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Low maternal retinol as a risk factor for schizophrenia in adult offspring

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Abstract

Background—Prenatal micronutrient deficiency has been linked to later development of schizophrenia among offspring; however, no study has specifically investigated the association between vitamin A and this disorder. Vitamin A is an essential nutrient which is required by the early embryo and fetus for gene expression and regulation, cell differentiation, proliferation and migration. Previous work suggests that vitamin A deficiency in the second trimester may be particularly relevant to the etiopathogenesis of neurobehavioral phenotypes some of which are observed in schizophrenia.

Methods—We examined whether low maternal vitamin A levels in the second trimester are associated with the risk of schizophrenia and other schizophrenia spectrum disorders (SSD) in the Prenatal Determinants of Schizophrenia study; third trimester vitamin A levels were also examined in relation to SSD. The cases were derived from a population-based birth cohort; all cohort members belonged to a prepaid health plan. Archived maternal serum samples were assayed for vitamin A in cases (N=55) and up to 2 controls per case (N=106) matched on length of

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Ms. Bao contributed to analytic study design, conducted the statistical analyses, and contributed to interpreting the findings and writing the manuscript. Ms. Ibram contributed to the literature searches, data interpretation, manuscript writing, and developing the tables. Dr. Blaner contributed to the protocol design, led the serologic assays for vitamin A, reviewed all vitamin A data, contributed to interpretation of the data, and to manuscript writing. Dr. Quesenberry contributed statistical expertise and manuscript writing. Dr. Shen contributed statistical expertise and manuscript writing. Dr. McKeague contributed to statistical analysis, interpretation of the findings, and manuscript writing. Dr. Schaefer contributed to protocol design, study implementation, and manuscript writing. Dr. Susser contributed to protocol design, study implementation, and manuscript writing. Dr. Brown contributed to protocol design, study implementation, interpretation of the findings, and manuscript writing.

Conflict of interest
Nothing to report.

membership in the health plan, date of birth (±28 days), sex, and gestational timing and availability of archived maternal sera.

Results—For the second trimester, low maternal vitamin A, defined as values in the lowest tertile of the distribution among controls, was associated with a greater than threefold increased risk of SSD, adjusting for maternal education and age (OR=3.04, 95% CI=1.06, 8.79, p=.039). No association between third trimester maternal vitamin A and SSD was observed.

Conclusions—Although further investigations are warranted, this is the first birth cohort study to our knowledge to report an association between low maternal vitamin A levels and SSD among offspring.

Keywords

Vitamin A; Retinoids; Schizophrenia; Nutrition; Micronutrient; Neurodevelopment; Epidemiology; Risk factors; Environment

1. Introduction

Vitamin A is an essential nutrient which is required by the early embryo and fetus for gene expression and regulation, cell differentiation, proliferation and migration (Morris-Kay, 1993; Maden, 1999; Nau and Elmazar, 1999). As elaborated by Goodman (1995, 1996, 1998), convergent evidence has implicated vitamin A and other retinoids in schizophrenia. First, there are similarities with respect to physical anomalies, including craniofacial anomalies, enlarged ventricles, and other defects in patients exposed to prenatal retinoid excess and deficiency and anomalies found in patients with schizophrenia spectrum disorders (SSD) (Goodman, 1995). Second, retinoids have been linked to neurotransmitter and neurobiological systems proposed to be disrupted in schizophrenia (Goodman, 1995, 1998). Retinoic acid (RA), an active form of vitamin A, is essential for the effects of vitamin A on vertebrate embryonic development. The targets of RA transcriptional regulation include dopamine and glutamate receptors, which have been widely implicated in the neurochemistry of schizophrenia. This suggests that retinoids play a significant role in the control of gene expression of these neurotransmitters (Samad et al., 1997; Goodman, 1998; Krezel et al., 1998). Third, retinoid pathways regulate the growth and differentiation of cells affecting many aspects of brain development including neurogenesis, axon path finding, activity-dependent plasticity (Goodman, 1998; LaMantia, 1999; Maden, 2000), cell survival, neurite outgrowth (Aggarwal et al., 2006; Alique et al., 2006; Ertesvag et al., 2007), and neurodevelopmental events that have been implicated in schizophrenia (Thornberg and Saklad, 1996; Krezel et al., 1998). Moreover, in a recent genome-wide screening study of schizophrenia, disruptions in the gene for the retinoid X receptor alpha (RXRA) by copy number variants (CNVs) were associated with schizophrenia (Lee et al., 2010).

While few epidemiologic studies have examined the relationship between low maternal vitamin A intake or serum levels and congenital malformations, two studies have reported that vitamin A supplementation lowers the risk of congenital abnormalities (Mills et al., 1997; Czeizel and Rockenbauer, 1998), and another reported significantly lower vitamin A blood levels in newborn infants with myelomeningocele (Drott and Meurling, 1992). Also, evidence suggests that low vitamin A concentration in maternal serum is associated with diminished birth weight (Brandt, 1978; Shenai et al., 1981; Navarro et al., 1984; Ghebremeskel et al., 1994; Gazala et al., 2003), a risk factor for schizophrenia (Abel et al., 2010). In addition, low vitamin A in cord serum has been associated with diminished motor developmental quotient (DQ) at age 2 (Chen et al., 2009).

In a previous study on early postnatal mice, a period thought to be broadly equivalent to the second trimester in humans, RA administration during this time period was shown to have particular sensitivity on development of the hippocampus and anterior cingulate (Luo et al., 2004). In addition, defects in synaptic plasticity in the adult hippocampus, including impaired long term potentiation (LTP) and long term depression (LTD), can be replicated by vitamin A deprivation in early postnatal mice, and reversed by RA administration (Misner et al., 2001). Moreover, this developmental period in mice is accompanied by rapid increases in the expression of retinaldehyde dehydrogenases (RALDH), which synthesize RA in the forebrain (Wagner et al., 2002). The RALDH expression patterns bear interesting spatiotemporal parallels to neuroanatomic abnormalities found in schizophrenia.

In spite of the potential importance of retinoids in neurodevelopmental and neurochemical processes relevant to schizophrenia, previous birth cohort studies have not directly examined the relationship between maternal vitamin A intake during pregnancy and schizophrenia among the offspring. For this study, we measured retinol, the form of vitamin A that is delivered to peripheral tissues to serve as a source of retinoic acid, which is needed to regulate vitamin A-dependent responses (Maden et al., 1996, 1999; Nau and Elmazar, 1999); consequently, “vitamin A” will be used to denote retinol throughout the manuscript. Following on the findings reviewed in the above paragraph, we examined whether low maternal vitamin A levels in the second trimester are associated with schizophrenia and other schizophrenia spectrum disorders (SSD) in offspring members of the Prenatal Determinants of Schizophrenia (PDS) study (Susser et al., 2000); given the overlap in certain vitamin A-related developmental processes between trimesters, we also assessed the relationship between vitamin A in the third trimester and SSD. Analyses were not conducted for the first trimester given the small number of maternal serum specimens from that gestational period. The study capitalized on the availability of prenatal serum specimens, which were collected in this cohort and were archived for future use (Brown et al., 2004, 2007).

2. Methods

2.1. Description of the cohort

The PDS study has been described in detail previously (Susser et al., 2000; Brown et al., 2007), and will therefore be only summarized here. The cohort members were derived from the Child Health and Development Study (CHDS) (van den Berg, 1979) which took place from 1959 through 1966. The CHDS recruited nearly all pregnant women who were receiving obstetric care from the Kaiser Permanente Medical Care Plan (KPMCP) in Alameda County, California. All of their 19,044 live births were automatically enrolled in KPMCP. Data were prospectively collected from maternal medical records, maternal interviews, and other sources of information.

Serum samples during pregnancy were obtained for the vast majority (91.6%) of the subjects. In general, the samples were drawn in the morning; however, the protocol did not require the subjects to be fasting. All samples were uniformly handled and stored following a strict protocol.

The cohort consisted of the 12,094 (96%) members of the CHDS followed through KPMCP registries from January 1, 1981 to December 31, 1997 (Susser et al., 2000). Case ascertainment began on January 1, 1981 when electronic registries for psychiatric diagnoses in KPMCP were established and continued over a 17-year period of continuous follow-up.

2.2. Screening and diagnosis of schizophrenia and other schizophrenia spectrum disorders

Cases were defined as individuals who were treated for schizophrenia and other SSD including any of the following related diagnoses: schizophrenia, schizoaffective disorder, delusional disorder, psychotic disorder not otherwise specified, and schizotypal personality disorder. This definition was based on precedents indicating familial aggregation between these disorders (Kendler et al., 1995). In later analyses, schizophrenia was analyzed apart from other SSD. Screening procedures for potential cases with SSD were conducted on the inpatient, outpatient, and pharmacy registries of KPMCP and involved three steps: 1) ascertainment of potential cases from computerized record linkages between CHDS and KPMCP, 2) chart review of potential cases to confirm diagnostic eligibility for assessment, and 3) diagnostic interview (or chart review) and consensus diagnosis. Subjects from the inpatient registry were screened for potential SSD based on registry diagnosis codes 295 through 299 from *the International Classification of Diseases, Ninth Revision (ICD-9)*. Subjects from the outpatient registry screened positive if they were assigned ICD-9 diagnoses of 295, 297, 298, or 299; subjects from the pharmacy registry screened positive based on a history of antipsychotic treatment. There were 389 subjects who screened positive. Following this step, the psychiatric and medical records of these subjects were reviewed for evidence of psychotic symptoms by an experienced, board-certified research psychiatrist. Among these subjects, 183 screened positive for potential SSD. Using state death certificate data, it was determined that 13 of these subjects were deceased. Among the 170 remaining potential cases, 146 (86%) were contacted to schedule a diagnostic interview.

The Diagnostic Interview for Genetic Studies (DIGS) was administered by clinicians with a minimum of a master's degree in a mental health field who were fully trained to administer this interview to potential cases (Nurnberger et al., 1994). A consensus diagnosis was assigned by three experienced research psychiatrists using DSM-IV criteria based on the interview information, medical records, and discussions with the interviewer. The DIGS was completed by 107 of the 146 (73%) contacted potential subjects with SSD. For the 76 potential cases that were not interviewed, DSM-IV diagnoses were made by chart review and confirmed by a research psychiatrist. A total of 71 cases of SSD were identified by full diagnostic assessment, 44 of whom received the DIGS 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 reviewed and approved by the institutional review boards of the New York State Psychiatric Institute and KPMCP.

2.3. Eligible cases

Among the 71 SSD cases, 55 had at least one available prenatal serum sample. 44 (80%) of the 55 cases with prenatal sera were diagnosed with schizophrenia (n=32) or schizoaffective disorder (n=12). The remaining cases consisted of 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, in whom a diagnosis of a specific schizophrenia spectrum disorder could not be made). Sera were available for the first trimester in 20 cases, for the second trimester in 44 cases, and for the third trimester in 47 cases. As noted above, analyses were conducted only on second and third trimester serum specimens due to the small sample of subjects with first trimester sera.

2.4. Selection of controls

Two matched controls were selected for each case from eligible birth cohort members representing the population at risk at the time the case was ascertained. The pool of eligible

controls excluded the 71 SSD cases and 318 individuals with other psychotic and major mood disorders.

Controls (N=106) were matched to cases on five variables: 1) membership in KPMCP at the time the case was ascertained, 2) date of birth (±28 days), 3) sex, 4) gestational timing (±28 days) of the first maternal serum sample taking during the index pregnancy, 5) number of maternal blood samples drawn during the index pregnancy (Susser et al., 2000; Brown et al., 2004). Hence, the controls were selected from a representative sample of offspring from the birth cohort who did not have psychotic or mood disorders requiring hospitalization and who had available maternal sera from comparable periods of gestation.

Serum specimens were provided by the CHDS for up to 2 matched controls per case and were selected at random from each matched set of controls. For improved accuracy of matching the gestational timing of the serum drawn between cases and controls, the control serum samples were matched to case samples on trimester and within each trimester, the sera from the controls were drawn within 42 days of the blood drawn for the corresponding cases. Serum availability was based on when the gravidas attended prenatal visits.

2.5. Serologic assay

In this study, all archived maternal sera from the cases and matched controls were assayed for vitamin A. The same assay method was used for both cases and controls. All assays were performed blind with respect to case and control status. Serum vitamin A was analyzed by a reverse-phase HPLC procedure (Mills et al., 1992; Redlich et al., 1996) using a 250×4.6 mm Beckman Ultrasphere C18 (5 mcm) column (Beckman Instruments, Inc.). The low limit of detection for vitamin A is 2 ng/ml for serum. The assay variability is between 3 and 6% (Burger et al., 1997; Redlich et al., 1998).

2.6. Statistical analysis

The analysis was based on a nested case–control design in which the matched controls for each case were selected from the population at risk at the time the case was ascertained (the first date of diagnosis for SSD), eliminating the need to obtain exposure data for the entire population at risk. 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 (Susser et al., 2006; Rothman et al., 2008).

Analyses of maternal vitamin A in relation to schizophrenia risk in offspring were conducted separately for the second trimester (98–188 days after the last menstrual period), and the third trimester (189 days after the last menstrual period until delivery). In the main analysis, subjects with low maternal vitamin A were defined as those belonging to the lowest tertile group for the particular trimester, based on cutoff points for that trimester as defined among controls. The use of tertiles in the primary analysis was based on evidence that maternal levels of two other micronutrients—homocysteine and vitamin D—do not show a continuous relationship with schizophrenia (Brown et al., 2007; McGrath et al., 2010). Finer grades of exposure (i.e. quartiles, quintiles) were not used given the modest sample size. In this analysis, the OR represents the effect of vitamin A levels during the second, and the third, trimesters in the lowest tertile group compared to the highest (reference) tertile group on SSD risk. The ranges of vitamin A levels in these tertile groups are provided in Table 1.

Covariates were selected a priori to be potential confounders based on the literature on maternal variables related to schizophrenia and vitamin A (Affenito et al., 2007; West and Mehra, 2010; Brown, 2011). These covariates included 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 (≤1 previous pregnancies, ≥2 previous pregnancies); maternal smoking; maternal homocysteine level; maternal hemoglobin level; and maternal infection [maternal influenza during first half of gestation, elevated maternal toxoplasma antibody (IgG titer ≥1:128), genital/reproductive infection in periconceptional period, or second trimester maternal respiratory infection]. Covariates were entered into the logistic models based on $p<0.20$ for bivariate relationships with both case and vitamin A exposure status, in accord with standard epidemiologic methods (Rothman et al., 2008). Given that the availability of sera differed by trimester, the samples of subjects were not identical for each trimester. Thus, these bivariate analyses were conducted separately for the second and third trimesters for the pools of cases and matched controls.

In a supplementary analysis, we examined, using conditional logistic regression, whether the slope corresponding to the change in maternal retinol between the second and third trimesters, taking the gestational day of the serum drawn into account, predicted case status among subjects with retinol levels in both trimesters. In this model, every subject has a unique slope and intercept, where slope is the change of serum retinol (3rd trimester retinol–2nd trimester retinol) per unit time. The intercept and slope were then averaged separately for cases and controls.

3. Results

3.1. Associations between demographic factors and case status

The only demographic covariates that met the a priori criteria for inclusion in the logistic models were maternal education and maternal age for the cases and matched controls with second trimester sera. Maternal education was lower in cases than controls [proportion with <H.S. education was 9/44 (22%) in cases and 4/70 in controls, p=.058] and mothers of cases were older than mothers of controls [mean (SD) was 28.9 (5.7) in cases and 27.2 (6.9) in controls, p=.17]. Low second trimester maternal vitamin A was found in mothers with decreased education [mean (SD) vitamin A level was 33.5 (12.9) for <H.S. education and 39.1 (13.5) for some college/college graduate, p=.066]. Second trimester maternal vitamin A levels were positively correlated with maternal age (p=.005). Maternal ethnicity, smoking, parity, birthweight, and gestational age did not meet these criteria for cases/matched controls in any trimester, nor did maternal age and education for the third trimester sample of cases and matched controls.

With regard to specific micronutrients, second trimester hemoglobin, which was related to schizophrenia in our previous study of this cohort (Insel et al., 2008), was not related to second trimester vitamin A (parameter estimate=1.15, p=0.40). Third trimester hemoglobin was related to third trimester vitamin A (parameter estimate=2.62, p=.036). With respect to homocysteine, a marker of folic acid deficiency, third trimester, but not second trimester levels of this analyte were previously shown to be related to schizophrenia in this cohort (Brown et al., 2007). However, neither second trimester (parameter estimate=0.31, p=0.32) nor third trimester (parameter estimate=−. 03, p=0.89) homocysteine was related to second or third trimester vitamin A, respectively. There was no association between maternal infection and maternal retinol for the second trimester [mean (SD) retinol=1.94 μg/dL (0.89) for infection exposed, 1.96 μg/dL (0.83) for infection unexposed, p=0.92] or for the third trimester [mean (SD) retinol=2.14 (0.88) for infection exposed, 1.90 (0.79) for infection unexposed, p=0.12)].

Hence, the logistic models for the second trimester analysis were adjusted only for maternal education and maternal age, and for the third trimester analysis, the model was adjusted for these covariates and maternal hemoglobin. Paternal age was also considered as a potential covariate but given that its relation to maternal vitamin A levels would have been mediated

by maternal age it was not necessary to adjust for this covariate once maternal age was included in the model.

3.2. Maternal vitamin A levels in SSD cases and matched controls

The numbers and proportions of SSD cases and matched controls by maternal vitamin A tertile status for each trimester are displayed in Table 1. For the second trimester, the proportion of SSD cases in the lowest tertile (45.5%) was increased compared to the highest (reference) tertile (31.8%).

In the conditional logistic regression analysis, second trimester exposure to maternal vitamin A levels in the lowest tertile was associated with a greater than threefold increased risk of SSD among offspring (OR=3.05; 95% CI=1.06–8.79, p=.039), adjusting for maternal education and maternal age, the a priori confounders (see Table 2). Third trimester exposure to maternal vitamin A levels in the lowest tertile was associated with no increased risk of SSD in offspring (OR=1.59, 95% CI=0.47, 5.38, p=0.46).

In a supplementary analysis, we re-analyzed vitamin A defined as <20 μg/dL, given that this cut-off point has been used to define moderate and severe deficiency. The odds ratio, adjusting for maternal education and age, for the second trimester was 2.92 (95% CI=0.48–17.7), p=0.25 and for the third trimester was 1.99 (95% CI=0.52–7.7), p=0.32. While non-significant, these findings are similar in magnitude to those of the main analysis.

We also analyzed whether a change in serum retinol between the second and third trimesters was related to case status. There was no significant difference in the slopes corresponding to the change in maternal retinol between the two trimesters (p=.66), although there was a mean (SD) decrease of 0.56 μg/dL (12.44) in cases, while in controls there was a decrease of 4.01 μg/dL (11.85), consistent with the findings of the main analysis.

3.3. Maternal vitamin A levels in schizophrenia

In a secondary analysis, we investigated the relationship between maternal vitamin A levels and schizophrenia apart from other SSD. Second trimester exposure to vitamin A levels in the lowest tertile was associated with a greater than fourfold increased risk of schizophrenia in offspring (OR=4.30, 95% CI=0.71, 25.8, p=0.11). Third trimester to exposure to low vitamin A was not related to schizophrenia (OR=1.32, 95% CI=0.37, 4.74, p=.66).

4. Discussion

We found that low maternal vitamin A levels, prospectively documented during the second trimester, are associated with a threefold, significant increase in risk of schizophrenia and other schizophrenia spectrum disorders in offspring from a large birth cohort. To our knowledge, this is the first time that maternal vitamin A levels have been related to schizophrenia in a birth cohort study, substantiating the previous literature on vitamin A and neurodevelopment in relation to this disorder (Goodman, 1998; Lamantia, 1999). The finding was observed only in the primary analysis, in which subjects in the lowest tertile of vitamin A levels were compared to those in the highest (reference) tertile. No associations were observed for third trimester vitamin A and schizophrenia in offspring.

Vitamin A plays an essential role in brain development. Following uptake by the brain and other tissues, vitamin A is converted to retinoic acid (RA), the active metabolite required for proper morphogenesis (Nau and Blaner, 1999). Unlike vitamin A, retinoic acid is not a stable metabolite and is present at very low levels in sera (Nau and Blaner, 1999). As reviewed in Section 1, minor physical anomalies, a prominent pathomorphologic feature of schizophrenia, occur following retinoid abnormalities during embryonic development

(Goodman, 1995, 1998). Animal studies have shown that rat embryos which have been deprived of vitamin A manifest an underdeveloped hindbrain, loss of posterior cranial nerves, lack of differentiation of neuronal populations in the brain, and other congenital anomalies (Dickman et al., 1997; Antipatis et al., 1998; White et al., 1998). Further, a recent study has demonstrated that RA is needed in order to promote development of neural progenitors in mouse embryonic stem cells (Engberg et al., 2010). Wong et al. (2006) have provided the first in vivo demonstration that upregulating a RA receptor leads to the induction of nerve regeneration and functional recovery.

Rodent models have demonstrated that disruption of retinoid signaling pathways plays a role in regulating synaptic plasticity and associated learning and memory behaviors (Lane and Bailey, 2005). Specifically, in mutant mice which lack mesenchymal cells that produce RA, neuromorphologic anomalies found in schizophrenia, including enlarged ventricles, and altered lamination of the thalamus, hippocampus, and neocortex were observed (Lamantia, 1999; Luo et al., 2004).

Animal studies suggest that in humans, midgestation may be an especially critical period for the effects of RA on brain development, including a significant impact on neuronal differentiation, maturation, and migration (Luo et al., 2004). In a study of mice made vitamin A deficient during parturition, broadly equivalent to the second trimester in the human, deficits in LTP and LTD in the hippocampus were observed, compared to mice given a vitamin A sufficient diet (Misner et al., 2001). These defects were reversed when a vitamin A sufficient diet was instituted at week 17. Rapid increases in retinaldehyde dehydrogenases (RALDHs), RA-synthesizing enzymes (Wagner et al., 2002), occur during the early postnatal period, which is analogous to the second trimester of fetal development. Expression of RALDH type 3 (RALDH3) during this period reflects the colonization of the nucleus accumbens and olfactory bulbs by neuronal precursors and cortical maturational processes including formation of dendritic arbors in postmigratory layer II/III neurons and transport across the corpus callosum. RALDH3 is strongly expressed in most of the limbic lobe. These and other enzymes involved in vitamin A metabolism are synthesized by the fetus, given that corresponding mRNA has been detected in the embryo. The expression and function of these enzymes have been demonstrated in the early embryo and throughout fetal life and their activity throughout the brain is spatiotemporally regulated over the course of development, depending upon the maturation of specific brain regions in which they play morphogenic roles.

These findings add to a growing body of literature indicating that low prenatal nutrient levels are related to schizophrenia. In a previous study from this birth cohort, elevated third trimester maternal homocysteine, an amino acid which is inversely correlated with folate, was associated with a greater than twofold increased risk of schizophrenia (Brown et al., 2007). In a second study from this birth cohort, low maternal hemoglobin, a proxy for iron deficiency, was related to a nearly fourfold increased risk of schizophrenia. However, the findings of the present study are unlikely to have been confounded by either of these exposures, since no association with schizophrenia was observed for second trimester homocysteine (Brown et al., 2007) and, as presented above (see Section 3), there was no association between vitamin A and hemoglobin in the second trimester. It is also worth noting that the present study, as well as other cited studies, could not examine nutritional deficiency in early gestation, which has been related to schizophrenia risk in famine studies (Susser et al., 1996; St Clair et al., 2005; Xu et al., 2009). Nonetheless, the findings are not mutually exclusive; the sensitive period in gestation depends on the particular nutritional deficiency. Folic acid supplements, for example, are particularly relevant to neurodevelopment in early gestation (Milunsky et al., 1989; MRC Vitamin Study Research Group, 1991; Roth et al., 2011). Similarly, neonatal vitamin D was related to later risk of

schizophrenia (McGrath et al., 2010). That study demonstrated a curvilinear effect with an increased risk of schizophrenia observed in the lowest three quintiles and the highest quintile of the vitamin D distribution, and animal models of vitamin D deficiency support the biological plausibility of this micronutrient in schizophrenia (McGrath et al., 2011).

The present study has several notable methodologic strengths. First, vitamin A exposure was prospectively documented. Second, biomarkers of the exposure, rather than maternal reports, which tend to be unreliable, were utilized. Third, the birth cohort was continuously followed up for schizophrenia, and the statistical model accounted for loss to follow-up, which can produce bias if not properly addressed. Fourth, the controls were representative of the source population which gave rise to the cases, further minimizing selection bias. Finally, the cases were diagnosed using structured research interviews, diminishing the likelihood of diagnostic misclassification.

There are some limitations that need to be considered. First, the sample size was modest, diminishing the precision of the effect size estimates. Second, the finding may have been confounded by associations with certain prenatal nutrients, such as vitamin D, which may co-vary with vitamin A, but were not measured in the cohort of the present study. However, as noted above, it is not likely that deficiencies of folic acid or iron, two nutrients that have been associated with schizophrenia, accounted for the observed association. Third, the observed vitamin A levels were considerably lower than those observed in fresh sera; the lowest tertile is in the range considered by the World Health Organization to be moderately vitamin A deficient (WHO, 2009). This may have been secondary to degradation of the molecule over time, given that the maternal sera utilized in the study were stored for over 30 years. However, degradation of vitamin A is most likely to have caused conservative error since the case and control serum samples were matched by date of birth of offspring and there is no reason to believe that any degradation would be differential with regard to case status. Second, it is possible, though less likely, that intake of vitamin A containing foods or supplements was lower during the years of the pregnancies (1959–1966) than in the present day. Fourth, maternal rather than fetal levels of vitamin A were quantified. However, cord serum retinol in infants is correlated with maternal retinol at the time of birth. In a previous study, 45% of mothers with serum retinol <0.7 μmol/L, compared to only 2% of mothers with serum retinol ≥0.7 μmol/L delivered infants with cord retinol <0.7 μmol/L (Gazala et al., 2003). The correlation between maternal and cord serum retinol was highly statistically significant ($r=0.37$, $p<.0001$). In another study, maternal serum retinol was highly correlated with fetal liver retinol at gestational age <24 weeks ($r=0.59$, $p<.01$), and this correlation increased to 0.83 ($p<.001$) for >24 weeks (Shah et al., 1987).

If replicated in independent cohorts, this study has potential implications for public health, specifically for the prevention of schizophrenia (McGrath et al., 2011). Fortunately, vitamin A supplementation is relatively inexpensive and can be scaled up to large populations given sufficient resources. However, such a public health recommendation must proceed with appropriate caution, as excess vitamin A intake has been associated with teratogenicity in offspring (Rothman et al., 1995). Consequently, the WHO recommends that a daily vitamin A supplement taken during any part of the fertile period be limited to 10,000 IU while in the USA, the Teratology Society recommends that total vitamin A intake not exceed 8000 IU/day (Teratology Society, 1987).

A second important implication of the study is the identification of pathogenic mechanisms that may increase susceptibility to schizophrenia. As noted above, there is intriguing evidence that retinoids play key roles in morphogenesis and patterning of the central nervous system and in regulating expression of genes for receptors implicated in schizophrenia through interactions with transcription factors. Hence, the study findings may lend further

traction to both human and animal studies for the elucidation of molecular and cellular pathogenic mechanisms that underlie schizophrenia, and to potentially identify candidate genes for this disorder.

Nonetheless, given the small sample size, the novelty of the study, and the fact that the findings emerged following case–control comparisons for two trimesters, the finding should be considered as exploratory, and replication in larger and independent samples will be necessary. Previous findings from this cohort suggest that a broad array of exposures, including other nutritional, as well as inflammatory, toxic, and other insults is associated with schizophrenia (Brown, 2011) and all of the analyses in this cohort were directed by careful a priori hypotheses. Taking all of these factors into consideration, the present study provides intriguing though not conclusive evidence that diminished maternal vitamin A status is a risk factor for schizophrenia, and may provide valuable clues for future studies, including models of schizophrenia that incorporate prenatal nutritional factors and maternal and fetal genetic susceptibility.

5. Conclusion

In summary, low second trimester vitamin A, prospectively quantified by analysis of archived maternal sera, was associated with a greater than threefold increased risk of schizophrenia and other schizophrenia spectrum disorders in a large, population-based birth cohort. Given that this is the first report of such an association, replications in independent birth cohorts are necessary. Although considerable work remains to clarify the biological mechanisms underlying these findings, vitamin A deficiency represents a plausible candidate risk factor for schizophrenia, and if the findings are replicated, this work may have implications for the prevention of schizophrenia cases, and the elucidation of etiopathogenic mechanisms.

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References

Abel KM, Wicks S, Susser ES, Dalman C, Pedersen MG, Mortensen PB, Webb RT. Birth weight, schizophrenia, and adult mental disorder: is risk confined to the smallest babies? Arch. Gen. Psychiatry. 2010; 67(9):923–930. [PubMed: 20819986]

Affenito SG, Thompson DR, Franko DL, Striegel-Moore RH, Daniels SR, Barton BA, Schreiber GB, Schmidt M, Crawford PB. Longitudinal assessment of micronutrient intake among African American and white girls: the National Heart, Lung, and Blood Institute Growth and Health Study. J. Am. Diet. Assoc. 2007; 107(7):1113–1123. [PubMed: 17604740]

Aggarwal S, Kim SW, Cheon K, Tabassam FH, Yoon JH, Koo JS. Nonclassical action of retinoic acid on the activation of the cAMP response element-binding protein in normal human bronchial epithelial cells. Mol. Biol. Cell. 2006; 17(2):566–575. [PubMed: 16280361]

Alique M, Moreno V, Kitamura M, Xu Q, Lucio-Cazana FJ. Kinase-dependent, retinoic acid receptor-independent upregulation of cycloxygenase-2 by all-trans retinoic acid in human mesangial cells. Br. J. Pharmacol. 2006; 149(2):215–225. [PubMed: 16894348]

Antipatis C, Ashworth CJ, Grant G, Lea RG, Hay SM, Rees WD. Effects of maternal vitamin A status on fetal heart and lung: changes in expression of key developmental genes. Am. J. Physiol. 1998; 275(6 Pt 1):L1184–L1191. [PubMed: 9843856]

Brandt, I. Growth dynamics of low-birth-weight infants with emphasis on the perinatal period. In: Falkner, F.; Tanner, JM., editors. Human Growth and Postnatal Growth. New York: Plenum; 1978. p. 557-617.

Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Bresnahan M, Babulas VP, Susser ES. Serologic evidence of prenatal influenza in the etiology of schizophrenia. Arch. Gen. Psychiatry. 2004; 61(8):774–780. [PubMed: 15289276]

Brown AS, Bottiglieri T, Schaefer CA, Quesenberry CP Jr, Liu L, Bresnahan M, Susser ES. Elevated prenatal homocysteine levels as a risk factor for schizophrenia. Arch. Gen. Psychiatry. 2007; 64(1): 31–39. [PubMed: 17199052]

Brown AS. The environment and susceptibility to schizophrenia. Prog. Neurobiol. 2011; 93(1):23–58. [PubMed: 20955757]

Burger H, Kovacs A, Weiser B, Grimson R, Nachman S, Tropper P, van Bennekum AM, Elie MC, Blaner WS. Maternal serum vitamin A levels are not associated with mother-to-child transmission of HIV-1 in the United States. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 1997; 14(4): 321–326. [PubMed: 9111473]

Chen K, Zhang X, Wei XP, Qu P, Liu YX, Li TY. Antioxidant vitamin status during pregnancy in relation to cognitive development in the first two years of life. Early Hum. Dev. 2009; 85(7):421–427. [PubMed: 19286336]

Czeizel AE, Rockenbauer M. Prevention of congenital abnormalities by vitamin. A Int. J. Vitam. Nutr. Res. 1998; 68(4):219–231.

Dickman ED, Thaller C, Smith SM. Temporally-regulated retinoic acid depletion produces specific neural crest, ocular and nervous system defects. Development. 1997; 124(16):3111–3121. [PubMed: 9272952]

Drott P, Meurling S. Plasma concentrations of fat-soluble vitamins A and E in neonates with myelomeningocele. Eur. J. Pediatr. Surg. 1992; 2(5):265–268. [PubMed: 1420069]

Engberg N, Kahn M, Petersen DR, Hansson M, Serup P. Retinoic acid synthesis promotes development of neural progenitors from mouse embryonic stem cells by suppressing endogenous, Wnt-dependent nodal signaling. Stem Cells. 2010; 28(9):1498–1509. [PubMed: 20665854]

Ertesvag A, Aasheim HC, Naderi S, Blomhoff HK. Vitamin A potentiates CpG-mediated memory B cell proliferation and differentiation: involvement of early activation of p38MAPK. Blood. 2007; 109(9):3865–3872. [PubMed: 17209053]

Gazala E, Sarov B, Hershkovitz E, Edvardson S, Sklan D, Katz M, Friger M, Gorodischer R. Vitamin A concentration in maternal and cord serum: its relation to birth weight in healthy mother–infant pairs. Early Hum. Dev. 2003; 71(1):19–28. [PubMed: 12614947]

Ghebremeskel K, Burns L, Burden TJ, Harbige L, Costeloe K, Powell JJ, Crawford M. Vitamin A and related essential nutrients in cord blood: relationships with anthropometric measurements at birth. Early Hum Dev. 1994; 39(3):177–188. [PubMed: 7712952]

Goodman AB. Chromosomal locations and modes of action of genes of the retinoid (vitamin A) system support their involvement in the etiology of schizophrenia. Am. J. Med. Genet. 1995; 60(4):335–348. [PubMed: 7485272]

Goodman AB. Congenital anomalies in relatives of schizophrenic probands may indicate a retinoid pathology. Schizophr. Res. 1996; 19(2–3):163–170. [PubMed: 8789914]

Goodman AB. Three independent lines of evidence suggest retinoids as causal to schizophrenia. Proc. Natl. Acad. Sci. U.S.A. 1998; 95(13):7240–7244. [PubMed: 9636132]

Insel BJ, Schaefer CA, McKeague IW, Susser ES, Brown AS. Maternal iron deficiency and the risk of schizophrenia in offspring. Arch. Gen. Psychiatry. 2008; 65(10):1136–1144. [PubMed: 18838630]

Kendler KS, Neale MC, Walsh D. Evaluating the spectrum concept of schizophrenia in the Roscommon Family Study. Am. J. Psychiatry. 1995; 152(5):749–754. [PubMed: 7726315]

Krezel W, Ghyselinck N, Samad TA, Dupe V, Kastner P, Borrelli E, Chambon P. Impaired locomotion and dopamine signaling in retinoid receptor mutant mice. Science. 1998; 279:863–867. [PubMed: 9452386]

LaMantia AS. Forebrain induction, retinoic acid, and vulnerability to schizophrenia: insights from molecular and genetic analysis in developing mice. Biol. Psychiatry. 1999; 46(1):19–30. [PubMed: 10394471]

Lane MA, Bailey SJ. Role of retinoid signalling in the adult brain. Prog. Neurobiol. 2005; 75(4):275–293. [PubMed: 15882777]

Lee CH, Liu CM, Wen CC, Chang SM, Hwu HG. Genetic copy number variants in sib pairs both affected with schizophrenia. J. Biomed. Sci. 2010; 17(1):2. [PubMed: 20064257]

Luo T, Wagner E, Crandall JE, Dräger UC. A retinoic-acid critical period in the early postnatal mouse brain. Biol. Psychiatry. 2004; 56(12):971–980. [PubMed: 15601608]

Maden M, Gale E, Kostetskii I, Zile M. Vitamin A-deficient quail embryos have half a hindbrain and other neural defects. Curr. Biol. 1996; 6(4):417–426. [PubMed: 8723346]

Maden, M. Retinoids in neural development. In: Nau, H.; Blaner, WS., editors. The Biochemical and Molecular Basis of Vitamin A and Retinoid Action. Germany: Springer-Verlag; 1999. p. 399-442.

Maden M. The role of retinoic acid in embryonic and post-embryonic development. Proc. Nutr. Soc. 2000; 59(1):65–73. [PubMed: 10828175]

McGrath J, Eyles DW, Pedersen CB, Anderson C, Ko P, Burne TH, Norgaard-Pedersen B, Hougaard DM, Mortensen PB. Neonatal vitamin D status and risk of schizophrenia: a population-based case–control study. Arch. Gen. Psychiatry. 2010; 67(9):889–894. [PubMed: 20819982]

McGrath J, Brown A, St Clair D. Prevention and schizophrenia—the role of dietary factors. Schizophr. Bull. 2011; 37(2):272–283. [PubMed: 20974747]

Mills JL, Tuomilehto J, Yu KF, Colman N, Blaner WS, Koskela P, Rundle WE, Forman M, Toivanen L, Rhoads GG. Maternal vitamin levels during pregnancies producing infants with neural tube defects. J. Pediatr. 1992; 120(6):863–871. [PubMed: 1593344]

Mills JL, Simpson JL, Cunningham GC, Conley MR, Rhoads GG. Vitamin A and birth defects. Am. J. Obstet. Gynecol. 1997; 177(1):31–36. [PubMed: 9240579]

Milunsky A, Jick H, Jick SS, Bruell CL, MacLaughlin DS, Rothman KJ, Willett W. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. JAMA. 1989; 262(20):2847–2852. [PubMed: 2478730]

Misner DL, Jacobs S, Shimizu Y, de Urquiza AM, Solomin L, Perlmann T, De Luca LM, Stevens CF, Evans RM. Vitamin A deprivation results in reversible loss of hippocampal long-term synaptic plasticity. Proc. Natl. Acad. Sci. U.S.A. 2001; 98(20):11714–11719. [PubMed: 11553775]

Morris-Kay G. Retinoic acid and craniofacial development: molecules and morphogenesis. Bioessays. 1993; 15(1):9–15. [PubMed: 8385449]

MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council vitamin study. Lancet. 1991; 338(8760):131–137. [PubMed: 1677062]

Nau, H.; Blaner, WS. Retinoids: The Biochemical and Molecular Basis of Vitamin A and Retinoid Action. Vol. Vol. 139. USA: Springer; 1999.

Nau, H.; Elmazar, M. Retinoid receptors, their ligands and teratogenesis: synergy and specificity of effects, Retinoids. In: Nau, H.; Blaner, WS., editors. The Biochemical and Molecular Basis of Vitamin A and Retinoid Action. Germany: Springer-Verlag; 1999. p. 465-487.

Navarro J, Causse MB, Desquilbet N, Hervé F, Lallemand DJ. The vitamin status of low birth weight infants and their mothersJPediatr. Gastroenterol. Nutr. 1984; 3(5):744–748.

Nurnberger JI Jr, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J, Severe JB, Malaspina D, Reich T. Diagnostic interview for genetic studies: rationale, unique features, and training. NIMH Genetics Initiative. Arch. Gen. Psychiatry. 1994; 51(11):849–859. [PubMed: 7944874]

Redlich CA, Grauer JN, Van Bennekum AM, Clever SL, Ponn RB, Blaner WS. Characterization of carotenoid, vitamin A alpha-tocopherol levels in human lung tissue and pulmonary macrophages. Am. J. Respir. Crit. Care Med. 1996; 154(5):1436–1443. [PubMed: 8912761]

Redlich CA, Blaner WS, Van Bennekum AM, Chung JS, Clever SL, Holm CT, Cullen MR. Effect of supplementation with beta-carotene and vitamin A on lung nutrient levels. Cancer Epidemiol. Biomarkers Prev. 1998; 7(3):211–214. [PubMed: 9521435]

Roth C, Magnus P, Schjolberg S, Stoltenberg C, Suren P, McKeague IW, Smith GD, Reichborn-Kjennerud T, Susser E. Folic acid supplements in pregnancy and severe language delay in children. JAMA. 2011; 306(14):1566–1573. [PubMed: 21990300]

Rothman, JK.; Sander, G.; Lash, TL. Modern Epidemiology. 3rd edn. Boston: Lippincott Williams & Wilkins; 2008.

Rothman KJ, Moore LL, Singer MR, Nguyen US, Mannino S, Milunsky A. Teratogenicity of high vitamin A intake. N. Engl. J. Med. 1995; 333(21):1369–1373. [PubMed: 7477116]

Samad TA, Krezel W, Chambon P, Borrelli E. Regulation of dopaminergic pathways by retinoids: activation of the D2 receptor promoter by members of the retinoic acid receptor-retinoid X receptor family. Proc. Natl. Acad. Sci. U.S.A. 1997; 94(26):14349–14354. [PubMed: 9405615]

Shah RS, Rajalakshmi R, Bhatt RV, Hazra MN, Patel BC, Swamy NB, Patel TV. Liver stores of vitamin A in human fetuses in relation to gestational, age, fetal size and maternal nutritional status. Br J. Nutr. 1987; 58(2):181–189. [PubMed: 3676240]

Shenai JP, Chytil F, Jhaveri A, Stahlman MT. Plasma vitamin A and vitamin A-binding protein in premature and term neonates. J. Pediatr. 1981; 99(2):302–305. [PubMed: 7195934]

St Clair D, Xu M, Wang P, Yu Y, Fang Y, Zhang F, Zheng X, Gu N, Feng G, Sham P, He L. Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959–1961. JAMA. 2005; 294(5):557–562. [PubMed: 16077049]

Susser E, Neugebauer R, Hoek HW, Brown AS, Lin S, Labovitz D, Gorman JM. Schizophrenia after prenatal famine. Further evidence. Arch. Gen. Psychiatry. 1996; 53(1):25–31. [PubMed: 8540774]

Susser ES, Schaefer CA, Brown AS, Begg MD, Wyatt RJ. The design of the prenatal determinants of schizophrenia study. Schizophr. Bull. 2000; 26(2):257–273. [PubMed: 10885629]

Susser, E.; Schwartz, S.; Morabia, A.; Bromet, EJ. Psychiatric Epidemiology: Searching for the Causes of Mental Disorders. New York: Oxford University Press; 2006.

Teratology Society. [Accessed 18 May, 2011] Teratology society position paper: recommendations for vitamin A use during pregnancy. Teratology Society. 1987. http://www.teratology.org/pubs/vitamina.htm1987

Thornberg SA, Saklad SR. A review of NMDA receptors and the phencyclidine model of schizophrenia. Pharmacotherapy. 1996; 16(1):82–93. [PubMed: 8700797]

van den Berg BJ. The California Child Health and Development Studies: twenty years of research. World Health StatQ. 1979; 32(4):269–286.

Wagner E, Luo T, Dräger UC. Retinoic acid synthesis in the postnatal mouse brain marks distinct developmental stages and functional systems. Cereb. Cortex. 2002; 12(12):1244–1253. [PubMed: 12427676]

West KP Jr, Mehra S. Vitamin A intake and status in populations facing economic stress. J. Nutr. 2010; 140(1):201s–207s. [PubMed: 19939993]

White JC, Shankar VN, Highland M, Epstein ML, DeLuca HF, Clagett-Dame M. Defects in embryonic hindbrain development and fetal resorption resulting from vitamin A deficiency in the rat are prevented by feeding pharmacological levels of all-trans-retinoic acid. Proc. Natl. Acad. Sci. U.S.A. 1998; 95(23):13459–13464. [PubMed: 9811822]

Wong LF, Yip PK, Battaglia A, Grist J, Corcoran J, Maden M, Azzouz M, Kingsman SM, Kingsman AJ, Mazarakis ND, McMahon SB. Retinoic acid receptor beta2 promotes functional regeneration of sensory axons in the spinal cord. Nat. Neurosci. 2006; 9(2):243–250. [PubMed: 16388307]

World Health Organization. Global Prevalence of Vitamin A Deficiency in Populations at Risk 1995–2005: WHO Global Database on Vitamin A Deficiency. Geneva: World Health Organization; 2009.

Xu MQ, Sun WS, Liu BX, Feng GY, Yu L, Yang L, He G, Sham P, Susser E, St Clair D, He L. Prenatal malnutrition and adult schizophrenia: further evidence from the 1959–1961 Chinese famine. Schizophr. Bull. 2009; 35(3):568–576. [PubMed: 19155344]

Table 1

Maternal vitamin A tertile levels (μg/dL) for offspring with schizophrenia and other schizophrenia spectrum disorder (SSD) cases and controls in the second and third trimesters.

Trimester 2	**Cases (N=44)**		**Controls (N=70)**	
	N (%)	**Range**	**N (%)**	**Range**
Lowest tertile	20 (45.5)	12.5–29.2	23 (32.9)	15.3–29.5
Middle tertile	10 (22.7)	30.2–38.7	23 (32.9)	30.0–41.0
Highest tertile	14 (31.8)	42.7–70.0	24 (34.2)	41.4–66.8
Trimester 3	Cases (N=47)		Controls (N=87)	
	N (%)	Range	N (%)	Range
Lowest tertile	18 (38.3)	15.8–26.4	29 (33.3)	11.6–26.6
Middle tertile	14 (29.8)	26.8–33.4	29 (33.3)	27.1–34.9
Highest tertile	15 (31.9)	35.0–69.5	29 (33.3)	35.4–80.2

Table 2

Conditional logistic regression analysis of low maternal vitamin A levels and schizophrenia and other schizophrenia spectrum disorders (SSD) in the second and third trimesters.

	Unadjusted			Adjusted		
	OR	**95% CI**	**p value**	**OR[a]**	**95% CI**	**p value**
Trimester 2	1.52	0.62, 3.72	0.36	3.05	1.06, 8.79	0.039
Trimester 3	1.19	0.50, 2.84	0.69	1.29	0.51, 3.32	0.59

[a]As defined a priori, odds ratios (OR) represent the effect of vitamin A levels during the second, and the third, trimesters in the lowest tertile group compared to the highest (reference) tertile group on SSD risk. Second trimester analysis adjusted for maternal education and maternal age. Third trimester analysis adjusted for maternal education, maternal age, and third trimester hemoglobin