ORIGINAL ARTICLE

# Elevated Prenatal Homocysteine Levels as a Risk Factor for Schizophrenia

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**Context:** Elevated prenatal homocysteine level is a plausible risk factor for schizophrenia because of its partial antagonism of *N*-methyl-D-aspartate receptors under physiologic glycine concentrations and its association with abnormal placental function and pregnancy complications.

**Objective:** We examined whether elevated maternal levels of homocysteine during the third trimester were associated with adult schizophrenia risk.

**Design:** Nested case-control study of a large birth cohort, born from 1959 through 1967 and followed up for schizophrenia from 1981 through 1997.

**Setting:** Population-based birth cohort and health plan.

**Participants:** Cases (n=63) were diagnosed with schizophrenia and other spectrum disorders (mostly schizophrenia and schizoaffective disorder). Controls (n=122) belonged to the birth cohort; had not been diagnosed with 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 samples.

**Main Measures:** Archived maternal serum samples were assayed for homocysteine levels during pregnancies of cases and matched controls.

**Results:** In a model that tested for a threshold effect of third-trimester homocysteine levels, an elevated homocysteine level was associated with a greater than 2-fold statistically significant increase in schizophrenia risk (odds ratio, 2.39; 95% confidence interval, 1.18-4.81; *P*=.02).

**Conclusions:** These findings indicate that elevated third-trimester homocysteine levels may be a risk factor for schizophrenia. Elevated third-trimester homocysteine levels may elevate schizophrenia risk through developmental effects on brain structure and function and/or through subtle damage to the placental vasculature that compromises oxygen delivery to the fetus. If future studies both replicate this association and support a causal link, then the use of folic acid supplementation would merit evaluation as a strategy for prevention of schizophrenia in offspring.

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COMPELLING EVIDENCE SUGgests that a disruption of neurodevelopment during fetal life plays a role in the etiology of schizophrenia.[1,2] Risk factors that are known to produce neurodevelopmental insult and that have been implicated in schizophrenia include prenatal viral infection,[3,4] prenatal nutritional deficiency,[5] fetal hypoxia,[6] and perinatal insults.[2] Despite significant advances, these and other prenatal exposures have not been definitively linked with neurochemical perturbations proposed in this illness. The identification of such a risk factor could help to unify current hypotheses on neurodevelopmental mechanisms and neurotransmitter function in schizophrenia.

Homocysteine, a sulfur-containing amino acid, may represent such a candidate. Although homocysteine has been shown to act as an *N*-methyl-D-aspartate (NMDA) receptor agonist when glycine levels are pathologically elevated, this amino acid acts as a partial antagonist at the glycine site of the NMDA receptor when glycine levels are in the physiologic range.[7] A wealth of evidence supports a role for glutamatergic dysfunction in the etiology and pathophysiology of schizophrenia. As reviewed by Goff and Coyle,[8] abnormalities of glutamate receptor function, particularly regarding the NMDA receptor, may explain several features of the illness. In animal studies, perinatal administration of the NMDA receptor antagonist phencyclidine induces

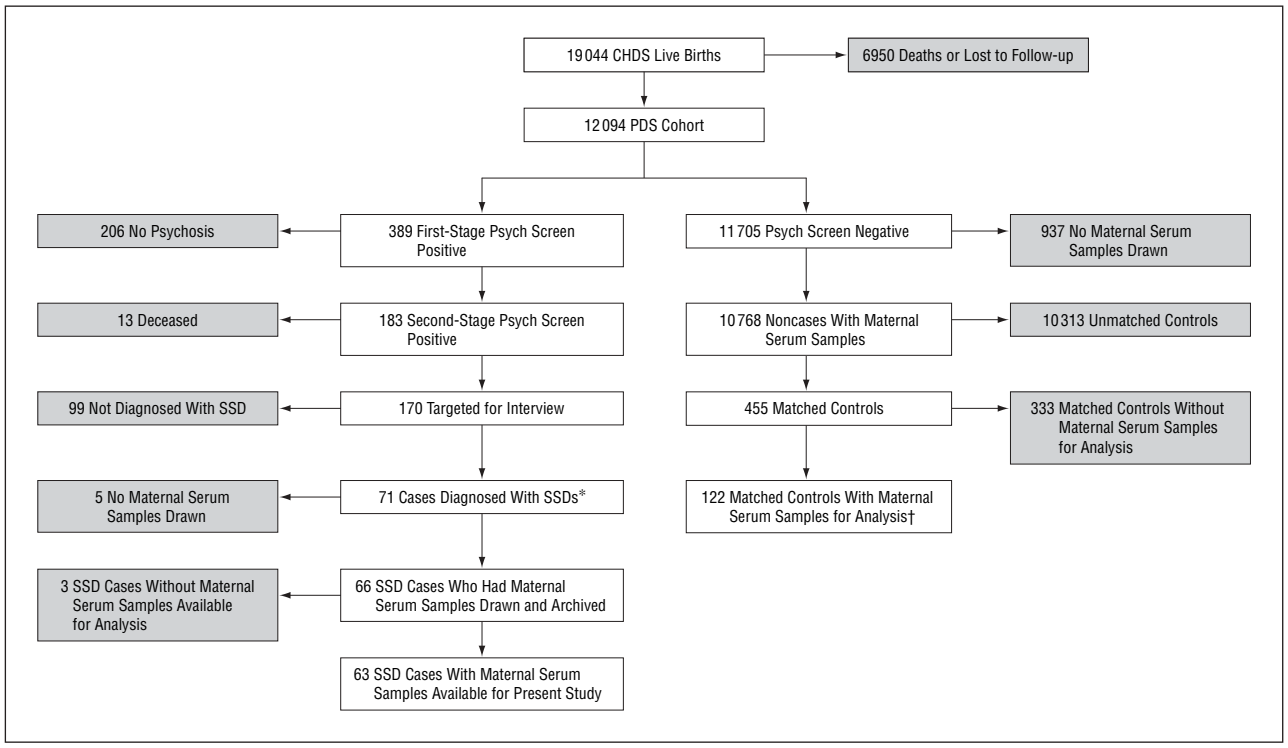


**Figure.** Flow diagram for the Prenatal Determinants of Schizophrenia (PDS) study. * Indicates schizophrenia and other schizophrenia spectrum disorders (SSDs). † Indicates that the serum samples were provided for up to 2 matched controls per case to conserve serum samples. These controls were randomly selected from the pool of matched controls for each case. The matching criteria are specified in the text (see the "Methods" section). The controls were matched 2:1 for 59 cases and 1:1 for 4 cases. CHDS indicates Child Health and Development Study.

abnormalities implicated in schizophrenia, including impaired synaptogenesis,[9] a deficit in prepulse inhibition,[9] and a sensitized locomotor response to phencyclidine challenge.[9] A second mechanism by which homocysteine might cause developmental perturbations that increase risk for schizophrenia is through the induction of placental vasculopathy,[10-15] which may lead to fetal hypoxia and adverse consequences for fetal development, including impaired brain growth[16] and disturbances of neurotransmitter systems.[17-21]

Therefore, we examined the relationship between prenatal levels of homocysteine and risk of schizophrenia in the birth cohort of the Prenatal Determinants of Schizophrenia (PDS) study.[22] For this purpose, we used prenatal serum specimens that were collected in this cohort and were archived for future use. We hypothesized that elevated homocysteine during the third trimester of fetal development will be related to increased schizophrenia risk for the following reasons. First, most previous studies of developmental effects of NMDA receptor blockade have focused on the equivalent of late pregnancy in humans.[9,23,24] Second, placental blood flow increases most rapidly during the third trimester.[25] Third, most of the complications of pregnancy that have been associated with elevated maternal homocysteine levels, including preeclampsia, placental abruption, and premature delivery, occur during late gestation.[20] Fourth, maternal hyperhomocysteinemia has also been associated with abnormal birth outcomes, including low birthweight and stillbirth[26]; these effects are largely determined by late gestational influences. In exploratory analyses, we also examined the relation of prenatal homocysteine levels in the first and second trimesters to risk of schizophrenia.

## METHODS

### DESCRIPTION OF THE COHORT

The development of the sample for the present study is summarized in a flowchart (**Figure**). The PDS study was described in a previous publication.[22] The cohort members were derived from the Child Health and Development Study (CHDS).[27] During 1959 through 1966, the CHDS recruited virtually all pregnant women receiving obstetric care from the Kaiser Permanente Medical Care Plan (KPMCP), Northern California Region, in Alameda County, California. Their liveborn offspring (N = 19 044) were automatically enrolled in KPMCP. Comprehensive data were prospectively collected from maternal medical records, maternal interviews, and other sources. Approximately 30% of the population of the county received their health care by KPMCP. The Kaiser membership was diverse: racially, educationally, and occupationally, it was largely representative of the population of the Bay Area of California at the time. It has been noted in previous studies of this cohort that there was some underrepresentation of the extremes of income.[28]

Maternal blood was drawn, and serum samples were obtained during pregnancy in the vast majority (91.6%) of these subjects. The samples were generally drawn in the morning, although the protocol did not require the subjects to be fasting; however, the effect of food intake on serum homocysteine levels is minimal.[29] The serum specimens were frozen immediately and archived at −20°C in a single repository. All samples were handled and stored following a uniform, strict protocol.

**Table 1. Comparison of Maternal Characteristics Between Subjects in the CHDS Cohort and PDS Cohort by Serum Availability***

| Characteristic | CHDS With Serum Samples (n = 17 445) | CHDS Without Serum Samples (n = 1599) | PDS Study With Serum Samples (n = 11 126) | PDS Study Without Serum Samples (n = 968) |
|---|---|---|---|---|
| Maternal race, %† | | | | |
| White | 65.9 | 69.7 | 60.6 | 65.7 |
| Black | 23.8 | 21.5 | 28.1 | 23.3 |
| Other | 10.3 | 8.9 | 11.2 | 11.1 |
| Maternal education, %‡ | | | | |
| Less than high school graduate | 18.7 | 23.7 | 18.3 | 23.0 |
| High school graduate | 38.0 | 39.5 | 39.1 | 38.5 |
| Some college/college graduate | 43.3 | 36.8 | 42.6 | 38.5 |
| Maternal age, mean, y§ | 27.0 | 27.6 | 27.6 | 28.4 |
| Parity, %‖ | | | | |
| 0 Previous live births | 31.7 | 23.3 | 28.2 | 19.5 |
| 1 Previous live birth | 27.0 | 25.5 | 26.6 | 24.5 |
| ≥2 Live births | 41.3 | 51.2 | 45.2 | 56.0 |
| CHDS or PDS study | 91.6 | 8.4 | 92.0 | 8.0 |

Abbreviations: CHDS, Child Health and Development Study; PDS, Prenatal Determinants of Schizophrenia.
*Values are percentages and means from known data.
†Values for maternal race were unknown for 284 in the CHDS cohort and 148 in the PDS cohort.
‡Values for maternal education were unknown for 2788 in the CHDS cohort and 1595 in the PDS cohort.
§Values for maternal age were unknown for 176 in the CHDS cohort and 105 in the PDS cohort.
‖Values for parity were unknown for 136 in the CHDS cohort and 74 in the PDS cohort.

The at-risk cohort comprised the 12 094 live births who were members of KPMCP between January 1, 1981 (the year in which computerized registries became available), and December 31, 1997.[22] This cohort is heretofore termed the "PDS cohort."

We compared maternal characteristics between all original CHDS cohort members (N = 19 044) and the 12 094 PDS cohort members (**Table 1**). The CHDS and PDS cohort members were stratified by the presence and absence of prenatal serum samples. As the table illustrates, there were no appreciable differences between the groups except that there was a mild increase in parity for subjects in the PDS cohort without prenatal serum samples. There were slight differences with regard to education and race such that individuals without prenatal serum samples tended to have less education, and there was a slight overrepresentation of African-American individuals in the PDS cohort with prenatal serum samples.

We found that of the 6950 subjects who were lost to follow-up, 441 died in infancy/childhood, and 5862 left KPMCP by age 10 years; thus, the vast majority of those who left KPMCP did so before age 10 years.[22] Although premorbid manifestations of schizophrenia may have been apparent at that age, prospective birth cohort studies of individuals destined to develop schizophrenia in adulthood have not demonstrated prodromal symptoms during childhood that would have been sufficiently disruptive to have affected a family's membership in KPMCP.[30-32]

## SCREENING PROTOCOL FOR SCHIZOPHRENIA AND OTHER SCHIZOPHRENIA SPECTRUM DISORDERS

The main outcome was schizophrenia and other schizophrenia spectrum disorders (SSDs). This definition was based on previous studies[33] and included any of the following related diagnoses: schizophrenia, schizoaffective disorder, delusional disorder, psychotic disorder not otherwise specified, and schizotypal personality disorder.

To identify potential SSD cases, we conducted a screening procedure that was applied to the inpatient, outpatient, and pharmacy registries of KPMCP. In the first stage of screening, computerized record linkages between CHDS and KPMCP identifiers were performed. Subjects from the KPMCP inpatient registry were screened for potential SSD based on registry diagnoses of codes 295 through 299 from the *International Classification of Diseases, Ninth Revision (ICD-9)*; potential cases from the outpatient registry were considered to screen positive if *ICD-9* diagnoses of 295, 297, 298, or 299 were assigned. Subjects from the pharmacy registry screened positive based on a history of antipsychotic treatment. There were 389 subjects who screened positive in the first stage.

In the second stage of screening, psychiatric and medical records among the 389 subjects who screened positive in the first stage were reviewed for evidence of psychotic symptoms by an experienced, board-certified research psychiatrist. Among these subjects, 183 screened positive for potential SSD based on evidence of psychotic symptoms. Of these, 13 were deceased. Among the 170 remaining potential cases, 146 (86%) were contacted to schedule a diagnostic interview.

## DIAGNOSTIC PROTOCOL

Clinicians with a minimum of a master's degree in a mental health field and trained to reliability administered the Diagnostic Interview for Genetic Studies to potential cases.[34] Diagnoses from the *DSM-IV* were assigned by consensus of 3 experienced research psychiatrists, based on the interview information, medical records, and discussions with the interviewer. The Diagnostic Interview for Genetic Studies was completed by 107 of the 146 contacted potential cases (73%). Potential cases not interviewed (n = 76) were diagnosed by medical-record review by experienced clinicians, and all medical record diagnoses were confirmed by a research psychiatrist. This protocol resulted in 71 total SSD cases, 44 of whom received the Diagnostic Interview for Genetic Studies and 27 of whom were diagnosed by medical record review.

All subjects 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 and KPMCP.

**Table 2. Demographics of the Schizophrenia and Other Spectrum Disorder Cases and Controls***

| Characteristic | Cases | Controls | *t* or $\chi^2$ Value | *P* Value |
|---|---|---|---|---|
| Subject age, mean ± SD, y | 24.1 (4.1) | 24.3 (4.9) | | |
| Subject sex, male, No. (%) | 42 (66.7) | 83 (68.0) | | |
| Maternal age, mean ± SD, y | 28.2 ± 6.3 | 28.4 ± 8.8 | 0.26 | .61 |
| Maternal education, No. (%) | | | | |
| Less than high school graduate | 14 (24.6) | 12 (10.4) | 7.06 | .07 |
| High school graduate | 24 (42.1) | 51 (44.4) | | |
| Some college/college graduate | 19 (33.3) | 52 (45.2) | | |
| Maternal race, No. (%) | | | | |
| White | 31 (50.0) | 65 (53.2) | 2.09 | .35 |
| Black | 27 (43.5) | 43 (35.2) | | |
| Other | 4 (6.5) | 14 (11.5) | | |
| Maternal smoking, No. (%) | | | | |
| No | 23 (41.8) | 50 (45.5) | 0.20 | .65 |
| Yes | 32 (58.1) | 60 (54.6) | | |
| Gestational age of serum samples, Mean ± SD, d | | | | |
| First trimester | 72.7 ± 11.5 | 70.9 ± 11.6 | 0.31 | .58 |
| Second trimester | 130.3 ± 23.5 | 134.1 ± 27.6 | 1.90 | .17 |
| Third trimester | 229.7 ± 28.7 | 224.8 ± 28.2 | 1.60 | .21 |

*These data are not reported by matched sets; they are presented for descriptive purposes only.

## ELIGIBLE CASES

Among the 71 SSD cases, 66 had prenatal serum drawn. For the present study, 3 subjects had no remaining available sera, leaving 63 SSD cases with at least 1 available prenatal serum sample. A diagnostic breakdown of these cases indicates that 52 (83%) of the 63 cases with prenatal sera had either schizophrenia (n=37) or schizoaffective disorder (n=15). In addition, 1 case had delusional disorder, 5 cases had schizotypal personality disorder, and 5 cases had another schizophrenia spectrum psychosis. There were 59 cases with third-trimester serum samples, all of which were analyzed. In further analyses, we assayed all of the available sera from the first trimester (n=17 cases) and the second trimester (n=47 cases).

## MATCHED CONTROLS IN THE PDS STUDY

We first describe the method for selecting eligible matched controls for the PDS nested case-control studies, which preceded the selection of controls for the present study. There were 11 705 members of the cohort who screened negative for potential SSD; that is, they had no previous treatment for major psychiatric disorders and/or no evidence of psychotic symptoms (see the section "Screening Protocol for Schizophrenia and Other Schizophrenia Spectrum Disorders"). After we excluded subjects without prenatal sera (n=937), 10 768 eligible controls remained. Among these, we selected controls matched to SSD cases on several criteria. First, to ensure that each case and the corresponding controls were followed for equal lengths of time from birth until first treatment of the case, controls were matched to cases on membership in KPMCP at the time the case was ascertained. Controls were also matched to cases on date of birth (±28 days), sex, gestational timing (±28 days) of the first maternal serum sample taken during the index pregnancy,[4,22] and the number of serum samples. We matched on the timing of the first serum sample because 6% of the cohort had only 1 sample drawn, and the timing of the first blood draw was a strong predictor of the gestational timing of all blood draws throughout the pregnancy. We matched for the number of serum samples to ensure that serum samples were available in cases and controls from the same periods of pregnancy.

The control selection procedure consisted of first enumerating for every case the individuals who met these 5 matching criteria. Those subjects assigned to a matched set were systematically removed from the potential control pool for subsequent cases until all matched sets were completed. Finally, we corrected the matched sample to include only 1 sibling per family since siblings represented nonindependent observations. This protocol yielded 455 matched controls; among the 66 cases with prenatal serum samples, 10 were matched to 1 to 4 controls and 56 were matched to 5 to 8 controls. Each case and their corresponding matched controls were termed a "matched set."

## MATCHED CONTROLS IN THE PRESENT STUDY

To conserve the sera, the CHDS provided serum specimens for up to 2 matched controls per case; these controls were selected at random from each matched set of controls. To ensure more precise matching of the gestational timing of serum draws between cases and controls, we also required that the control serum samples be matched to case serum samples on trimester and that for each trimester, the sera from the controls be drawn within 42 days of the blood draw for the corresponding cases. This process yielded a total of 122 matched controls for the present study; each of these controls had at least 1 available prenatal serum sample. Among these 122 matched controls, 112 had third-trimester serum, 30 had first-trimester serum, and 79 had second-trimester serum. The mean gestational ages (in days) of serum samples from cases and controls did not differ significantly from one another in any of the trimesters (**Table 2**).

## LABORATORY ASSAY

The same assay method was used for cases and controls and all assays were performed blind to case and control status. Total homocysteine was measured using a high performance liquid chromatography method with coulometric electrochemical detection.[35] The method is highly sensitive and well suited for quantitative analysis on low volumes of serum samples (5-10 µL). The method consists of reduction of the sample with tris-2-carboxyethyl-phosphine followed by precipitation of serum proteins with trichloroacetic acid. The trichloroacetic acid precipitates were directly injected into the high-performance liquid chromatography system, and detection of total homocysteine was performed by oxidation at +900 mV on an electrode surface (ESA 5010 electrochemical cell; ESA Inc, Chelmsford, Mass). The within-day and between-day coefficients of variation are 6.3% and 6.9%, respectively. Homocysteine levels are reported as µmol/L.

## STATISTICAL ANALYSIS

The analysis was based on a nested case-control design,[36] in which the controls for each case are randomly selected from the population at risk when the case was ascertained (the first date of medical attention for SSD). Appropriate to the nested case-control study design, point and interval estimates of odds ratios (ORs) were obtained by fitting conditional logistic regression models for matched sets.[37] Statistical significance was judged at $\alpha = .05$ (2-tailed).

In the first analysis, we examined whether there was a graded effect of third-trimester homocysteine on SSD risk. Third-trimester homocysteine was analyzed in tertile groups with cutoff points as defined among controls. The ranges of homocysteine levels (micromoles per liter) for each tertile group were as follows: lowest, 3.2 to 8.7; middle, 8.8 to 11.4; highest, 12.1 to 33.7. In this analysis, the ORs represent the effect of third-trimester homocysteine levels in the highest tertile group, compared with the lowest (reference) tertile group, on SSD risk and of third-trimester homocysteine levels in the middle tertile group, compared with the reference tertile group, on SSD risk.

In the second analysis, we sought to test whether our data were compatible with a "threshold" effect in which the third-trimester homocysteine level was associated with an increased risk of SSD only for the highest tertile group of the homocysteine distribution. In this analysis, the lowest and middle tertile groups of third-trimester homocysteine levels were combined to form the reference category, and the OR represents the effect of homocysteine levels in the highest tertile group vs the reference category so defined on SSD risk. This analysis was justified by the precedent of 2 prominent previous studies of adverse birth outcomes secondary to hyperhomocysteinemia. In the first, neonates born to mothers in the highest homocysteine tertile group had lower birth weights than those born to mothers in the low-medium tertile groups.[26] In the second study, only mothers in the highest quartile of homocysteine, but not in any of the lower quartiles, were significantly more likely to have suffered from preeclampsia and to have offspring with premature delivery, low birth weight, and intrauterine growth retardation, all of which are putative risk factors for schizophrenia.[20] These previous studies lend biologic plausibility for the use of a cut point based on the distributions of homocysteine in the sample to define a threshold for hyperhomocysteinemic pregnancies. This analysis also afforded greater statistical power by increasing the numbers of subjects in the reference category.

In both analyses, we considered adjustment for several potential confounders after first obtaining unadjusted estimates of the association between prenatal homocysteine and SSD. The criterion for inclusion in the final model was that the covariates were required to change the β coefficient of the association by more than 10%. The covariates were maternal age (in years); maternal ethnicity (white [reference], African-American, other); socioeconomic status, defined as maternal education (less than high school graduate, high school graduate [reference], some college/college graduate); parity; maternal smoking; maternal body mass index (calculated as weight in kilograms divided by height in meters squared); and gestational age of the serum sample (in days after last menstrual period).

### POWER ANALYSES

Power calculations were based on the likelihood ratio test and a logistic regression model, as presented by Self et al[38] and as implemented in the software package EGRET.[39] These analyses were conducted for the first, second, and third trimesters. In the first power analysis, we presented minimum detectable relative odds across tertile groups of a continuous variable. We assumed a graded linear trend in relative odds across tertile groups, but power calculations were conservatively based on treating the risk factor as a categorical variable in the regression model (ie, testing a series of indicator variables rather than a test for linear trend). The minimum detectable relative odds detectable with 80% power, $\alpha = 0.05$ (2-sided) for tertile groups 1, 2, and 3, respectively, were as follows: trimester 1: 1.0 (reference), 4.0, 16.0; trimester 2: 1.0 (reference), 2.12, 4.50; trimester 3: 1.0 (reference), 1.90, 3.60. In the second power analysis, we presented minimum detectable relative odds at 80% power, $\alpha = 0.05$ (2-sided), comparing the highest tertile group vs the combined lowest 2 tertile groups. For the first, second, and third trimesters, respectively, the minimum detectable relative odds were 6.06, 2.87, and 2.51.

**Table 3. Third-Trimester Homocysteine Levels Categorized by Tertile Group Among Schizophrenia and Other Schizophrenia Spectrum Disorder Cases and Controls***

| Tertile Group (Range of Homocysteine Level, μmol/L) | No. (%) | |
|---|---|---|
| | Cases (n = 59) | Controls (n = 112) |
| Lowest (3.2-8.7) | 18 (30.5) | 37 (33.0) |
| Middle (8.8-11.4) | 11 (18.6) | 39 (34.8) |
| Highest (12.1-33.7) | 30 (50.9) | 36 (32.1) |

*These data are not reported by matched sets; they are presented for descriptive purposes only.

**Table 4. Conditional Logistic Regression Analysis of Third-Trimester Homocysteine Levels and Schizophrenia by Tertile of Distribution: Middle and Highest Tertile Groups Compared With Lowest Tertile Group**

| Tertile Group | Parameter Estimate ± SE | $\chi^2$ Value | Odds Ratio (95% CI) | *P* Value |
|---|---|---|---|---|
| Lowest (ref) | NA | NA | NA | NA |
| Middle | −0.52 ± 0.45 | 1.35 | 0.59 (0.25-1.43) | .25 |
| Highest | 0.61 ± 0.42 | 2.14 | 1.84 (0.81-4.16) | .14 |

Abbreviations: CI, confidence interval; NA, not applicable; ref, reference category.

## RESULTS

### DEMOGRAPHICS

The demographic data are presented in Table 2. There were no differences between cases and controls with regard to maternal age, race, smoking, or gestational age of the serum samples. There was a trend-level effect of maternal education: a greater proportion of cases had less than a high school education. However, we found no relationship between maternal homocysteine levels and maternal education ($P = .91$). Age and sex of the cases and controls were matched by design.

### MATERNAL HOMOCYSTEINE LEVELS IN SSD CASES AND MATCHED CONTROLS

The results for third-trimester homocysteine levels by tertile group and by case-control status are presented in **Table 3**. The proportion of cases in the highest tertile group was 30 (50.9%) of 59.

The results of the conditional logistic regression analyses are presented in **Table 4** and **Table 5**. In the analysis that tested for a graded effect of third-trimester homocysteine on risk of SSD (Table 4), the OR was elevated for the highest tertile group as compared with the refer-

**Table 5. Conditional Logistic Regression Analysis of Third-Trimester Homocysteine Levels and Schizophrenia: Highest Tertile Group Compared With Lowest Plus Middle Tertile Groups**

| Tertile Group | Parameter Estimate ± SE | $\chi^2$ Value | Odds Ratio (95% CI) | *P* Value |
|---|---|---|---|---|
| Lowest + middle (ref) | NA | NA | NA | NA |
| Highest | 0.87 ± 0.36 | 5.89 | 2.39 (1.18-4.81) | .02 |

Abbreviations: CI, confidence interval; NA, not applicable; ref, reference category.

**Table 6. Homocysteine Levels in Cases and Controls by Trimester***

| Trimester | Cases, Mean ± SD, µmol/L | Controls, Mean ± SD, µmol/L |
|---|---|---|
| Trimester 1 (17 cases, 30 controls) | 11.0 ± 4.6 | 10.3 ± 5.2 |
| Trimester 2 (47 cases, 79 controls) | 9.3 ± 2.9 | 10.4 ± 4.5 |
| Trimester 3 (59 cases, 112 controls) | 11.9 ± 5.1 | 10.9 ± 4.6 |

*These data are not reported by matched sets; they are presented for descriptive purposes only.

ence tertile group, although the result was not statistically significant. The unadjusted OR for the highest tertile group was 1.84 (95% confidence interval [CI], 0.81-4.16; *P*=.14). There were greater than 10% changes in the β coefficients when maternal education and maternal race were entered into the models; the remaining covariates, including maternal smoking, maternal age, parity, and gestational age of the serum samples, had no appreciable effects on the association between elevated maternal homocysteine and risk of schizophrenia and were therefore not included in the final model. Adjustment for maternal education and race had a modest effect on the OR (1.57; 95% CI, 0.62-4.01; *P*=.34). There was no increase in risk of schizophrenia for homocysteine levels in the middle tertile group. Adjustment for maternal education and race did not appreciably affect the OR (0.51; 95% CI, 0.18-1.46; *P*=.21).

In the analysis that tested for a threshold effect of third-trimester homocysteine on SSD risk (Table 5), elevated maternal homocysteine was associated with a significant, greater than 2-fold increased risk of SSD (unadjusted OR, 2.39; 95% CI, 1.18-4.81; *P*=.02). Separate adjustment for maternal education, race, smoking, age, parity, and gestational age of the sera had no appreciable effects on the association between elevated maternal homocysteine and risk of schizophrenia (<10% change in β coefficients) and thus did not meet our a priori criterion for confounding. Nonetheless, for further confirmation, we conducted a supplementary analysis that adjusted for maternal education and maternal race in the same model. The results were not appreciably changed in this model with only a 7.2% reduction in the β coefficient (OR, 2.18; 95% CI, 0.98-4.86; *P*=.06).

To assess whether the findings were specific to the third trimester, we conducted secondary analyses of elevated first- and second-trimester homocysteine levels (highest tertile group vs lowest 2 tertile groups combined) in relation to schizophrenia risk. For elevated second-trimester homocysteine levels, there was no increase in risk of schizophrenia (OR, 0.49; 95% CI, 0.19-1.31; $\chi^2$=2.02; *P*=.16). For elevated first-trimester homocysteine levels, there was a numerical increase in risk of schizophrenia but no significant association (OR, 1.91; 95% CI, 0.52-7.06; $\chi^2$=0.95; *P*=.33). Mean levels of homocysteine by case and control status and trimester are reported in **Table 6**.

## COMMENT

We observed an association between elevated third-trimester maternal homocysteine levels and risk of schizophrenia and other SSDs. Specifically, we found that third-trimester homocysteine above a predefined threshold—the highest tertile of the distribution—was associated with a greater than 2-fold statistically significant increase in risk of SSD.

Strengths of the study included direct measurement of homocysteine obtained prospectively and face-to-face diagnostic assessments. In addition, selection bias was minimized by use of a representative sample from a defined birth cohort, continuous follow-up of cases, and controls selected from the source population that gave rise to the cases at the time of diagnosis.

The analysis that tested for a graded effect of third-trimester homocysteine on schizophrenia risk revealed that the point estimate for the middle tertile group was appreciably below 1 (Table 4). However, the 95% CI for the point estimate included 1, indicating that no significant association was found between the middle tertile group and risk of schizophrenia. Thus, these results provide no definitive support for a curvilinear effect.

As noted earlier, animal studies indicate that homocysteine has partial NMDA receptor anagonist effects when glycine levels are in the physiologic range.[7] Although no studies have been conducted on neurobehavioral or neurocognitive effects of perinatal homocysteine levels, these studies have shown that perinatal administration of the NMDA receptor antagonist phencyclidine has long-lasting effects on locomotor response, working memory, and prepulse inhibition that are analogous to abnormalities observed in schizophrenia.[23] Perinatal administration of several NMDA antagonists[9,23,40,41] also causes apoptosis, which has been proposed in the pathogenesis of schizophrenia.[42] In a recent review of maternal folate and homocysteine levels in the etiopathogenesis of schizophrenia, Picker and Coyle[43] elaborated on putative teratogenic mechanisms by which elevated maternal homocysteine levels might increase schizophrenia risk.[43]

Animal studies have also demonstrated that hyperhomocysteinemia disturbs placental function by inducing activation of coagulation factors,[44,45] endothelial damage,[46] and apoptosis in the trophoblast,[15,47] an effect that is reversed by folate. The resulting placental pathology

may act to increase the risk of schizophrenia by compromising delivery of oxygen to the fetus.[6] Numerous studies have implicated fetal hypoxia in the etiology of schizophrenia[2] and elevated homocysteine levels are also associated with several obstetric complications that have been linked with schizophrenia in offspring.[2,48,49] To further examine this hypothesis, studies examining placental tissue pathology from pregnancies giving rise to cases with schizophrenia and matched controls would be required.

Deficiencies of several nutrients may give rise to maternal hyperhomocysteinemia. Folate is a prime candidate because this B vitamin donates a methyl group to homocysteine, permitting its transformation to methionine,[50] and folate levels are inversely related to homocysteine levels.[51] Human pregnancy is a period of increased susceptibility to folate deficiency because of an increased maternal requirement, particularly in later gestation.[52] This suggests that elevated homocysteine levels may have been a marker of low folate concentrations. However, because folate is light-sensitive and not stable in stored serum samples, it could not be quantified. Interestingly, low plasma folate levels accompanied by normal homocysteine levels have been found in schizophrenia.[53] Other studies have reported elevated homocysteine levels in patients with schizophrenia[54,55]; in the latter of these 2 studies, the elevation was only in young men. In a pilot study, we demonstrated an increase in plasma homocysteine levels in schizophrenia cases with low, but not high, folate levels.[56] Other nutritional deficiencies that may be responsible for elevated homocysteine levels include vitamin $B_{12}$, which acts as a cofactor in the conversion of homocysteine to methionine, and vitamin $B_6$, a cofactor in the conversion of homocysteine to cystathionine and cysteine.[50] Elevated homocysteine levels also result from genetic influences. One of the more promising candidates is the C677T polymorphism in the gene for methylenetetrahydrofolate reductase (*MTHFR*). Homozygosity for this thermolabile mutation is related to elevated homocysteine levels and increased risks of cardiovascular[57] and cerebrovascular disease.[14,58] This mutation has been associated with schizophrenia in some[59] but not all[60] studies. Deficiencies of other enzymes, such as methionine synthase and cystathionine β-synthase, may also disrupt the metabolism of homocysteine and lead to an accumulation of homocysteine. In future work, we seek to examine prenatal serum levels of these other nutrients in our subjects and conduct genotyping for the *MTHFR* mutation.

There were some limitations worth noting. First, the sample size of the study was modest. Second, the study used sera that had been frozen for more than 30 years. Although it is worth considering whether lengthy storage time may have altered the serum homocysteine levels, it is unlikely that it would have significantly affected the findings because homocysteine has been shown to be very stable in stored frozen sera and visual inspection of the samples did not indicate prior freeze-thawing, which is the most likely factor to cause degradation of serum proteins. Moreover, careful matching of controls to cases on date of birth and gestational timing and uniform handling and storage of the samples indicates that length of storage would not have biased the associations in the direction of a spurious result.

An additional limitation is the use of maternal homocysteine as a proxy measure for fetal homocysteine. It is worth noting that the maternal serum homocysteine level has been robustly correlated with fetal cord homocysteine at all stages of pregnancy.[26,61] However, there has yet to be a study of maternal serum homocysteine and homocysteine concentration in the fetal brain.

Finally, we had inadequate power to demonstrate a statistically significant association between first-trimester homocysteine and risk of schizophrenia (see the section "Power Analyses"). For elevated first-trimester homocysteine, the risk of schizophrenia was numerically increased, but the finding was not statistically significant. Nonetheless, this hypothesis is tenable given the findings of the Dutch Hunger Winter, in which Susser et al[5] observed an increased risk of schizophrenia coincident with an increase in risk of neural tube defects in the population exposed to severe famine during the periconceptional period. This finding has been replicated in a recent study from China.[62] Given these findings, and the well-documented association between periconceptional folate deficiency and neural tube defects,[63] we should consider whether early gestational folate deficiency, which is inversely related to homocysteine, or subtle neurotoxic effects of homocysteine in early pregnancy may be associated with schizophrenia. A further reason that a first-trimester association between elevated homocysteine levels and schizophrenia may not have been observed is that the first-trimester serum samples were generally drawn during the third month of pregnancy, approximately 2 months following the periconceptional period. As a result, the measured homocysteine levels may not adequately reflect periconceptional folate. We aim to address the limitation of low statistical power for the first-trimester homocysteine level analysis in future studies with larger numbers of schizophrenia cases.

## CONCLUSIONS

The findings suggest that an elevated third-trimester homocysteine level may be a risk factor for adult schizophrenia. Potential mechanisms for the effect include partial antagonism of the NMDA receptor and subtle placental vascular damage that interferes with oxygen delivery to the fetus. These findings warrant replication in independent samples with larger numbers of cases. Folic acid supplements in pregnant women are not uncommonly stopped after the first trimester, and homocysteine levels rise in the third trimester in women not taking folic acid.[64] Thus, if future studies both replicate this association and support a causal link, then the continuation of folic acid supplementation into the second and third trimesters would merit evaluation as a strategy for prevention of schizophrenia in offspring.

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