ORIGINAL ARTICLE

Neonatal Vitamin D Status and Risk of Schizophrenia

A Population-Based Case-Control Study

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Context: Clues from the epidemiology of schizophrenia suggest that low levels of developmental vitamin D may be associated with increased risk of schizophrenia.

Objective: To directly examine the association between neonatal vitamin D status and risk of schizophrenia.

Design: Individually matched case-control study drawn from a population-based cohort.

Setting: Danish national health registers and neonatal biobank.

Participants: A total of 424 individuals with schizophrenia and 424 controls matched for sex and date of birth.

Main Outcome Measures: The concentration of 25 hydroxyvitamin D_3 (25[OH]D3) was assessed from neonatal dried blood samples using a highly sensitive liquid chromatography tandem mass spectroscopy method. Relative risks were calculated for the matched pairs when examined for quintiles of 25(OH)D3.

Results: Compared with neonates in the fourth quintile (with 25[OH]D3 concentrations between 40.5 and 50.9 nmol/L), those in each of the lower 3 quintiles had a significantly increased risk of schizophrenia (2-fold elevated risk). Unexpectedly, those in the highest quintile also had a significantly increased risk of schizophrenia. Based on this analysis, the population-attributable fraction associated with neonatal vitamin D status was 44%. The relationship was not explained by a wide range of potential confounding or interacting variables.

Conclusions: Both low and high concentrations of neonatal vitamin D are associated with increased risk of schizophrenia, and it is feasible that this exposure could contribute to a sizeable proportion of cases in Denmark. In light of the substantial public health implications of this finding, there is an urgent need to further explore the effect of vitamin D status on brain development and later mental health.

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In recent decades we have learned a great deal about the epidemiology of schizophrenia.[1] While many aspects remain to be clarified, several findings appear relatively robust. One of the most consistent features relates to season of birth—people born in winter or spring have a small but significantly increased risk of developing schizophrenia.[2] The effect size of this association increases with distance from the equator.[3,4] Of those with schizophrenia, season of birth is also associated with symptom profile (ie, summer birth is associated with deficit syndrome).[5] There is also robust evidence showing that the offspring of some migrant groups have increased risk of schizophrenia.[6,7] The effect is most prominent in those with dark skin[7] such as Afro-Caribbean and African migrants to England.[8] Finally, there is robust evidence showing that, compared with rural settings, individuals born and raised in an urban setting are at increased risk of schizophrenia.[9] Because hypovitaminosis D is more prevalent during winter and early spring in dark-skinned migrants living in cold climates and in urban vs rural settings, these convergent clues led to the hypothesis that developmental vitamin D deficiency may be a risk factor for schizophrenia.[10] The ecological evidence in support of this hypothesis has been summarized elsewhere.[4,10,11]

The production of vitamin D_3 depends on the action of sunlight on the skin.[12] Ultraviolet B radiation, acting on a cholesterol metabolite in the epidermis, leads to the production of vitamin D. Subsequent hydroxylations in the liver to 25-hydroxyvitamin D_3 (25[OH]D3), and then in the kidney, result in the active moiety

1,25-dihydroxyvitamin D_3 (1,25[OH]2D3), a potent secosteroid hormone. Vitamin D production is strongly and consistently associated with the duration of the photoperiod (which is influenced by latitude and season), and the prevalence of hypovitaminosis D is particularly prominent in dark-skinned people who live in cold climates.[13,14]

While profound deficiencies have been associated with conditions such as rickets and osteomalacia, there is now widespread recognition that hypovitaminosis D may contribute to a broad range of adverse health outcomes[13] including neurological and neuropsychiatric disorders.[15] Of particular interest to the hypothesis linking vitamin D deficiency and schizophrenia, the enzyme required for the production of 1,25(OH)D3 has now been identified in the human brain,[16] and there is evidence from rodent models demonstrating that transient prenatal vitamin D deficiency results in persistent changes in adult brain structure, neurochemistry, and behavior.[17-23]

While the vitamin D hypothesis has received some indirect support from a Finnish birth cohort study,[24] a small pilot study[25] that examined 25(OH)D3 in third-trimester maternal sera was inconclusive. To date, no study has directly examined the association between neonatal vitamin D status and risk of schizophrenia. We had the opportunity to examine this hypothesis in a large, population-based Danish case-control study. We predicted that neonates with low concentrations of 25(OH)D3 would be at increased risk of schizophrenia.

METHODS

PARTICIPANTS

The study was based on record linkage between the Danish Psychiatric Central Register[26] and the Danish Civil Registration System[27] as well as dried blood spots from the Newborn Screening Biobank.[28] These dried blood spots have been systematically stored for individuals born in Denmark since May 1, 1981. The Danish registers used in this study have near-complete coverage of the entire population, and register-derived diagnoses of schizophrenia have good validity compared with research-derived diagnoses.[29] We identified all singletons who had been diagnosed with schizophrenia (*International Statistical Classification of Diseases, 10th Revision [ICD-10]* code F20) up to September 2005 and who were born in Denmark May 1, 1981, or later. For each case we identified 1 control who was individually time-matched for sex, exact date of birth, birth in Denmark, and being alive with no history of schizophrenia on the date of the first diagnosis of schizophrenia in the matched case. The risk set included 923 cases and controls born between 1981 and 1994. From the risk set, we identified 430 case-control matched pairs who had sufficient biological material to allow at least 4 punches to be sampled (each disc is 3.2 mm in diameter, which corresponds to 3.3 μL of whole blood). Of the 430 case-control pairs, the level of 25(OH)D3 was successfully measured in 424 pairs. This study was approved by the Danish Data Protection Agency and the ethics committees of Aarhus University and the University of Queensland.

ASSESSMENT OF VITAMIN D STATUS

For the main hypothesis, we examined 25(OH)D3 as the main circulating form of vitamin D.[13] In addition, we also measured the related ergosterol-derived form, 25 hydroxyvitamin D_2 (25[OH]D2), which can be obtained from certain dietary sources (eg, mushrooms, fortified foods, vitamin supplements).

For each individual, one 3.2-mm disc of dried blood was used. The assay method is highly sensitive and uses minimal sample cleanup to reduce sample loss during extraction, chemical derivatization to enhance 25(OH)D2 and 25(OH)D3 ionization, and liquid chromatography tandem mass spectroscopy coupled with multiple reactant monitoring.[30] Based on samples from Australia and Denmark, we have previously demonstrated that the assay can reliably detect seasonal (within-year) fluctuations and is strongly correlated with neonatal cord blood (r=0.86).[31] Moreover, appropriate 25(OH)D3 concentrations can still be detected after prolonged storage time (eg, greater than 20 years).[30] The concentration of 25(OH)D3 is reported in nmol/L and, as the protein-bound molecule is excluded from erythrocytes, results are reported as sera concentrations adjusted to account for the increased hematocrit in capillary blood.

STATISTICAL ANALYSIS

To validate the assay, we first examined 25(OH)D3 for variation by month of birth (we predicted it would be lower in winter/spring vs summer/autumn) and in the offspring of immigrants (we predicted it would be lower in the offspring of immigrants vs the offspring of native-born individuals).

The main analyses were based on quintiles for 25(OH)D3 in the control sample, using conditional logistic regression.[32] Owing to the matching scheme, this means that all relative risks were controlled for sex, date of birth, and age. Based on previous research using the same psychiatric case register and based on factors known to be associated with vitamin D status,[13] we included a range of variables as potential confounds in the analyses. These include maternal, paternal, and sibling history of mental illness subdivided hierarchically as schizophrenia (*ICD-8* code 295; *ICD-10* code F20), schizophrenialike psychoses (*ICD-8* codes 297, 298.39, 301.83, and *ICD-10* codes F21-F29), and any other diagnoses (any *ICD-8* or *ICD-10* diagnosis), sex, age at onset, year of birth, calendar year at onset, second-generation immigrant status, degree of urbanization at place of birth, maternal and paternal age at the time of the child's birth, gestational age, congenital malformations, Apgar score, birth weight, and birth length.

To examine 25(OH)D3 as a continuous variable, we also used second-degree fractional polynomials to explore the relationship between the variables of interest.[33] If the exposure-risk relationship was nonlinear, we planned further analyses to explore the nature of the relationship using more fine-grain quantiles and individually assessing the list of variables previously included as covariates for interaction effects with 25(OH)D3. Population-attributable fraction was calculated according to the recommendations of Bruzzi et al[34] (equation 10).

As described above, a relatively large proportion of cases were excluded owing to lack of the necessary amount of blood. Participants born early in the study period were significantly less likely to have sufficient biological material for inclusion in the study (results not shown). However, as date of birth is a matching criterion in this study, this cannot bias our results. Furthermore, to ensure that our sample was not subject to other selection bias related to potential confounders (ie, parental history of mental illness, migrant status of parents, age of parents, urbanization of place of birth), we systematically compared the influence of these variables in the 424 case-control pairs included in the study with the original risk set. We found very similar magnitude and direction of all associations when comparing these samples in separate analyses (results not shown).

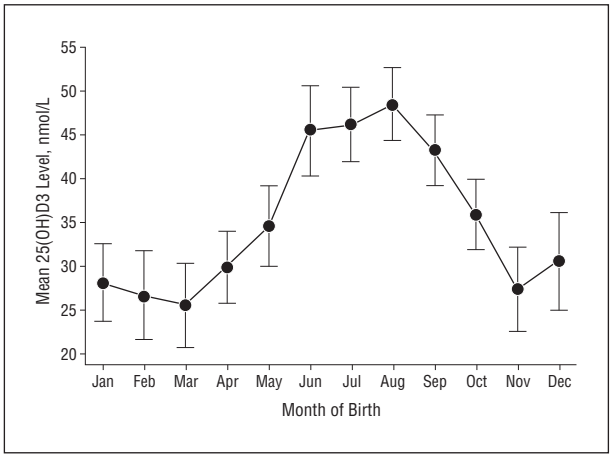

Figure 1. Mean level of 25 hydroxyvitamin D_3 (25[OH]D3) by month of birth. Vertical lines indicate 95% likelihood-based confidence intervals. Cases and controls are combined. The level of 25(OH)D3 depended significantly on month of birth ($\chi^2_{11}=140.2$; $P<.001$).

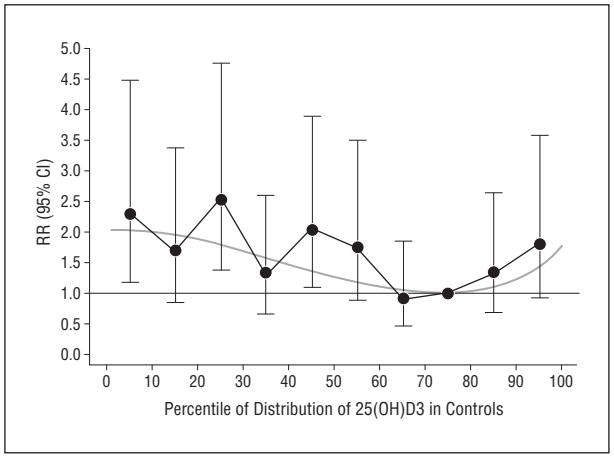

Figure 2. Relative risk (RR) of schizophrenia according to the level of 25(OH)D3, measured by the percentile of the distribution of 25(OH)D3 among the controls. Vertical lines indicate 95% confidence intervals (CI). Children in the 70th to 80th percentile of the distribution were chosen as the reference category for the categorical analyses. The gray line shows the fitted second-degree fractional polynomial with orders 3 and 3.

RESULTS

In the entire sample, the distribution of 25(OH)D3 was mildly skewed; the mean (SD) for all subjects was 35.9 (21.0) nmol/L (median, 32.3; interquartile range [IQR], 20.5-46.6). The dietary form of 25(OH)D2 was detected above the lowest level of assay quantification in less than 5% of samples. The mean (SD) for all subjects was 10.0 (9.0) nmol/L (median, 7.4; IQR, 4.5-12.9). As predicted, 25(OH)D3 showed significant monthly variation ($\chi^2_{11}=140.2$; $P<.001$) (**Figure 1**). Compared with the offspring of native-born parents, the offspring of immigrants had significantly lower 25(OH)D3 levels (mean, 36.8; 95% confidence interval [CI], 35.3-38.4 vs mean, 29.3; 95% CI, 25.4-33.3; $\chi^2_2=12.1$; $P=.002$).

The quintiles for 25(OH)D3 in the control group were less than 19.7, 19.7 to 30.9, 31.0 to 40.4, 40.5 to 50.9, and greater than 51 nmol/L. The distribution of 25(OH)D3 in the cases and controls is shown in the eFigure (available at http://www.archgenpsychiatry.com). Overall, the risk of schizophrenia was significantly associated with the level of 25(OH)D3 ($\chi^2_4=12.6$; $P=.01$). To best display the relationship between the variables of interests, neonates in the fourth quintile were chosen as the reference category. The relative risk was significantly increased for neonates in each of the 3 lowest quintiles of the distribution of 25(OH)D3. Compared with the reference category, neonates in the lowest quintile had a relative risk of 2.1 (95% CI, 1.3-3.5) while those in the second and third quintiles had relative risks of 2.0 (95% CI, 1.3-3.2) and 2.1 (95% CI, 1.3-3.4), respectively. Unexpectedly, neonates in the highest quintile of the distribution also had a significantly increased relative risk of later developing schizophrenia when compared with the reference category (relative risk, 1.71; 95% CI, 1.04-2.8).

Figure 2 shows that the U-shaped nature of the exposure-risk relationship was more prominent when the data were assessed in deciles (based on the control 25[OH]D3 values). The gray line shows the best fit analysis for the continuous data (a second-degree fractional polynomial with orders 3 and 3). This polynomial had a significantly better fit compared with both a straight line ($P=.046$) and the null model ($P=.003$). This model predicted the lowest relative risk of schizophrenia in the 73rd percentile (corresponding to 46.5 nmol/L of 25[OH]D3).

The U-shaped relationship between the level of 25 (OH)D3 and schizophrenia was not explained by interaction effects between 25(OH)D3 and a wide range of variables (ie, sex, age at onset, year of birth, calendar year at onset, second-generation immigrant status, degree of urbanization at place of birth, history of any mental illness in a family member, maternal and paternal age at the time of the child's birth, gestational age, congenital malformations, Apgar score, birth weight, and birth length [results not shown]).

With respect to the population-attributable fraction, shifting all subjects to the optimal level (fourth quintile) accounted for 43.6% of cases in this sample.

We have undertaken various post hoc analyses to further explore the findings. We examined the exposure-risk relationship when the fourth and fifth quintiles were combined as the reference category (ie, 25[OH]D3 above 40.5 nmol/L). Those in the lowest and second quintiles still had significantly increased risk of schizophrenia (OR, 1.7; 95% CI, 1.1-2.5 and OR, 1.8; 95% CI, 1.2-2.6, respectively), while those in the third quintile did not have a significantly increased risk (OR, 1.4; 95% CI, 0.9-2.0). We went back to the original data for all samples above 40.5 nmol/L (the 2 upper quintiles). Keeping the technicians blind to case-control status, we checked all aspects of the tandem mass spectroscopy readout and double-checked that the integration of the peak of interest was correct. We examined several additional variables post hoc to explain the nonlinear relationship including delays in collection of the sample (which could have introduced a differential opportunity to access postnatally derived 25[OH]D3 prior to sampling), admission to neonatal intensive care unit (which could have altered properties such as the hematocrit or vitamin D metabolism), and diagnosis of neonatal hyperbilirubinemia (UV light, which is used as a treatment for this

condition, may have resulted in higher vitamin D production). None of these analyses could account for the nonlinear exposure-risk relationship.

COMMENT

Neonatal vitamin D status was significantly associated with the risk of schizophrenia. The nature of this relationship was nonlinear. Compared with those in the fourth quintile, those in lower quintiles had a 2-fold increased risk of schizophrenia. Unexpectedly, we identified that those in the highest quintile of 25(OH)D3 levels also had a significantly increased risk of schizophrenia compared with those in the fourth quintile.

J-shaped or U-shaped exposure-risk relationships have been previously described in studies examining maternal 25(OH)D3 status and neonatal growth outcomes[35] as well as adult case-control studies exploring the relationship between 25(OH)D3 and cancer outcomes.[36] Two large studies that examined the association between vitamin D status and cardiovascular disease[37] and all-cause mortality[38] both found nonlinear exposure-risk relationships that were remarkably similar to the fractional polynomial shown in Figure 2. In addition, there is in vitro evidence that 1,25(OH)2D3 has a U-shaped relationship with a range of cellular responses including brain-related outcomes such as the expression of L-type voltage-sensitive calcium channels.[39] In the current study, however, the optimal range of neonatal vitamin D with respect to later schizophrenia risk (ie, the fourth quintile range, 40.5-50.9 nmol/L), appears somewhat low in light of recent recommendations.[40] While optimal neonatal 25(OH)D3 levels for brain-related outcomes have yet to be defined, based on bone health, levels in the range of 25-50 nmol/L have been defined as mild deficiency.[40]

We recommend caution in the interpretation of the absolute levels of 25(OH)D3. The samples had been archived for 14 to 27 years, and it is likely that some degradation of 25(OH)D3 occurs in the first few days after collection and during storage.[30] However, as date of birth was a matching criterion in our study, there is no reason to suspect that any degradation of 25(OH)D3 could bias our results. The findings that neonatal 25(OH)D3 levels fluctuated across month of birth and were significantly lower in the offspring of migrants lends additional weight to the validity of the assay.

It is also feasible that the nonlinear relationship identified in this study represents a summation of different underlying exposure-risk relationships that reflect genetic variation. Gene × environment studies relating vitamin D to cancer[41] and bone outcomes[42] suggest that single-nucleotide polymorphisms in vitamin D–related genes can influence the association between 25(OH)D3 and health outcomes. While speculative, it is feasible that a subgroup of the population could have single-nucleotide polymorphisms in genes that influence the conversion of 25(OH)D3 to the active hormone 1,25(OH)2D3. As a result, these individuals could have relatively high levels of 25(OH)D3 (ie, the main circulating form used in standard assays) but have lower levels of 1,25(OH)2D3. In other words, the seemingly high levels in some individuals may be accompanied by a relative deficiency in the biologically active form of vitamin D. We plan to examine the interaction between neonatal 25(OH)D3, single-nucleotide polymorphisms in candidate genes related to vitamin D metabolism, and later schizophrenia in larger, adequately powered samples.

The nonlinear nature of the relationship between neonatal 25(OH)D3 and risk of schizophrenia has implications from a public health perspective. For example, if we assume that future studies replicate the nonlinear relationship and indicate that this relationship is not a reflection of subgroups of the populations who are particularly sensitive or resistant to vitamin D, then different public health interventions would be required for those with low vs high 25(OH)D3. We feel it is premature to enter into these speculations based on this study alone. The levels of 25(OH)D3 found in the upper quintile of this study would not be generally regarded as toxic or associated with detrimental health outcomes.[40] From a public health perspective, there is a growing body of evidence showing that low prenatal vitamin D is associated with adverse bone outcomes in neonates and children.[35,43] Developmental vitamin D deficiency has also been proposed as a risk factor for type I diabetes[44] and multiple sclerosis.[45] It is feasible that different adverse health outcomes are associated with different optimal ranges. Clearly, the field will need more prospective studies to integrate the effect of low developmental vitamin D on a diverse range of childhood and adult outcomes.

There are several limitations to this study that warrant careful reflection. For example, the analysis based on quintiles does not suggest a dose-response curve across the lower 3 quintiles. While the fractional polynomial curve and the analysis based on deciles suggest that a dose-response relationship may underlie this part of the exposure-risk relationship, further scrutiny in studies based on larger samples is required. Our sample is still relatively young, and the relationship between neonatal vitamin D and later-onset schizophrenia will require revisiting in future years. Concerning biological plausibility, while there is good evidence showing that developmental vitamin D deficiency is associated with changes in brain development,[15] there is a lack of data with respect to the potential that 25(OH)D3 levels in the upper range found in this study may also be associated with adverse brain outcomes. Animal studies may be able to address these issues.[46]

While this study was based on a candidate risk factor that is parsimonious with respect to a diverse range of epidemiological clues, and one that is biologically plausible,[15] we believe that the results from observational epidemiology should still be treated cautiously. The results from even well-designed observational studies can be influenced by residual confounding.[47] For example, there may be a range of factors not considered by our study that could independently influence schizophrenia risk but also be associated with vitamin D status. For example, low levels of 25(OH)D3 may lead to increased risk of prenatal infection.[48,49] Factors related to heat stress, maternal fish intake, or maternal body mass index may also warrant closer inspection in future studies.

Schizophrenia is associated with a substantial burden of disability. In the absence of major advances in the efficacy of treatments, interventions that offer the prospect of reducing the incidence of the disorder should be pursued vigorously.[1,50] It is acknowledged that there is an urgent need to undertake more research that focuses on the environmental risk factors and gene × environmental associations with schizophrenia.[51-53] Mindful of these issues, we note that hypovitaminosis D is prevalent in many societies,[13] and is a particular concern in pregnant and lactating women.[54] From a public health perspective, the chance to prevent a serious disorder like schizophrenia via simple, safe, and cheap nutritional supplements is a scenario that has not previously seemed plausible. Mindful of the caveats of estimates of population-attributable fractions, there may be subgroups in which vitamin D supplementation may be particularly indicated. For example, in dark-skinned ethnic groups living in cold countries, there is a substantially increased risk of schizophrenia.[7,55] Kirkbride and Jones[56] have estimated that if the yet-to-be-identified risk factors underlying the increased risk of schizophrenia in black minority ethnic groups living in England could be identified and prevented, it may be feasible to reduce the incidence of schizophrenia in this group by a staggering 87%. While there is much more work to be done, if future studies confirm the association between developmental vitamin D deficiency and risk of schizophrenia, it raises the tantalizing prospect of the primary prevention of this disabling group of brain disorders in a manner comparable with folate supplementation and the prevention of spina bifida.

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