

Serologic Evidence of Prenatal Influenza in the Etiology of Schizophrenia

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Context: Some, but not all, previous studies suggest that prenatal influenza exposure increases the risk of schizophrenia. These studies used dates of influenza epidemics and maternal recall of infection to define influenza exposure, suggesting that discrepant findings may have resulted from exposure misclassification.

Objective: To examine whether serologically documented prenatal exposure to influenza increases the risk of schizophrenia.

Design: Nested case-control study of a large birth cohort, born from 1959 through 1966, and followed up for psychiatric disorders 30 to 38 years later.

Setting: Population-based birth cohort.

Participants: Cases were 64 birth cohort members diagnosed as having schizophrenia spectrum disorders (mostly schizophrenia and schizoaffective disorder). Controls were 125 members of the birth cohort, had not been diagnosed as having a schizophrenia spectrum or major affective disorder, and were matched to cases on date of

birth, sex, length of time in the cohort, and availability of maternal serum.

Main Outcome Measures: Archived maternal serum was assayed for influenza antibody in pregnancies giving rise to offspring with schizophrenia and matched control offspring.

Results: The risk of schizophrenia was increased 7-fold for influenza exposure during the first trimester. There was no increased risk of schizophrenia with influenza during the second or third trimester. With the use of a broader gestational period of influenza exposure—early to midpregnancy—the risk of schizophrenia was increased 3-fold. The findings persisted after adjustment for potential confounders.

Conclusions: These findings represent the first serologic evidence that prenatal influenza plays a role in schizophrenia. If confirmed, the results may have implications for the prevention of schizophrenia and for unraveling pathogenic mechanisms of the disorder.

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MANY STUDIES SUGGEST that prenatal exposure to influenza is associated with adult schizophrenia. In the first study of its kind, Mednick et al¹ demonstrated an increased risk of schizophrenia among Finnish individuals who had been in the second trimester of gestation during the 1957 influenza A2 epidemic. Thus far, among 26 investigations that sought to replicate the finding, about half reported positive associations, yet some of the more rigorously designed studies have shown no evidence of a relationship.²

This divergence of findings is likely explained, at least partly, by imprecise measurement of the exposure in the populations studied. These studies defined “exposure” on the basis of the dates of influ-

enza epidemics in the population, or on maternal recall of influenza infection after pregnancy. It has been argued that this question will remain unresolved until more sophisticated methods of documenting the exposure are applied.^{3,4}

To more definitively test this hypothesis, we conducted assays for influenza antibody in serum samples drawn from pregnant women whose offspring developed schizophrenia and in a matched comparison group of pregnant women whose offspring did not develop schizophrenia. These assays were conducted in the cohort of the Prenatal Determinants of Schizophrenia (PDS) study, which featured a large birth cohort of well-characterized pregnancies with archived maternal serum specimens prospectively collected throughout pregnancy, and rig-

orous diagnostic assessments of the schizophrenia outcome.⁵ The serum samples were tested for antibodies that were specific to the influenza strains previously documented as having been prevalent in the population during 1959 through 1966, the years of the pregnancies. To our knowledge, no previous study of influenza and schizophrenia has used serologic methods to document influenza exposure.

METHODS

DESCRIPTION OF THE COHORT

The PDS study was fully described in a previous publication⁵ and will therefore only be briefly summarized here. The mothers of the cohort members in the PDS study were enrolled in the Child Health and Development Study (CHDS),^{6,7} which was conducted from 1959 through 1966. During that period, the CHDS recruited nearly every pregnant woman who received obstetric care from the Kaiser Foundation Health Plan (KFHP) in Alameda County, California. Hence, all of the offspring of these women were automatically enrolled in KFHP. All subjects assessed in the PDS study provided written informed consent for human investigation. The study protocol was approved by the institutional review boards of the New York State Psychiatric Institute, New York, and the Kaiser Foundation Research Institute, Oakland, Calif.

The PDS study cohort consisted of the subsample of 12094 products of live births who were members of KFHP from January 1, 1981, through December 31, 1997. These dates correspond to the period of case ascertainment, which began in 1981 because computerized records did not exist in KFHP before that date. We have previously demonstrated that subjects who remained in KFHP and subjects lost to follow-up did not differ on a wide range of maternal and paternal characteristics^{5,8} and that the vast majority of subjects who left KFHP did so before the age of 10 years,⁵ suggesting that early manifestations of schizophrenia did not influence the likelihood of loss to follow-up.

COLLECTION OF MATERNAL SERUM

A special feature of the CHDS was the collection of maternal serum samples during pregnancy. The serum samples from these pregnancies were frozen immediately at -20°C and have been archived at that temperature or below in a single serum repository. All specimens were uniformly handled in accordance with a strict protocol. Serum samples were available from mid- to late gestation in virtually all pregnancies, and from early gestation in about half of the pregnancies.

ASCERTAINMENT AND DIAGNOSIS OF CASES

The main outcome was schizophrenia and other schizophrenia spectrum disorders (SSD), defined as schizophrenia, schizoaffective disorder, delusional disorder, psychotic disorder not otherwise specified, and schizotypal personality disorder, based on previous studies.⁹ Case ascertainment and screening were accomplished by means of a computerized record linkage between the CHDS and KFHP identifiers from inpatient, outpatient, and pharmacy registries. Subjects from the hospital registry were screened for potential SSD on the basis of their having been given registry diagnoses of codes 295 to 299 in the *International Classification of Diseases, Ninth Revision*, and were subsequently judged to be potential SSD cases after psychiatrist review of all psychiatric and medical records. For the outpatient registry, cases were considered positive on screening if they had

diagnosis codes of 295, 297, 298, or 299. For the pharmacy registry, subjects screened positive if they received treatment with antipsychotic medications. Among subjects who screened positive for potential SSD (n=183), 13 had died. Among the 170 remaining potential cases, 146 (86%) were successfully contacted to schedule a diagnostic interview.

Potential cases were administered the Diagnostic Interview for Genetic Studies¹⁰ by clinicians with at least a master's degree in a mental health field who were trained for reliability (A.S.B. and E.S.S.). Psychiatric diagnoses, by *DSM-IV* criteria, were made by consensus of 3 experienced research psychiatrists on the basis of the Diagnostic Interview for Genetic Studies narrative, medical records, and discussions between the interviewer and diagnosticians. Of the 146 contacted potential cases, 107 (73%) completed the Diagnostic Interview for Genetic Studies. For the 76 potential cases who were not interviewed, medical chart reviews by experienced clinicians were conducted. A research psychiatrist reviewed and confirmed all chart review diagnoses. These procedures yielded 71 total SSD cases, 44 of whom had received the Diagnostic Interview for Genetic Studies, and 27 of whom were diagnosed by chart review. Among these 71 SSD cases, 64 had available prenatal serum. The diagnoses of these cases were as follows: schizophrenia (n=38), schizoaffective disorder (n=15), delusional disorder (n=1), schizotypal personality disorder (n=5), and other schizophrenia spectrum psychosis (n=5) (the last category included subjects diagnosed by chart review who met criteria for either schizophrenia or schizoaffective disorder, but in whom there was insufficient information to definitively specify either condition). Therefore, 58 (91%) of the 64 cases with prenatal serum had either schizophrenia or schizoaffective disorder.

SELECTION OF CONTROLS

To select eligible controls, we first excluded the 71 SSD cases already diagnosed and 318 subjects with major psychiatric disorders other than SSD. Up to 8 matched controls were selected for each case. Controls were matched to cases on 5 characteristics: membership in KFHP at the time of case ascertainment (ie, the time of first treatment for SSD), date of birth (± 28 days), sex, number of maternal blood samples drawn during the index pregnancy, and number of weeks after the last menstrual period (referred to as the time post-LMP) of the first maternal blood draw during the index pregnancy (± 4 weeks). Matching for KFHP membership ensured that the controls for each case were representative of the population at risk at the time of case ascertainment. For this purpose, we used the KFHP membership registry, which enabled us to define the cohort members remaining in KFHP at the time that each case was first treated. Birth date was included as a matching factor to ensure that any degradation in the serum samples over time would be comparable between cases and controls. Controls were matched on sex. Controls were also matched by the number and timing of maternal blood draws to ensure sufficient and comparable serologic data for cases and matched controls. Further details on control selection in the PDS study were reported by Susser et al.⁵ Because of the need to conserve serum, we randomly selected 2 from the maximum 8 matched controls per case for the serologic analysis.

INFLUENZA ASSAY PROCEDURE

The antigens acquired for testing of serum subsets from the study population were those of the prevalent influenza strains from 1959 through 1966 in northern California. These antigens include A/H2N2/Japan/57, A/H2N2/Japan/62, A/H2N2/Taiwan/64, and B/Massachusetts/66.¹¹ The hemagglutination inhibi-

tion (HAI) method, following Good Laboratory Practice standards as previously described,^{12,13} was used to assay the serum for influenza antibody. All available serum samples from each subject were tested in duplicate on the same V-bottom plate in serial 2-fold dilutions (1:5, 1:10, 1:20, etc) after receptor-destroying enzyme treatment. The HAI duplicates that differed by more than a factor of 2 were repeated for that sample. Serum samples were tested with chicken red blood cells from 1 of 2 standardized chickens used by the Eastern Virginia Medical School laboratory, Norfolk. The HAI-determined titer was the greatest dilution of serum that completely inhibited agglutination of chicken red blood cells by the test virus. The test virus or viruses used for each assay were those of the specific circulating strains to each subtype that were prevalent during the period during which each serum sample was collected. If more than one influenza strain was prevalent in a given period, then separate HAI assays were conducted for each strain.

VALIDITY STUDY

Antibodies are known to be stable in stored frozen serum for long intervals. To verify the viability of measurements for influenza antibody in the serum specimens, we first conducted serologic assays, using the procedure specified in the previous section, in 51 CHDS pregnancies giving rise to neither cases nor matched controls in the present study, and who had serum available from each trimester. This permitted us to document the occurrence of seroconversion (ie, a 4-fold rise in influenza antibody titer in serial samples), which is diagnostic of influenza infection.¹⁴ Pregnancies that overlapped with known influenza epidemics evidenced a 2-fold increase in prevalence of seroconversion for influenza, compared with pregnancies that did not overlap with influenza epidemics. This provided further evidence of the feasibility of detecting influenza antibody in these archived serum samples by means of the HAI technique.

Although serum samples were obtained during gestation in virtually all pregnancies of the CHDS cohort, seroconversion could not be documented in most pregnancies because of an insufficient number with available serial samples and inconsistencies with regard to the gestational periods during which these samples were drawn. Thus, we developed a method for determining influenza infection status during pregnancy with the use of only a single antibody titer. For this purpose, we assessed the validity of an antibody titer threshold value (an antibody titer equal to or greater than a specified cutoff value) as a proxy for influenza infection, as documented by seroconversion (4-fold rise in influenza antibody titer). Maternal serum from the noncase, noncontrol sample described in the preceding paragraph was used for this purpose. The validity of a series of antibody titer threshold values (ranging from $\geq 1:10$ to $\geq 1:80$) as proxies for influenza infection were tested (a titer of 1:5 indicated no infection, and titers of $\geq 1:160$ were very rare). We demonstrated the following validity parameters for a strain-specific influenza antibody titer of 1:20 or greater in any serum sample during the pregnancy: sensitivity, 100% (7/7); specificity, 95% (42/44); positive predictive value, 78% (7/9); and negative predictive value, 100% (42/42). This supported the use of the 1:20 or greater antibody threshold titer in a single serum sample as an adequate proxy for influenza exposure during pregnancy.

DATA ANALYSIS

Key Analytic Variables

The primary outcome measure was case or control status (ie, whether a subject was classified as having SSD). The primary

exposure measure was influenza infection, which, in accordance with the results of our validity study, was defined as the first occurrence during pregnancy of an influenza antibody titer of 1:20 or greater. Antibody titers of 1:20 or greater occurring at any point in gestation after the first such titer for a given subject were designated as negative (unexposed) in the analysis. This criterion was justified for 3 reasons. First, each subject can be infected only once by a given influenza strain within any single year. Second, influenza is an acute infection, rarely lasting longer than 1 week,¹⁴ and the serum samples were always drawn at least 2 weeks apart from one another. Third, positive antibody titers to 2 different influenza strains at different points in time during a single pregnancy occurred rarely in our sample.

The analyses were conducted separately by trimester (see "Methods of Statistical Analysis" for a description of the analyses). We defined trimester 1 as 0 to 97 days post-LMP, but because of the delay in recognition of pregnancy, the actual range of first-trimester gestational days in which serum samples were available was 46 to 97 days post-LMP (or the latter half of the first trimester). We defined trimester 2 as 98 to 187 days post-LMP, and trimester 3 as 188 days post-LMP until 3 days after birth. Variables selected a priori as potential confounders included maternal age, paternal age, maternal education, and maternal ethnicity; all of these demographic data were acquired at or near the time of birth of the child.

Methods of Statistical Analysis

Matching via the nested case-control design ensured that cases and their corresponding controls were followed up for equal periods from birth until the date of first treatment for SSD (or until time of last follow-up for controls). Thus, having corrected by design for duration of follow-up, we were able to apply conditional methods for binary data to compare cases and controls on influenza exposure. As the first step in evaluating the potential association between influenza exposure in each period of pregnancy and SSD risk, we applied the Mantel-Haenszel methods for stratified data.¹⁵ This analysis designated each matched set as its own stratum, within which case-control status (SSD vs not) was cross-classified by the presence or absence of influenza infection. Separate analyses were conducted for each period of gestation. The Mantel-Haenszel methods yield an estimated odds ratio relating exposure and SSD and a test of the significance of the observed association. Subsequent analyses were conducted by means of conditional logistic regression¹⁶ to allow for control of other potential confounding variables (eg, maternal education). This model was conditioned on an identification variable that uniquely identified matched stratum membership. Like the Mantel-Haenszel analysis, the conditional logistic model provides an estimate of the odds ratio to measure the strength of the effect, as well as a significance test to formally assess the association between prenatal influenza exposure and SSD risk.

The population attributable risk was calculated on the basis of the following formula:

$$(RR - 1)P_1/RR,$$

where RR indicates relative rate and P_1 is the proportion of exposed cases.¹⁷ In accordance with the usual practice for case-control studies, we substituted the adjusted odds ratio for the RR. This measure provides a crude indication of the proportion of cases occurring in the studied population that might be prevented by removal of the exposure, if the exposure is indeed a cause.

Table 1. Characteristics of Matched Case-Control Sample, During Pregnancy, for Analysis of Prenatal Exposure to Influenza and Risk of SSD*

Characteristics of Sample	No. (%)	
	SSD Cases (n = 64)	Matched Controls (n = 125)
Maternal education		
Less than HS	13 (23)	12 (11)
HS graduate ± trade school	25 (44)	50 (44)
HS + some college	10 (18)	31 (27)
College graduate/nurse	9 (16)	20 (18)
Maternal age		
Mean (SD)	28.3 (6.2)	27.6 (6.1)
Median (IQR)	29 (24.0-32.5)	28 (22.0-32.5)
Paternal age		
Mean (SD)	33.5 (8.0)	31 (6.6)
Median (IQR)	32 (27-40)	31 (25-35)
Maternal race/ethnicity		
White/European origin	32 (51)	61 (50)
Black	27 (43)	47 (38)
Other	4 (6)	15 (12)
Sex†		
Male	42 (66)	83 (66)
Female	22 (34)	42 (34)
No. of maternal serum samples drawn†		
1	5 (8)	7 (6)
2	13 (20)	27 (22)
3	26 (41)	51 (41)
4	20 (31)	40 (32)

Abbreviations: HS, high school; IQR, interquartile range; SSD, schizophrenia-schizophrenia spectrum disorder.

*Table entries give sample size and column percentage, unless otherwise specified. Percentages are based on the number of nonmissing observations.

†These were matching criteria.

RESULTS

The distribution of potential confounding characteristics was compared between the matched samples of SSD cases and controls (**Table 1**). The only characteristics that differed appreciably were maternal education (larger proportion of cases with less than a high school education) and paternal age (increased in cases, as reported in a previous publication).¹⁸

Among subjects tested in the first trimester of pregnancy, 5 (25%) of SSD cases were influenza-exposed, compared with 4 (11%) of controls. For the second and third trimesters, the proportions of influenza-exposed cases and controls were similar to one another (**Table 2**).

The Mantel-Haenszel analysis, controlling for matched set membership, demonstrated that influenza exposure in the first trimester of pregnancy was associated with a 7-fold increased risk of SSD, although the result did not achieve statistical significance ($P=.08$). For influenza exposure in the second trimester of pregnancy, the risk of SSD was not appreciably elevated in cases as compared with controls. There was also no increase in risk of SSD after third-trimester influenza exposure (**Table 3**).

In the conditional logistic regression analysis, adjustment for maternal age, paternal age, maternal education, or maternal ethnicity had no appreciable impact

Table 2. Exposure Profiles for the 189 Subjects (64 Matched Sets) in Analysis of Prenatal Influenza and Risk of SSD*

Gestational Period	SSD Cases (n = 64)		Matched Controls (n = 125)	
	No. of Subjects	No. (%) Exposed	No. of Subjects	No. (%) Exposed
Trimester				
1	20	5 (25)	38	4 (11)
2	48	7 (15)	82	11 (13)
3	63	12 (19)	121	21 (17)
First half of pregnancy	43	9 (21)	75	7 (9)
Second half of pregnancy	64	13 (20)	125	30 (24)

Abbreviation: SSD, schizophrenia-schizophrenia spectrum disorder.

*Column percentages are based on the number tested.

Table 3. Mantel-Haenszel Analysis (Controlling for Matched Set Membership) of Association Between Prenatal Exposure to Influenza and Risk of Schizophrenia-Schizophrenia Spectrum Disorder*

Gestational Period	OR _{MH}	95% CI	χ^2	P Value
Trimester 1	7.0	0.7-75.3	3.00	.08
Trimester 2	1.1	0.3-3.9	0.05	.83
Trimester 3	1.1	0.5-2.6	0.09	.77
First half of pregnancy	3.0	0.9-10.1	3.77	.05†
Second half of pregnancy	0.7	0.3-1.6	0.64	.42

Abbreviations: CI, confidence interval; OR_{MH}, Mantel-Haenszel odds ratio.

*For each analysis, there are a maximum of 64 cases and 125 matched controls.

†Results are reported to 2 decimal places for all P values; using 3 decimal places, this P value was .052.

on the estimated effect of first-trimester influenza exposure on SSD risk (results available on request).

In a further analysis, we sought to assess whether the association between first-trimester influenza and SSD risk extended into the first part of the second trimester. Our rationale for this analysis was as follows. First, as noted previously (see "Data Analysis: Key Analytic Variables"), the serum samples from the first trimester were obtained during the latter half of that gestational period (thus bordering on the beginning of the second trimester). Second, the divisions between trimesters are defined arbitrarily, rather than being based on biological mechanisms. This suggests that alternative definitions of pregnancy intervals may yield valuable additional information. Third, although we did not observe an increased risk of SSD in the second trimester analyzed as a whole (see Table 3), previous ecologic studies demonstrated second-trimester specificity (see the introduction).

Hence, in the additional analysis, we divided pregnancy into 2 periods of exposure: the first half and the second half. The exposed period for the first half began on day 0 (as discussed earlier, however, the first available sample was drawn on day 46) and ended on day 142 post-LMP (the midpoint of pregnancy). In effect, this period consists of the latter half of the first trimester and the first half of the second trimester. The second half of pregnancy was defined as the period from day 143 until 3 days after the termination of pregnancy.

We then examined the association between SSD risk and influenza for subjects exposed during each of these 2 periods. Using this definition of exposure, we found that 9 (21%) of SSD cases, compared with 7 (9%) of controls, had been exposed to influenza in the first half of pregnancy (see Table 2). In the Mantel-Haenszel analysis, influenza exposure during the first half of pregnancy was associated with a 3-fold increased risk of SSD, which was at the margin of statistical significance ($P = .052$) (see Table 3). The findings were not appreciably altered after adjustment for maternal age, paternal age, maternal education, and maternal ethnicity (results available on request). There was no association between SSD risk and influenza exposure in the second half of pregnancy.

The population attributable risk associated with influenza exposure was 21% for the first trimester and 14% for the first half of pregnancy.

COMMENT

This study was the first, to our knowledge, to use maternal influenza antibody levels in individual pregnancies to examine the relationship between prenatal influenza exposure and schizophrenia. The data indicate that the risk of schizophrenia was increased by a factor of 7 after serologically documented influenza exposure during the first trimester of pregnancy. Prospective acquisition of the serum samples in a well-characterized, continuously monitored birth cohort and the use of a face-to-face psychiatric diagnostic assessment lend credence to this result. It should be noted, however, that this finding did not achieve statistical significance ($P = .08$) and is based on a small sample.

As noted in the introduction, nearly all previous positive studies of influenza and schizophrenia were specific to exposure during the second trimester.² An additional analysis permitted us to further examine the compatibility between our findings and those of the previous studies. This analysis showed that exposure to influenza from approximately the midpoint of the first trimester to the midpoint of the second trimester increased the risk of schizophrenia by a factor of 3. Although this finding fell slightly short of statistical significance ($P = .052$), it suggests significant overlap between the present and previous studies with regard to the gestational periods of influenza exposure that were associated with an increased risk of schizophrenia.

The fact that these gestational periods were not identical might be explained by differences in measuring the timing of influenza infection during pregnancy. In previous studies, the timing of exposure was defined by the dates of the peak periods of influenza epidemics in the population, not serologic assessment of infection in individual pregnancies. In addition, previous investigations used the date of birth of the offspring to define the trimester or month of pregnancy; in contrast, we had complete information on the date of the last menstrual period, permitting a more precise estimate of gestational age. Moreover, the influenza strains examined varied between the present and previous studies. Many of the previous findings were based on the 1957 influenza A2 epidemic.² In contrast, our study included not only the 1957

influenza A2 strain, but 3 additional influenza strains that differed significantly in antigenicity from the 1957 influenza A2 and other influenza strains examined in previous studies.

PATHOGENIC MECHANISMS

In a recent study, the offspring of pregnant mice infected with influenza virus on gestational day 9.5 had altered exploratory behavior, decreased contact with novel objects, and deficits in prepulse inhibition to acoustic startle, the last of which was corrected by antipsychotic medications.¹⁹ Each of these findings is analogous to abnormalities demonstrated in schizophrenia. In another study, the offspring of pregnant mice infected with influenza at gestational day 9 evidenced significant reductions at birth in reelin-positive Cajal-Retzius cells in the cortex and hippocampus, and diminished areas of these brain regions,²⁰ consistent with postmortem studies of schizophrenia.²¹⁻²³

Since influenza is believed to only rarely cross the placenta, an indirect effect on fetal brain development is the most plausible pathogenic mechanism linking it to an increased risk of schizophrenia.²⁴ One such mechanism, previously considered, is that maternal IgG antibodies elicited by influenza traverse the placenta and cross-react with fetal brain antigens by molecular mimicry, thereby disturbing fetal brain development and increasing vulnerability to schizophrenia.²⁵ Another potential immunologic mediator is an influenza-induced excess of maternal cytokines, which may damage the developing fetal brain.²⁶ This hypothesis is based in part on evidence that elevated levels of cytokines cause neurodevelopmental damage, such as periventricular leukomalacia.^{27,28} In addition, increased cytokines in cord blood have been reported in neonates who developed cerebral palsy and mental retardation in childhood.²⁹ Finally, in the preclinical study discussed at the beginning of this section,¹⁹ prenatal administration of synthetic double-stranded poly(I:C), which evokes a strong immune response including cytokine elevations, resulted in prepulse inhibition deficits similar to those found in mice prenatally infected with influenza.

Recent evidence, however, may argue instead for a direct effect of influenza infection on the fetal brain. Aronson et al³⁰ reported that mice that were prenatally infected with human influenza (A/WSN/33 strain) had influenza viral RNA in the brain. The viral RNA persisted for at least 90 postnatal days.

Other possible mediating factors²⁴ include hyperthermia, which is teratogenic to animals³¹ and possibly also to humans³²; fetal hypoxia, which has been previously associated with schizophrenia^{3,33}; and prescribed over-the-counter influenza remedies, including aspirin, which may cause central nervous system anomalies.³⁴

ATTRIBUTABLE RISK

Since influenza is common in the population, we used our data to calculate the population risk for schizophrenia attributable to influenza in early to midpregnancy. Our data suggest the possibility that up to 14% of

schizophrenia cases would not have occurred if influenza exposure during early to midpregnancy had been prevented.

RELATION TO PREVIOUS WORK

These findings add to a body of work suggesting a relationship between in utero exposure to infectious agents and risk of adult schizophrenia.³⁵ In a previous study on the National Collaborative Perinatal Project that used prenatal serum specimens, elevated maternal IgG antibody levels to herpes simplex type 2 virus were found in pregnancies giving rise to adults with psychosis, compared with matched controls.³⁶ Brown et al³⁷ reported that more than 20% of subjects who were clinically and serologically documented with in utero exposure to rubella were diagnosed as having schizophrenia and other SSDs. Maternal upper respiratory infections have been associated with an increased risk of schizophrenia in the offspring.³⁸ Finally, ecologic studies have demonstrated associations between schizophrenia and prenatal exposure to polio, varicella-zoster, and measles.³⁵ These findings suggest that common downstream effects from several infectious agents on neuronal function may be relevant to the etiopathogenesis of schizophrenia.

LIMITATIONS

We used a proxy measure of influenza exposure during pregnancy. As described earlier (see "Methods: Validity Study"), influenza during a defined period is classically assessed by the demonstration of seroconversion (ie, a 4-fold rise in antibody titer). In our primary analysis, we approximated influenza infection by using antibody titers measured at single points in time. This method, however, was well validated with the use of seroconversion as the "gold standard" in serum samples from our cohort.

It is also worth noting that if elevated influenza-specific IgG antibody is responsible for mediating the effects of influenza on the risk of schizophrenia, as previously postulated,²⁵ then antibody titers may provide a more direct and accurate exposure measure than seroconversion. To further address this question, we conducted an additional analysis in which exposure in each period of pregnancy was defined as an elevated antibody titer ($\geq 1:20$) during that period, irrespective of whether within-subject titers from samples drawn earlier in pregnancy were elevated. (In the primary analysis, infection was determined on the basis of the first occurrence during pregnancy of an elevated antibody titer, regardless of whether titers drawn later in pregnancy were elevated; see "Data Analysis: Key Analytic Variables" in the "Methods" section.) The respective magnitudes of associations between elevated influenza antibody titers and SSD for each period of pregnancy were similar to those of the primary analysis, suggesting that one cannot differentiate between the 2 effects (results available on request).

The serum samples used in the present study had been frozen for more than 30 years, raising the issue of the stability of the antibodies during that time. Several factors argue against compromised protein stability as a factor in this

study. First, careful visual inspection of our samples showed little evidence of previous freeze-thawing, a major cause of protein breakdown, or of evaporation, which might cause a spurious elevation of antibody levels. Second, in consultation with the lead CHDS investigators, we verified that throughout the entire storage period, these samples had been carefully and uniformly handled, and special efforts had been expended to ensure that they constantly remained at a temperature of -20°C or lower, which considerably protects against protein breakdown. Third, we demonstrated that seroconversion, as measured in our serum specimens, occurred twice as often in specimens drawn during influenza epidemics as in those from nonepidemic periods (see "Methods: Validity Study"). Fourth, controls were matched to cases on date of birth and gestational timing, and the maternal specimens of cases and controls were uniformly handled and stored, suggesting that these factors should not have biased the observed associations.

Our study also did not include data on family history of schizophrenia, which would have permitted the adjustment for possible confounding by this factor, and the examination of interaction between prenatal influenza exposure and genetic susceptibility to schizophrenia. We are presently conducting family history and molecular genetic assessments of our cases and matched controls to further address these questions.

Finally, because our sample sizes were small to modest, and the findings did not achieve statistical significance, independent replications are needed.

CONCLUSIONS

Using serologic methods, we demonstrated that exposure to influenza during early to midpregnancy may play a role in the etiology of schizophrenia. If confirmed by other studies, our findings may ultimately have implications for ameliorating, or possibly preventing, a significant portion of schizophrenia cases, for example, by administration of influenza vaccine to women of reproductive age. Although the precise mechanisms need to be delineated, it may be worth considering the question of routine vaccination of nonpregnant women, given the possibility that the antibody response to influenza, rather than direct infection, may be responsible for the observed increase in risk of schizophrenia. A further implication of the present findings is that they may stimulate further work on the pathogenesis of schizophrenia.

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