Prenatal Nutritional Deficiency and Risk of Adult Schizophrenia

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Converging evidence suggests that a neurodevelopmental disruption plays a role in the vulnerability to schizophrenia. The authors review evidence supporting in utero exposure to nutritional deficiency as a determinant of schizophrenia. We first describe studies demonstrating that early gestational exposure to the Dutch Hunger Winter of 1944–1945 and to a severe famine in China are each associated with an increased risk of schizophrenia in offspring. The plausibility of several candidate micronutrients as potential risk factors for schizophrenia and the biological mechanisms that may underlie these associations are then reviewed. These nutrients include folate, essential fatty acids, retinoids, vitamin D, and iron. Following this discussion, we describe the methodology and results of an epidemiologic study based on a large birth cohort that has tested the association between prenatal homocysteine, an indicator of serum folate, and schizophrenia risk. The study capitalized on the use of archived prenatal serum specimens that make it possible to obtain direct, prospective biomarkers of prenatal insults, including levels of various nutrients during pregnancy. Finally, we discuss several strategies for subjecting the prenatal nutritional hypothesis of schizophrenia to further testing. These approaches include direct assessment of additional prenatal nutritional biomarkers in relation to schizophrenia in large birth cohorts, studies of epigenetic effects of prenatal starvation, association studies of genes relevant to folate and other micronutrient deficiencies, and animal models. Given the relatively high prevalence of nutritional deficiencies during pregnancy, this work has the potential to offer substantial benefits for the prevention of schizophrenia in the population.

Key words: prenatal/schizophrenia/nutrition

Evidence from various domains of research indicates that a disturbance in early neurodevelopment may lead to a vulnerability to schizophrenia in adolescence or adulthood. Accumulating data have implicated the in utero environment in the etiology of this disorder.1 In this article, we review and discuss the sources of evidence for testing hypotheses about the relation of prenatal nutritional deficiency to offspring risk of schizophrenia. The long interval between an exposure in the prenatal period and the risk of schizophrenia in adulthood and the difficulty of obtaining precise data on prenatal nutritional intake are among the considerable challenges faced by researchers in this field. Nonetheless, successful studies have been built around historic events, a design sometimes referred to as a “natural experiment.”

We first describe studies linking prenatal exposure to the Dutch Hunger Winter of 1944–1945 with offspring schizophrenia and a recent worthy replication of this finding. We also discuss some of the candidate nutritional deficiencies that might explain the results from these studies. Next we describe how the intriguing findings from these studies can be pursued in birth cohorts followed up for schizophrenia, making use of archived biological specimens to measure prenatal nutritional status. Finally, we discuss the approaches being developed for more powerful tests of these hypotheses, focusing for illustrative purposes on the folate/homocysteine (hcy) pathway.

Natural Experiments

The strongest evidence linking prenatal starvation to schizophrenia derives from natural experiments. Natural experiments are perhaps best known in the context of genetic epidemiology where twin and adoption studies are classic examples. However, natural experiments of a different kind can be built around circumscribed historical events. Sometimes these are tragic events such as famines, as in the examples described below, but a beneficial event can also be the basis for a natural experiment. The various kinds of natural experiment share 2 defining features.2 First, unlike an ordinary observational study, people are selected into an exposed or unexposed group by an event largely outside of their control. Second, unlike an ordinary experiment, this event is not under the control of the investigator. As a result of these features, the design tends to be stronger than an ordinary...
observational study though not as strong as an ordinary experiment.

**Dutch Hunger Winter**

The first direct test of an association between prenatal starvation and schizophrenia arose as a result of the Dutch Hunger Winter of 1944–1945, one of the tragic events of World War II. The famine was precipitated by a Nazi blockade of occupied Holland in October 1944, in retaliation for the support by the Dutch resistance to the Allied command. Already compromised by food shortages at the onset of the blockade, the food situation worsened further due to an unusually severe winter, which froze the canals used to transport food. The famine grew steadily worse until it ended with liberation in May 1945. During the height of the famine in the 2–3 months prior to liberation, the daily food ration was mainly bread and potatoes (by April 1945 the ration provided less than 500 calories daily), supplemental food was scarce, and the population was nutritionally depleted. Mortality was more than double, and fertility (reflected in births 9 months later) was less than half that of the previous year. Most affected were the 6 cities of western Holland.

Although tragic, the famine has provided a unique opportunity to examine health effects throughout life of starvation during specific periods of gestation. This was made possible by the fact that the height of the famine was brief, clearly circumscribed in time, and afflicted a population that maintained excellent records on both food rations during the famine and on health outcomes for several decades hence. An early neurodevelopmental finding from the Dutch famine studies was an increase in congenital neural defects, especially neural tube defects including spina bifida and anencephaly, among a birth cohort conceived during the height of the famine. This finding of an effect on neurodevelopment bolstered the plausibility of prenatal famine as a cause of later schizophrenia. It also provided a key component of the rationale for the analytic design of the Dutch famine study of schizophrenia.

In the schizophrenia study, we examined whether the birth cohort with excess central nervous system (CNS) anomalies also had an increased risk of schizophrenia. The exposed cohort was defined by birth in the famine cities during October 15–December 31, 1945; the height of the famine corresponded to the periconceptional period or early gestation for this cohort. The Dutch psychiatric registry was used to compare psychiatric outcomes in adulthood for exposed and unexposed birth cohorts. The primary outcome was a diagnosis of narrowly defined schizophrenia by International Classification of Diseases, Eighth/Ninth Revision, criteria (295.1, 2, 3, 6), as categorized in the Dutch National Psychiatric Registry for the years 1970–1992, during which time the subjects were aged 24–48 years. The study found a significant, 2-fold increase in the cumulative risk of schizophrenia in the exposed birth cohort. Moreover, a subsequent study showed a 2-fold increased risk of schizoid personality disorder in the same exposed birth cohort. In this instance, the outcome data were obtained from military induction examinations conducted on all males when they reached age 18. Unlike the schizophrenia result, which was based on the psychiatric registry data, this finding was not limited to treated cases. It provides further evidence of an effect on schizophrenia spectrum disorders (SSDs) from an independent data source.

Inspection of the disease risks for successive birth cohorts of 1944–1946 revealed striking peaks in the incidence of schizophrenia, schizoid personality, and congenital neural defects in this same birth cohort. This occurred in the context of an otherwise stable incidence of these disorders among cohorts exposed to famine during other periods of gestation and cohorts who were completely unexposed to famine during pregnancy.

**Chinese Famine Study**

It proved difficult to identify a second natural experiment along the same lines in which the Dutch result could be replicated or refuted. While famine is not uncommon in the world, it is usually not clearly demarcated in time, and the resources for assessing health outcomes are not available. In a recent study, however, the relation of prenatal famine to risk of schizophrenia was successfully examined in a cohort in the Wuhu region of Anhui Province, China. In the late 1950s, a massive famine was precipitated in China by the marked social and economic upheaval known as the Great Leap Forward, which involved agricultural collectivization, use of flawed agricultural practices, and diversion of agricultural labor to other purposes. By some estimates, the famine caused 30–40 million deaths. Anhui Province was one of the most affected.

In the Chinese study, monthly data on caloric rations were not available. Nonetheless, the authors, based on the Dutch results, could examine whether the risk of schizophrenia was increased in the birth cohorts conceived during the height of this famine. Accordingly, the Wuhu birth cohorts of 1960 and 1961 were defined as the exposed group. These cohorts were conceived in the period of most severe famine for this region, as documented in historical records, and as reflected in birth rates for 1960 and 1961 that were less than one-third the average for 1956–1959.

Thus, the authors compared the cumulative risk of schizophrenia among the birth cohorts of 1960 and 1961 with that of birth cohorts prior to and subsequent to the famine. The schizophrenia outcomes were obtained from systematic review of the records of the sole...
psychiatric hospital in the Wuhu region over the period 1971–2001. The increased risk—approximately 2-fold—was similar to that of the Dutch famine. A key advantage of the study was its much larger sample size. Replication in a population of very distinct ethnicity and culture from the Netherlands is consistent with a biological causation hypothesis.\textsuperscript{11}

Several limitations of these studies need to be considered. We first consider the Dutch famine. First, as in all observational studies, there is the possibility that the results may have been confounded by certain known and unknown factors. One possible confounder that needs to be considered is prenatal stress. The exposed population was likely to have already been under severe stress due to the combination of the famine, war, and many other hardships, and prenatal stress has been associated with schizophrenia in some though not all studies, and there have been several limitations to this work.\textsuperscript{12–14} However, it is worth noting that other areas of the Netherlands, which were also exposed to war and moderate levels of starvation, did not evidence an increased risk of schizophrenia.\textsuperscript{15} A second potential confounder is social class of origin. We consider confounding by this factor to be unlikely because no associations have been demonstrated between schizophrenia and social class of origin in the Netherlands. Moreover, because the exposed cohort was weighted toward the upper classes due to greater fertility, this would tend to reduce the observed association between prenatal famine and schizophrenia.\textsuperscript{5} Other limitations of the study included group data to define exposure and the inability to tease apart the effects of different types of nutritional deficiencies or of other substances that may have been ingested that might have toxic potential. With regard to the former limitation, it should be emphasized that the exposure was documented in detail and was pervasive in the population.

While the Chinese famine study offered several strengths, one weakness is that famine exposure data were not available by month; hence, the precision of the periods of famine cannot be as accurately estimated as in the Dutch famine study. However, the pattern of increased risk of schizophrenia by birth year is consistent with an early gestational effect; this is discussed in more detail in St Clair et al.\textsuperscript{9}

### Candidate Nutritional Deficiencies

We first consider specific candidate micronutrients that might explain the association between early gestational famine and schizophrenia. Several of the most prominent candidates are as follows.

**Folate**

The coincidence of the peak in risk of schizophrenia and schizoid personality disorder with congenital neural defects in the Dutch famine cohort provides a potentially valuable clue. One of the most consistent findings in the literature on prenatal nutrition and CNS disturbances is the association between periconceptional folate supplementation and neural tube defects.\textsuperscript{16,17} Studies have shown convincingly that this simple dietary intervention diminishes the likelihood of neural tube defects by as much as 80%,\textsuperscript{17–19} although the biological mechanism for the protective effect remains unclear.

This intriguing coincidence led us to speculate early on that the folate pathway might also be important in the origins of schizophrenia.\textsuperscript{19,20} Later, genetic association studies of neural tube defects and of schizophrenia provided a further reason for considering folate as a candidate. Studies have suggested that mutations in known genes involved in folate-dependent pathways increase liability for neural tube defects. For example, a single-nucleotide polymorphism C677T of the gene coding for 5,10-methylenetetrahydrofolate reductase (MTHFR) has been associated with neural tube defects.\textsuperscript{21} Some, though not all, studies have also shown an increase in frequency of this variant allele in patients with schizophrenia (see Gilbody et al\textsuperscript{22} for meta-analysis).

In addition, much has been learned in the past decade about the key role of folate in health and development. Folate is a generic term for a family of chemically similar compounds that facilitate the transfer of one-carbon units in metabolic pathways.\textsuperscript{23} Because humans cannot synthesize folate, it must be obtained from the diet. Folate is particularly important in the synthesis of purines and pyrimidines, in the conversion of homocysteine to methionine, and in the interconversion of serine and glycine. Based on current knowledge, there are at least 3 pathways by which prenatal folate deficiency could plausibly influence the risk of offspring schizophrenia.\textsuperscript{24–28} First, folate deficiency can impede the synthesis and repair of DNA and might thereby increase the risk of de novo mutations (J. M. McClellan, MD, E. Susser, MD, DrPh, M. C. King, PhD, unpublished data, 2007). Second, folate deficiency can impede the production of methyl donors and the methylation of DNA and might thereby affect the expression of genes that regulate neurodevelopmental processes.\textsuperscript{29} Third, folate deficiency can impede the conversion of homocysteine and thereby lead to accumulation of homocysteine with adverse effects on fetal brain development.

**Essential Fatty Acids**

Essential fatty acids (EFAs) play critical roles in brain development. Humans do not have the ability to synthesize these fatty acids de novo and thus are largely dependent upon dietary sources.\textsuperscript{30} Docosohexaenoic acid (DHA), an omega-3 fatty acid, is the primary structural fatty acid in the brain, comprising 25%–30% of the structural fatty acids in the gray matter.\textsuperscript{30,31} Maternal supplementation with cod liver oil, which contains very
long-chain n-3 fatty acids, during pregnancy has been associated with higher IQ at age 4 years. In that study, umbilical plasma levels of DHA were correlated with increased age 4 IQ and umbilical eicosapentanoic acid was associated with increased mental processing skills in childhood. Umbilical vessel EFA levels, including DHA, have been correlated with decreased neonatal neurological abnormalities. In 2 additional studies, however, long-chain polyunsaturated fatty acid status at birth was not associated with cognitive function at age 4 and 7. Nonetheless, further investigation of maternal DHA and other EFAs during pregnancy in relation to adult outcomes, including risk of schizophrenia, may prove fruitful.

Retinoids

Retinol (vitamin A) and other retinoids are essential nutrients that are required by the early embryo and fetus for gene expression, cell differentiation, proliferation, and migration. Vitamin A deficiency in animals results in gross CNS malformations, including hydrocephalus, anencephaly, spina bifida, and an underdeveloped posterior hindbrain, including loss of the myelencephalon. These and other findings suggest that retinoid signaling plays a key role in morphogenesis of the CNS. Because retinoids also function as antioxidants, they may help protect the developing brain from a number of potential insults that generate free radicals.

Vitamin D

McGrath has hypothesized that prenatal exposure to vitamin D deficiency is a risk factor for schizophrenia. The plausibility of this hypothesis is supported by the role of this vitamin in cell growth and differentiation, the excess of winter births in schizophrenia (a period when vitamin D levels are low), and increased births of preschizophrenic subjects in urban areas, where vitamin D deficiency is higher. A preliminary study of maternal vitamin D levels in archived prenatal sera from the Collaborative Perinatal Project, however, showed no decrease in prenatal vitamin D in subjects who later developed schizophrenia.

Iron

Maternal iron deficiency is known to affect the development of the fetal brain. This may occur through several mechanisms during pregnancy. First, during pregnancy, the needs of the growing fetus and placenta, as well as the increasing maternal red blood cell mass, impose a substantial demand on maternal iron stores, reducing hemoglobin levels and increasing the incidence of anemia, which compromises oxygen delivery to the developing fetus. Indirect indicators of fetal hypoxia have been associated with increased susceptibility to schizophrenia;

the hippocampus is believed to be particularly vulnerable to hypoxic insult. Iron deficiency may also affect the development of brain structures and functions of relevance to schizophrenia, independent of anemia. Iron metabolism has a profound effect on the development and functioning of dopaminergic neurotransmission that has long been implicated in the pathophysiology of schizophrenia. Iron is also essential for normal myelination that affects neural connectivity and myelin deficits have been observed in schizophrenia.

We have recently demonstrated that prenatal exposure to low maternal hemoglobin, which is highly correlated with iron, is associated with an increased risk of schizophrenia in adult offspring.

Protein-Calorie Malnutrition

Although we consider it more likely that a micronutrient is involved, protein-calorie malnutrition (PCM) should also be considered as a potential explanation for the association between famine and schizophrenia. There is some support from preclinical studies for the biological plausibility of prenatal PCM as a risk factor for schizophrenia. Prenatal and early postnatal PCM are associated with neurotransmitter, cellular, electrophysiological, and behavioral disruptions that have been demonstrated in schizophrenia. These include increased dopamine and serotonin release and turnover and dysfunction in the hippocampus, including decreased cell numbers, reduced dendritic branching, establishment and maintenance of long-term potentiation, and behavioral deficits such as impaired spatial performance. Prenatal protein deprivation has also been shown to reduce prepulse inhibition in rats in early adulthood but not prepubertally. Many studies have demonstrated that prepulse inhibition is impaired in patients with schizophrenia. Furthermore, prenatally deprived rats had an increase in binding of the N-methyl-D-aspartic acid (NMDA) receptor, which has been implicated in the pathophysiology of schizophrenia, as well as increased sensitivity to MK-801, an NMDA receptor antagonist.

Archived Biological Specimens

To study specific micronutrients, the most direct approach is to use archived prenatal biological specimens from birth cohorts followed up for schizophrenia. The first large birth cohort studies to collect and archive biological specimens from pregnant women were the Child Health and Development Study (CHDS) and the National Collaborative Perinatal Project. These were contemporaneous studies initiated in the 1950s in the United States. Decades later, the archived sera from both these cohorts are being used to study prenatal nutrients and offspring risk of schizophrenia. The strategy is that of a nested case-control design, ie, the cases of
schizophrenia in the cohort are ascertained and are compared with a control group selected from the same cohort. Thus, the serologic study can be efficiently completed using a few hundred subjects rather than the many thousands enrolled in the original cohort.

Although these 2 cohorts were the first to archive prenatal specimens, many other cohorts established in later years have done so. We anticipate, therefore, that this strategy will be widely applied because these other cohorts pass through the age of risk for schizophrenia.

A prototype for this approach is the Prenatal Determinants of Schizophrenia (PDS) study (for a detailed description see PDS design article). The cohort members in the PDS study were derived from the CHDS. During 1959–1966, the CHDS recruited virtually all pregnant women receiving obstetric care from the Kaiser Permanente Medical Care Plan, Northern California Region (KPMCP) in Alameda County, CA. 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. KPMCP membership was diverse; racially, educationally, and occupationally, it was similar to the employed population of the Bay Area of California at the time, although there was underrepresentation of the extremes of income. 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. Following exclusion of subjects who did not receive a maternal interview including important demographic and lifestyle factors, and random selection of one subject per family in order to eliminate nonindependent observations, the final cohort consisted of 7,796 subjects.

Potential cases of schizophrenia and other SSDs were identified by a screening procedure involving computerized record linkages between CHDS and KPMCP identifiers from inpatient and outpatient registries based on diagnoses of International Classification of Diseases, Ninth Revision, 295–299, and by a pharmacy registry, based on prescriptions for antipsychotics. Psychiatric and medical records were then reviewed for evidence of psychotic symptoms by an experienced, board-certified research psychiatrist. Subjects who screened in for psychotic illnesses were administered the Diagnostic Interview for Genetic Studies (DIGS) and Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, diagnoses were assigned by consensus of 3 experienced research psychiatrists. Potential cases not interviewed were diagnosed by chart review by experienced clinicians, and all chart diagnoses were confirmed by a research psychiatrist. This protocol resulted in 71 total SSD cases, 44 of whom received the DIGS and 27 of whom were diagnosed by chart review.

In the CHDS, a wealth of data were collected on prenatal, perinatal, and neonatal exposures, and a subsample were followed up through childhood. A maternal interview included a detailed reproductive history; health-related behaviors in mother and father; lifestyle habits, including smoking, alcohol, and weight before and during pregnancy; and detailed sociodemographic information. Detailed data on medical conditions and prescribed medications were abstracted from obstetric and medical records for all enrolled mothers. Extensive data on labor and delivery records were abstracted. Additional data included blood groups and placental morphology.

As noted earlier, a remarkable feature of the study that has made it possible to test hypotheses regarding specific prenatal micronutrient deficiencies was the availability of archived maternal sera, which were drawn during pregnancy, frozen, and archived for the past 40 years. Prenatal sera are available in virtually all pregnancies in the cohort for later gestation and for about 40% during the first trimester. The PDS study is well suited for both cohort and nested case-control designs. The latter design is particularly appropriate for serologic analyses, given the prohibitive costs and logistics involved with analyses of prenatal sera on a large cohort. In the nested case-control design, the controls for each case were selected to represent the population at risk at the time the case was ascertained. Matching criteria for controls included membership in KPMCP, date of birth, sex, number of maternal serum samples drawn during the index pregnancy, and timing of the first maternal blood draw during the index pregnancy. Matching for time of KPMCP membership ensured that controls represented the population at risk at the time of case ascertainment. Matching on birth date ensured control of potential confounding by season of birth. Gender was matched to allow for assessment of different effects for men and women. Matching on maternal blood draws was conducted to permit sufficient and comparable data for serologic analyses.

Serologic analyses are presently underway to examine the micronutrients described earlier. Both the potential and the limitations of this approach are illustrated by a recent study from our group. Hcy levels are a reliable marker of serum folate, which is not measurable in archived sera. Hcy has long been known to increase folate deficiency states secondary to the critical role of folate in the methylation of hcy and its conversion to methionine.

Using the archived sera of the PDS cohort, we found that elevated hcy in the first trimester was associated with a nearly 2-fold increase in risk of schizophrenia. We had only a small number of subjects with available first trimester sera, however, and the finding fell well short of statistical significance. Moreover, in these subjects, the sera were generally drawn during the latter period of the first trimester, approximately 2 months or longer after the periconceptional period. Thus, the first trimester
results were inconclusive, though suggested that further studies with a larger number of cases with first trimester samples might substantiate the folate deficiency hypothesis.

The sample size did offer sufficient power to test the hypothesis that elevated third trimester hcy was associated with risk of schizophrenia because maternal archived serum specimens drawn during late pregnancy were available for virtually all cohort members. We found that subjects with elevated third trimester hcy, defined as the highest tertile of the distribution, was associated with a significant, greater than 2-fold increased risk of schizophrenia in the offspring; the findings persisted following adjustment for several potential confounders including maternal education, race, smoking, and age.

The result for third trimester hcy does not necessarily support an effect of folate deficiency in early gestation. At physiologic glycine concentrations, hcy has NMDA receptor antagonist properties, and dysfunction of the NMDA receptor has been implicated in schizophrenia. Moreover, perinatal administration of phencyclidine, a known NMDA receptor antagonist, induces a disruption in synaptogenesis, prepulse inhibition, and working memory. hcy is also known to cause placental vasculopathy through several mechanisms which might lead to fetal hypoxia, a putative risk factor for schizophrenia. We had therefore hypothesized that elevated third trimester hcy would be associated with risk of schizophrenia, based on the late gestational effects of NMDA receptor blockade on endophenotypes of schizophrenia and on the increase in placental blood flow during the third trimester. Because this is the first time that elevated prenatal hcy has been associated with schizophrenia, this finding requires replication in an independent sample.

Discussion

In this section, we raise several issues relevant to the plausibility of prenatal nutrition in the pathogenesis of schizophrenia in the broader context of epidemiologic studies of schizophrenia and of previous research on effects of malnutrition on neurodevelopment.

We first consider the argument that one might expect an increased incidence of schizophrenia in developing countries, in which chronic malnutrition is widespread, if prenatal nutritional deficiency is a bona fide risk factor for schizophrenia. Two points are worth noting in this regard. First, it is unclear whether the incidence of schizophrenia is higher in countries that have more malnutrition because to date no incidence studies have been conducted in regions of developing countries that have experienced chronic malnutrition. Second, such studies may not reveal an effect of chronic or severe malnutrition on risk of schizophrenia because the consequent effects on child mortality will reduce survival to the age of risk for schizophrenia. A second issue that requires consideration is whether there are particular periods of gestational exposure to malnutrition that confer greater vulnerability to schizophrenia. Based on the Dutch and China famine studies, it appears likely that exposure during the first trimester plays an especially important role, though studies of more specific exposures in non-famine-exposed populations may yield different findings. In our view, what is most important in the formulation of gestational specific hypotheses is the interaction of the nutritional exposure with the specific neurodevelopmental events within each window of gestation. Although there are relatively few examples in the literature on prenatal nutritional factors as causes of developmental disorders, one of the most salient examples is periconceptual folic acid deficiency as a cause of neural tube defects. This is concordant with our findings on the Dutch famine, and supports the biological plausibility of first trimester exposure to famine and schizophrenia.

In 2 previous studies, our group demonstrated that exposure to famine during the second and third trimester was associated with a significantly risk of affective psychosis, suggesting that the timing of exposure may have specificity to the type and severity of disorder that ensues. In the Dutch famine, caloric rations of <1000 kcal were associated with an increased risk of schizophrenia, while exposure to lesser severity of famine (1000–1500 kcal) was not related to an increased risk of schizophrenia, suggesting that the degree of nutritional deficiency may also be relevant.

Third, we consider the relative effects of nutritional deficiency during the fetal period, compared with childhood. Clearly, both micro- and macronutrient deficiencies during childhood are associated with neuropsychiatric sequelae. With regard to schizophrenia, vitamin D supplementation during the first year of life was associated with reduced risk of the disorder in males but not females.

A fourth consideration is the degree to which the findings are concordant with other epidemiologic findings of schizophrenia. A markedly increased risk of schizophrenia has been found among first- and second-generation immigrant populations. Conceivably, this finding might be accounted for by greater prenatal malnutrition that would be expected in immigrant groups due to lower socioeconomic status and other factors. However, as argued by Cantor-Graae and Selten, evidence relating low social class of origin to schizophrenia is inconsistent, and no effect of neighborhood levels of socioeconomic status was found in migrant studies of schizophrenia. Hence, at least insofar as social class differences are concerned, there is as yet no compelling evidence that dietary differences among immigrants explain the association between migrant status and schizophrenia. A similar argument may be made with regard to the well-replicated
association between urbanicity and schizophrenia, in which dietary deficiencies linked to social deprivation prominent among inner city neighborhoods would be a potential cause of the association.

**Summary and Future Directions**

In summary, evidence from 2 independent natural experiments supports the hypothesis that prenatal nutritional deficiency is related to the development of schizophrenia. Given that micronutrient deficiencies are common during pregnancy, even in well-fed populations, this work could stimulate public health efforts to ensure adequate nutritional intake during pregnancy, which may not only facilitate the prevention of schizophrenia but also result in additional improvements in health outcomes for offspring throughout the life course.

We envision 5 strategies to further test this hypothesis. The first is the direct test for associations between prenatal nutritional deficits and schizophrenia. This is being accomplished through specific micronutrient assays of archived maternal sera in birth cohorts that have been followed through the age of risk for schizophrenia. One caveat of this work in the PDS study is the relatively low statistical power to detect small effects due to a modest number of subjects with prenatal sera, particularly during the first trimester. However, this limitation can be potentially overcome by combining comparably designed cohorts for selected analyses. Moreover, new cohorts with high-quality data including archived biological prenatal specimens will be coming of age for risk of schizophrenia over the ensuing years and may be used for larger and more definitive studies. An example of this strategy is a study of the impact of folate deficiency in a birth cohort of 100,000 being collected in Norway. In this cohort, the investigators are collecting samples for genetic analyses on mother, father, and child, as well as archiving prenatal biological specimens for analysis of nutrients. The neurodevelopmental outcomes are being traced over the life course. Although it will be 2 decades before it is possible to study schizophrenia in this cohort, the findings on earlier outcomes may yield insights into the spectrum of manifestations consequent to prenatal nutritional deficiency.

A second strategy is to assess the individuals who developed schizophrenia after prenatal exposure to famine. The study in China now provides a large enough sample for this purpose. The homogeneous environmental exposure makes this a particularly good strategy for identifying genes that play a role in schizophrenia. For example, this homogeneity increases the statistical power for detecting genetic variants hypothesized to interact with prenatal nutritional deficiency to cause schizophrenia. With the advance of genomic technology, this design may also permit exploration of whether prenatal starvation induced genetic mutations or epigenetic effects that predispose to schizophrenia. For example, hcy appears to act as a methyl donor following activation to S-adenosylmethionine, influencing DNA methylation, which could alter regulation of genes.

A third approach is to investigate in general populations the genes that are salient to metabolic pathways involving these nutrients. One such example is the C677T variant in the *MTHFR* gene described above. Genetic association studies of this variant have already been conducted, and large family-based genetic association studies are now underway in the hope of attaining more definitive results. Complexities in these studies include the possibility that either the fetus or the mother’s genes or both could be important to the in utero environment and that the genetic variant may only be associated with disturbed development in the presence of the relevant prenatal nutritional deficiency (eg, folate deficiency for a *MTHFR* variant).

A fourth approach is the development of animal models of prenatal malnutrition. In an accumulating body of research, investigators have explored the biological mechanisms of action linking prenatal nutritional deficiency to abnormalities of brain function that are salient to the pathophysiology of schizophrenia. These include, but are not limited to, studies of prenatal vitamin D depletion and PCM. Further translational research on nutritional animal models are expected to identify new molecular targets on which future interventions might be based. These types of studies are expected to flourish with the rapid development of modern neuroscience and molecular genetic approaches.

**Funding**

The National Institute of Mental Health (NIMH) 1K02MH65422-01 to A.S.B.; NIMH 1R01MH 63264-01 to A.S.B.; National Alliance for Research on Schizophrenia and Depression (NARSAD) Independent Investigator Award to A.S.B.; NIMH 1R01MH 053147 to E.S.S.; Independent Investigator Award to E.S.S.; National Institute on Aging P01AG023028 to E.S.S.; National Institute of Child Health and Human Development (NIMH) N01-HD-1-3334 to B. Cohn; NICHD NO1-HD-6-3258 to B. Cohn.

**Acknowledgments**

We wish to thank Catherine Schaefer, PhD, Barbara Cohn, PhD, Barbara van den Berg, MD, Michaeline Bresnahan, PhD, and Justin Penner, MA, for their contributions to this work.

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