring. Although for certain cytokines, notably IL-6 and IL-8, there were considerable differences in magnitude between cases and controls, overall the lack of statistically significant findings is not consistent with the hypothesis that maternal pro-inflammatory cytokines are elevated in case offspring with BD relative to control offspring in the second trimester, or during any trimester of pregnancy. There were also no appreciable associations for most cytokines when the analyses were restricted to BD cases with psychotic features. Lower levels of first trimester IL-4 and third trimester IL-6 were significantly associated with BD without psychotic features.

In earlier studies we found that clinically and serologically documented maternal influenza infection was related to BD in offspring. Given this association we may have expected that maternal cytokines, which become activated following infection with influenza and other pathogens, would have been elevated during pregnancies with cases (17, 18). In an exploratory comparison of cytokine levels between subject mothers with and without prenatal influenza diagnoses (Supplementary Table 4), we observed lower levels of first trimester IFN- γ , IL-10, and IL-4; and of second trimester IL-1 β in samples from mothers who had been diagnosed with influenza. Second and third trimester levels of IL-8 were also notably increased among mothers diagnosed with influenza, although the differences fell short of statistical significance. This is interesting in light of our prior finding that increased maternal IL-8 in the second and third trimesters is associated with increased risk of schizophrenia in the same cohort (13). Thus, it may be that additional immune activity related to influenza infection, rather than elevation of IL-8 alone, is related to the increase in risk for BD. However, because the increase in cytokines following influenza is transient, it is also likely that elevations were not accurately captured at the time of the serum collection. The numbers of maternal influenza diagnoses from our study were insufficient to support comparisons based on the specific timing of diagnosis. Additionally, patterns of cytokine

elevation associated with influenza infection have been observed to vary with virus strain and symptom severity (32). It is also possible that maternal infection influences the risk of BD in offspring through mechanisms other than changes in cytokine levels, such as increases in oxidative stress, or iron and zinc deficiencies (reviewed in (33)).

In a previous study we found that cases with schizophrenia from the same cohort had elevated second trimester maternal IL-8 levels relative to controls (13). Though not statistically significant, our estimates of association between second trimester maternal IL-8 and BD were consistent with a potential positive association. If there is a true difference in maternal IL-8 between BD cases and controls, however, our results suggest that the magnitude of the association is lower than that for IL-8 and schizophrenia. This may have contributed to a lack of statistical power to observe a significant association. In addition, the different methods for measurement of cytokines that were used in our study of schizophrenia (ELISA) and the present study (Luminex) may have contributed to study differences. Misclassification of cytokine levels due to the imprecision in the Luminex measures (see below) may have biased our estimate of association towards the null. Nonetheless, it is also possible that our findings reflect true differences in the immunological abnormalities associated with BD versus schizophrenia.

The only significant associations that we observed following adjustment for confounders were between lower levels of first trimester IL-4 and of third trimester IL-6 and BD without psychotic features. While this finding may have been due to chance given the multiple comparisons, this is not consistent with findings from animal studies, which suggest that the effects of maternal immune activation are mediated largely by excess IL-6 (34). However, IL-6 also plays important developmental and physiological roles including the function of sensory neurons, pain, emotional reactivity, sickness behavior, learning, and memory (35), and thus deficient levels may conceivably contribute to psychopathology. A recent study reported that overall maternal levels of a different pro-inflammatory cytokine, TNF- α , were negatively associated with odds of offspring depression, although there was effect modification by sex when considering the ratio of TNF- α to the anti-inflammatory IL-10 (30). Consistent with the direction of our finding for IL-4, a prior study reported that elevated maternal levels of anti-inflammatory Th2 cytokines including IL-4 were associated with a reduced risk of psychosis (36). Conversely, our finding for IL-4 occurred only in the cases with BD without psychotic features. It should be noted that there were a high percentage of excluded observations for IL-4 due to unreliable measurement; consequently, the results for this cytokine should be considered preliminary.

Another possibility is that the effect of prenatal maternal cytokine levels on the risk of BD is modified by additional exposures, such as genetic risk for the disorder (29) or subsequent exposure to trauma (37). For example, in a mouse model of prenatal immune activation, exposure to unpredictable stress around the time of puberty led to a synergistic increase in behaviors with relevance to human neuropsychiatric disease (38). While we did not find evidence for effect modification by maternal psychiatric history according to our broad definition, we note that we had limited power for this analysis due to the relative infrequency of the exposure (15 cases and 26 controls total). We were unable to assess effect modification by more narrowly defined history of psychiatric disorders, and did not have the

data available to examine childhood trauma or stress in relation to prenatal cytokine levels. These may be fruitful directions for future study.

The study had several strengths including prospective collection of maternal serum samples from each trimester during pregnancy, validation of BD diagnosis by clinical interview, and selection of cases and matched controls from a population-based birth cohort.

There are the following limitations. Luminex multiplex assays, such as those used here, are generally believed to be less accurate and reliable for the measurement of cytokines than are ELISA tests performed separately for each analyte (39). Nonetheless, the comparison of commercially available ELISA versus Luminex assays in clinical samples has yielded high correlation for most cytokines (40), and Luminex assays for cytokines have been successfully applied in numerous clinical and population-based studies focusing on pregnancy (41–44). Moreover, these assays offer the advantages of reduced sample volume and higher throughput relative to ELISA (40). Additionally, we limited our statistical analyses for each cytokine-trimester combination to the observations that met an a priori minimum level of agreement between replicate measurements.

The maternal serum samples that were available for any given subject in our study represent a maximum of only 3 time points during the pregnancy. Thus, as discussed above, we may not have been able to detect transient elevations in maternal cytokines if they occurred solely between blood draws. Also, because of the differences in the numbers of subjects with maternal serum samples for each trimester, we were not able to conduct analyses of change over time within pregnancies or to assess correlations between individual cytokine levels. Finally, it is possible that the long-term storage of the samples resulted in degradation of cytokines or changes in their immune-reactivity, which may have reduced our ability to observe modest differences between cases and controls. However, our previous finding in a study of schizophrenia from the same cohort (13) indicates reasonable preservation of the samples.

In conclusion, we did not find clear evidence to support the hypothesis that higher levels of maternal prenatal pro-inflammatory cytokines are associated with increased risk of BD. If replicated in a larger sample, with even more reliable assays, these findings may indicate differing etiologies for BD and schizophrenia with respect to the role of prenatal maternal immune factors. Nonetheless, attempts to replicate these findings in independent birth cohorts with larger sample sizes are essential.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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- Cases with bipolar disorder (BD) and matched controls were identified
- Nine cytokines were measured in archived prenatal maternal serum samples
- Among cases overall, the cytokine levels were not associated with risk of BD
- Lower levels of IL-4 and IL-6 were observed in BD cases without psychotic features
- Findings varied by trimester

Table 1Demographic characteristics of cases with bipolar disorders and controls from full study population^a.

Characteristics	Cases (N=85)	Controls (N=170)	P value ^b
Maternal age (years), mean (SD)	27.5 (6.6)	28.2 (6.0)	0.37
Sex of subject, N (%)			
Male	27 (31.8)	54 (31.8)	1.00
Female	58 (68.2)	116 (68.2)	
Maternal race, N (%)			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, N (%)			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)	
Total annual family income, N (%)			0.93
Less than \$5000	9 (13.1)	17 (12.8)	
\$5000 to \$10000	45 (65.2)	90 (67.7)	
Greater than \$10000	15 (21.7)	26 (19.5)	
Maternal psychiatric history (broad) ^c , N (%)			0.66
Yes	15 (17.7)	26 (15.5)	
No	70 (82.3)	142 (84.5)	
Maternal psychiatric history (narrow) ^d , N (%)			0.60
Yes	2 (2.4)	2 (1.2)	
No	83 (97.6)	166 (98.8)	
Maternal smoking, N (%)			0.04
Yes	39 (53.4)	60 (39.0)	
No	34 (46.6)	94 (61.0)	
Gestational age at blood draw (days), mean (SD)			
First trimester (51 cases, 102 controls)	89.4 (25.5)	83.6 (19.0)	0.15
Second trimester (73 cases, 146 controls)	168.4 (25.5)	166.7 (19.9)	0.62
Third trimester (62 cases, 124 controls)	249.5 (17.2)	250.3 (13.4)	0.74

^aPercentages were calculated based on observations with non-missing values. Data were missing for the following numbers of observations: maternal race, 1 case; maternal education, 6 cases and 9 controls; family income, 16 cases and 37 controls; maternal psychiatric history, 2 controls; maternal smoking, 12 cases and 16 controls.

^bp-values from t-tests (continuous variables); chi-square tests (categorical values); or Fisher's Exact test (categorical values with cell counts <=5).

^cDefined as: psychoses, schizophrenia, affective disorder, anxiety, alcohol and/or substance abuse, mental deficiency, and other mental disorders.

^dDefined as: psychoses, schizophrenia or depression.

Table 2

Median prenatal maternal cytokine levels and conditional logistic regression analysis of log-transformed cytokine levels and risk of bipolar disorder, by trimester.

	N		Median maternal cytokine level (pg/ml)		Controls	Unadjusted		Adjusted for potential confounders ^a	
	Cases	Controls	Cases	Controls		OR (95% CI) ^b	OR (95% CI) ^b		
First trimester									
IL-1 β	48	83	0.03	0.03	0.03	0.98 (0.80, 1.20)	1.06 (0.85, 1.33)		
IL-5	47	87	0.12	0.10	0.10	1.16 (0.91, 1.47)	1.22 (0.95, 1.57)		
IL-6	42	80	2.35	2.93	2.93	0.88 (0.67, 1.16)	0.88 (0.65, 1.20)		
IL-8	45	87	77.0	103.0	103.0	1.04 (0.83, 1.30)	1.06 (0.82, 1.36)		
IL-10	48	91	8.53	8.85	8.85	1.00 (0.72, 1.38)	0.98 (0.69, 1.37)		
TNF- α	48	94	4.50	4.47	4.47	1.03 (0.55, 1.95)	1.29 (0.62, 2.67)		
Second trimester									
IL-1 β	63	108	0.03	0.03	0.03	0.89 (0.74, 1.07)	1.01 (0.82, 1.24)		
IL-5	70	131	0.11	0.10	0.10	1.05 (0.87, 1.27)	1.10 (0.89, 1.36)		
IL-6	67	130	2.49	4.06	4.06	0.82 (0.65, 1.04) [*]	0.82 (0.62, 1.08)		
IL-8	67	127	110.5	80.8	80.8	1.14 (0.94, 1.37)	1.14 (0.92, 1.42)		
IL-10	70	124	8.43	8.32	8.32	0.97 (0.75, 1.25)	1.12 (0.85, 1.48)		
TNF- α	71	137	3.90	4.48	4.48	0.80 (0.50, 1.29)	1.03 (0.59, 1.79)		
Third trimester									
IL-1 β	54	94	0.03	0.03	0.03	0.95 (0.78, 1.17)	0.97 (0.77, 1.21)		
IL-5	58	108	0.08	0.09	0.09	0.97 (0.76, 1.25)	0.96 (0.75, 1.23)		
IL-6	53	104	3.05	4.62	4.62	0.72 (0.51, 1.00) ^{**}	0.74 (0.52, 1.07)		
IL-8	56	105	98.8	82.0	82.0	1.01 (0.81, 1.26)	1.06 (0.83, 1.36)		
IL-10	51	94	10.7	9.11	9.11	1.01 (0.77, 1.32)	0.95 (0.70, 1.28)		
TNF- α	58	113	4.33	4.84	4.84	0.74 (0.39, 1.41)	0.83 (0.40, 1.71)		

^aMaternal age and smoking.

^bOR, odds ratio; CI, confidence interval.

^{**}p<0.05;

*
 $p < 0.10$

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Table 3

Conditional logistic regression analysis^a of log-transformed trimester-specific cytokine levels and risk of bipolar disorder, with and without psychotic features.

	<u>Bipolar disorder with psychotic features</u>		<u>Bipolar disorder without psychotic features</u>	
	OR (95% CI)		OR (95% CI)	
<i>First trimester</i>				
IL-1β	1.10	(0.77, 1.58)	0.92	(0.71, 1.20)
IL-4	0.84	(0.61, 1.17)	0.76	(0.58, 0.98) **
IL-5	1.10	(0.76, 1.60)	1.20	(0.87, 1.64)
IL-6	1.02	(0.58, 1.78)	0.85	(0.62, 1.16)
IL-8	1.00	(0.71, 1.40)	1.07	(0.79, 1.44)
IL-10	1.08	(0.65, 1.80)	0.95	(0.63, 1.44)
IFN-γ	0.60	(0.27, 1.33)	0.87	(0.62, 1.21)
GM-CSF	0.98	(0.54, 1.76)	0.98	(0.64, 1.51)
TNF-α	1.00	(0.33, 2.97)	1.05	(0.48, 2.31)
<i>Second trimester</i>				
IL-1β	0.97	(0.74, 1.28)	0.82	(0.62, 1.08)
IL-4	0.84	(0.62, 1.15)	1.01	(0.81, 1.26)
IL-5	1.45	(0.92, 2.28)	0.97	(0.78, 1.20)
IL-6	0.85	(0.57, 1.27)	0.81	(0.60, 1.07)
IL-8	1.07	(0.82, 1.39)	1.21	(0.93, 1.57)
IL-10	1.02	(0.66, 1.59)	0.94	(0.69, 1.29)
IFN-γ	0.93	(0.57, 1.51)	0.97	(0.74, 1.27)
GM-CSF	1.07	(0.61, 1.87)	0.87	(0.64, 1.20)
TNF-α	0.91	(0.38, 2.17)	0.76	(0.42, 1.36)
<i>Third trimester</i>				
IL-1β	0.91	(0.65, 1.27)	0.98	(0.76, 1.27)
IL-4	0.99	(0.69, 1.41)	0.83	(0.66, 1.04)
IL-5	0.66	(0.34, 1.30)	1.08	(0.82, 1.43)
IL-6	0.94	(0.50, 1.75)	0.64	(0.42, 0.98) **
IL-8	0.84	(0.58, 1.22)	1.12	(0.84, 1.50)
IL-10	1.06	(0.69, 1.63)	0.97	(0.68, 1.39)
IFN-γ	0.79	(0.50, 1.25)	0.67	(0.44, 1.03) *
GM-CSF	0.89	(0.58, 1.37)	1.06	(0.71, 1.58)
TNF-α	0.34	(0.09, 1.23) *	1.01	(0.47, 2.18)

^aAdjusted for maternal age and smoking.

^bOR, odds ratio; CI, confidence interval.

**
p<0.05;

*
p<0.10.