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Neonatal Vitamin D Status in Relation to Autism Spectrum Disorder and Developmental Delay in the CHARGE Case-Control Study

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SCIENTIFIC ABSTRACT

Vitamin D appears essential for normal neurodevelopment and cognitive and behavioral function. We examined neonatal vitamin D in relation to the child's later diagnosis of autism spectrum disorder (ASD) or developmental delay (DD). Children aged 24–60 months enrolled in the population-based CHARGE case-control study were evaluated clinically for ASD (n=357), DD (n=134), or typical development (TD, n=234) at the MIND Institute (Sacramento, CA) using standardized assessments. Total 25-hydroxyvitamin D (25[OH]D) was measured using sensitive isotope dilution liquid chromatography-tandem mass spectrometry (LC/MS/MS) in archived dried blood spots collected for the California Department of Public Health's Newborn Screening Program. Multinomial logistic regression was used to calculate ORs as measures of the associations between 25 nmol/L change in 25(OH)D and ASD and DD. Associations between 25(OH)D and scores on Mullen Scales of Early Learning (MSEL) and Vineland Adaptive Behavior Scales (VABS) were assessed using robust linear regression. Effect modification was examined using stratified models and interaction product terms.

Unadjusted mean (SD) 25(OH)D was lower for DD (73.2 [37.6]) than for TD (82.7 [39.3]) and ASD (80.1 [37.4]). After adjustment for maternal pre-pregnancy body mass index and education, a

AUTHORS' CONTRIBUTIONS

RJS conceived of and designed the study, helped secure funding, helped guide statistical analyses, interpreted results and drafted the manuscript; QN conducted preliminary statistical analysis; DE conducted measurements of dried blood spot vitamin D. RLH provided clinical oversight for the study and contributed clinical diagnoses; AMI designed the analytical plan and oversaw the preliminary statistical analyses and conducted final analyses. All authors reviewed and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

Dr. Schmidt has received lodging for The Baby Siblings Research Consortium Meeting; travel and lodging for invited talks at the University of Sherbrooke, Sherbrooke, Québec, Canada; the University of California Santa Cruz. Santa Cruz, California (Lodging); Epigenomics 2016, Puerto Rico (Lodging); Neurotoxicity Society & International Neurotoxicology Association, Florianópolis, Brazil; RISE 2017 Second International Meeting on Environmental Health in Strasbourg. Strasbourg, France. Dr. Schmidt also received Autism Speaks grant funding to develop an online autism environmental questionnaire. No other authors declare potential conflicts of interest.

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25 nmol/L increase in total 25(OH)D was not associated with ASD (OR=0.97; CI: 0.87–1.08) or DD (OR=0.90; 95% CI: 0.78–1.06). Neonatal 25(OH)D was associated with significantly reduced ASD only in females (adjusted OR=0.74; 95% CI: 0.55–0.99, $P_{interaction}$=0.03, and significantly reduced DD only in non-Hispanic white children (adjusted OR=0.79; 95% CI: 0.63–0.98, $P_{interaction}$=0.11 for Hispanic, $P_{interaction}$=0.31 for other), driven by DD children with trisomy 21. This study provides evidence that neonatal vitamin D could be associated with ASD in females and with DD in non-Hispanic white children.

LAY SUMMARY

Vitamin D appears essential for brain development and function. We examined neonatal total 25-hydroxyvitamin D (25[OH]D) measured in dried blood spots in relation to later diagnoses of autism spectrum disorder (ASD) or developmental delay (DD) and related assessment scores. Higher neonatal 25(OH)D was associated with a 26% reduction in the odds for ASD only in females. After taking into account factors that could contribute to vitamin D status, a significant association with 21% reduced odds for DD was found only in non-Hispanic white children. Though results were non-significant overall, certain subgroups might benefit from higher neonatal vitamin D.

Keywords

Autistic Spectrum Disorder; Vitamin D; Child Development Disorders; Down Syndrome; Prevention; Infant; Newborn

Autism spectrum disorder (ASD) consists of a range of neurodevelopmental disorders characterized by the presence of social deficits, language impairments, and stereotyped or repetitive behaviors and interests. The etiology of ASD in most cases remains unclear, though combinations of multiple genetic and environmental factors are likely to play a role. Vitamin D deficiency was hypothesized to contribute to the increase in the incidence of ASD based on studies showing increased rates of autism among dark-skinned immigrants displaced into northern latitudes, and differences in autism prevalence across season and latitude (Cannell 2008), potentially reflecting changes in sunlight exposure and absorbed vitamin D. A large register-based study in Sweden found an association between maternal lifetime vitamin D deficiency diagnosis and increased ASD with intellectual disability in the child, especially in non-immigrants (Magnusson et al. 2016). These findings have been attributed to a potential effect of *maternal* vitamin D status on the child's risk for ASD. The biologic plausibility for a link between vitamin D and autism is ample, as previously reviewed (Cannell 2008; Eyles et al. 2012). Animal studies show long-lasting neurodevelopmental effects of transient vitamin D deficiency during gestation leading to autism-relevant structural and functional changes in the brain and behaviors of the offspring (Levenson and Figueiroa 2008; Eyles et al. 2009; Grecksch et al. 2009). Several studies have recently found associations between gestational vitamin D status and ASD diagnosis (Fernell et al. 2015; Chen et al. 2016; Vinkhuyzen et al. 2017) and autism-related traits (Vinkhuyzen et al. 2018), but findings have been inconsistent, and the number of children diagnosed with ASD has been relatively small and did not allow for investigation of effect modification by race and sex. There is biological potential for heterogeneous effects across

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sex given that sex hormones like estrogen and testosterone have very different effects on calcitriol's metabolism (Cannell 2008), and males are more prone to vitamin D deficiency (Tonnesen et al. 2016). Serum vitamin D status differs by race, given the biologic role for pigmentation and melanin in vitamin D production (Jablonski 2004; Bonilla et al. 2014), which could also influence associations with developmental outcomes. This study examined neonatal vitamin D in relation to the child's later diagnosis of autism spectrum disorder (ASD) or developmental delay (DD).

METHODS

Participants, Eligibility and Diagnostic Criteria

Individuals included in this study were participants in the CHARGE (*CH*ildhood *A*utism *R*isks from *G*enetics and the *E*nvironment) case-control study (Hertz-Picciotto et al. 2006). Eligibility criteria for children were: 1) age of 24 to 60 months at time of enrollment, 2) birth in California, 3) residence with at least one biologic parent who speaks English or Spanish, and 4) residence in the catchment areas of a specified list of California Regional Centers that coordinate services for persons with developmental disabilities. Children with autism, intellectual disability, or developmental delay were identified through the California Regional Centers as having received services for one or more of these conditions. Autism cases were also referred from the MIND Institute and other health or service providers, or self-referred from the CHARGE Study website. General population controls identified from state birth files were frequency matched to the age and catchment area distribution of the autism cases, and a 4:1 male-to-female ratio reflective of that seen for ASD. We retained the first child in the family enrolled in the study and excluded 18 siblings. We also excluded 89 children enrolled and assessed at the UCLA Neuropsychiatric Institute due to differences in assessment protocols and scores.

All children were assessed for cognitive function using the Mullen Scales of Early Learning (MSEL) (Mullen 1995) and for adaptive function using the Vineland Adaptive Behavior Scales (VABS) (Sparrow et al. 1984). For children with autism, the primary caregiver completed the Autism Diagnostic Interview–Revised (ADI–R) (Lord et al. 1994), and children were assessed using the Autism Diagnostic Observation Schedule–Generic (ADOS) (Lord et al. 2000) to confirm the child's diagnosis. The children of families recruited from the general population or with developmental delay/intellectual disability were screened for evidence of ASD using the Social Communication Questionnaire (SCQ) (Rutter et al. 2003) and if they scored above 15, were evaluated for autism. Final ASD case status was defined as 1) scoring at least 7 on ADOS Module 1 or at least 8 on ADOS Module 2; 2) meeting the cutoff value for section A or B and scoring above or within 2 points of the cutoff value on A or B (whichever did not meet cutoff value) in ADI–R; and 3) meeting the cutoff value on section D in ADI-R. Typical development (TD) required being recruited from the general population, screening negative for evidence of ASD on the SCQ, and scoring 70 or above on both the MSEL and VABS. These analyses included only the first child per family recruited into the study. The University of California (UC) Davis Institutional Review Board and the State of California Committee for the Protection of Human Subjects approved this study and

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the CHARGE Study protocols. Neither data nor specimens were collected until written informed consent was obtained from the parents.

Archived Newborn Dried Blood Spot Vitamin D Measurement

Blood spots collected on filter paper for the California Department of Public Health's (CDPH) Genetics Disease Branch Newborn Screening Program were obtained for CHARGE participants. These 1.5 cm diameter bloodspots were punched into 3/16 (3.2 mm diameter) punches that were shipped to Dr. Eyles' laboratory for vitamin D measurement. Typically, about 12–14 3 mm punches are obtained from a blood spot. Blood spots and punches were stored in laboratories protected from light exposure at −20C (CDPH) or −70C (UC Davis).

We report total 25-Hydroxyvitamin D (25[OH]D) concentrations as the sum of 25(OH)D2 and 25(OH)D3. 25(OH)D was measured by an isotope dilution liquid chromatography-tandem mass spectrometry (LC/MS/MS) assay validated for dried blood spots (Eyles et al. 2009; Kvaskoff et al. 2016) by a participatory laboratory of the Vitamin D External Quality Assessment Scheme. Assay accuracy was assessed using NIST sera calibrants (SRM 972a Levels 1–4) in replicates of 3 for each run. Interassay measures of imprecision were also examined by running 1 high and 1 low 25(OH)D3 sample every 50 samples giving 8 replicates spread across each plate. Three separate 384 well plates containing samples were required to analyze the total study population. We also examined sample variance across plates. Hematocrit (Hct) corrections were applied to obtain sera equivalent values: 25(OH)D × (1/(1-Hct)), where Hct=0.61 (Eyles et al. 2009; Eyles et al. 2010). Dried blood spot 25(OH)D concentrations are comparable to matched sera values (Heath et al. 2014).

Statistical Analysis

The exposure of interest, 25(OH)D, was analyzed both as a continuous variable and by clinically relevant categories of sufficient (75+ nmol/L, reference), insufficient (50-<75 nmol/L), or deficient (<50 nmol/L) (Gallagher and Sai 2010). In post-hoc analyses, we also divided the deficient category into severely deficient (< 25 nmol/L) and deficient (25 - < 50 nmol/L). The linearity of the relationship between the log-odds of ASD and DD and 25(OH)D (using the original scale and log-transformed) was assessed using empirical logit plots.

Characteristics of the children with ASD, DD, and TD and their parents were compared using multinomial logistic regression. Associations between child and maternal characteristics in the TD children and neonatal sera equivalent vitamin D values were estimated using linear robust regression. For the categorical exposure, we used Mantel-Haenszel Chi-Square test for associations with categorical child and maternal characteristics and Spearman correlation for continuous ones.

Odds ratio (OR) and 95% confidence intervals (CI) were estimated for associations between neonatal vitamin D and both ASD and DD, adjusted for confounders, using multinomial logistic regression analysis. For continuous 25(OH)D we report crude and adjusted OR corresponding to 25 nmol/L increases. Confounders were identified through a three-pronged strategy involving: review of the literature, a hypothesized causal model, and an empirical screen of measured factors. A directed acyclic graph (DAG) (Greenland et al. 1999; Hernan

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et al. 2002) was constructed based on the biology and known/hypothesized relationships among predictor, outcome and covariates (Supplemental Figure S1). Potential confounders considered based on the DAG included: child sex, race and ethnicity (categorized as white non-Hispanic, Hispanic, and other), gestational age at delivery, birth season and year, maternal age at the time the child's birth, maternal education, pre-pregnancy body mass index (BMI), and intake of prenatal vitamins). These nine potential confounders selected for adjustment from the DAG were then empirically screened for associations with the outcomes (ASD or DD) and with 25(OH)D in the bivariate analyses. We selected as candidates for the multivariate model only those that were broadly associated ($p < 0.2$) with both 25(OH)D and outcome (ASD or DD) and caused ≥ 10% change in the parameter estimate for neonatal vitamin D in association with either outcome of ASD or DD. We started building a multivariate model by including all confounders that met these criteria. We then fitted a series of nested models, starting with this full model and eliminating each potential confounder one at a time (starting with the one that caused the smallest beta change) to assure that with the other variables in the model, each still met criteria as a confounder with a 10% change in the parameter estimate for the outcome (either ASD or DD, or the continuous scores). A similar model-building strategy was employed for each of the two continuous outcomes, using robust linear regression. Effect modification was examined for the association between neonatal vitamin D and child case status and continuous outcome measures by child race and ethnicity and child sex using stratified models and product terms. Effect modification by case status was also examined for the association between neonatal vitamin D and continuous outcome measures.

Five of the potential confounders and one of the assessment scores had missing data, ranging from 1 (0.1%) to 51 (7%) observations. Multiple imputation with 10 imputations was used. All analyses, including model building, were conducted on each imputed data set; results were combined using standard rules (Rubin 1987). Primary results using imputed data were compared with those obtained from complete and all available data. All analyses were implemented in SAS 9.4 (SAS Institute Inc. 2002–2012).

RESULTS

The CHARGE participants included for this analyses included 725 with neonatal 25(OH)D concentrations measured on a DBS obtained before the child was 400 hours (17 days) old and had a clinically confirmed diagnosis of ASD, DD, or TD. Of the 723 included children with information on race/ethnicity, 566 (78.3%) children included were white, 19 (2.6%) were black, 32 (4.4%) were Asian, and 106 (14.7%) were of mixed or other races; 223 (30.8%) children were Hispanic. The study sample was predominantly male (595 [82.1%] male and 130 [17.9%] female).

Characteristics by Neurodevelopmental Outcomes

Children with ASD were more likely to be born in earlier years than children with TD (Table 1). Children with DD were less likely than children with TD to be male, more likely to be Hispanic, and were on average older at the time of newborn screening blood spot collection and at time of diagnosis. Parents of children with ASD and DD were less likely to own their

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homes and mothers of children with ASD and DD were less likely to have a bachelor degree, more likely to have higher pre-pregnancy BMI and BMI in the overweight or obese NIH categories and less likely to take a prenatal vitamin in the months from 3 months before through the first month of pregnancy (Table 1).

MSEL and VABS composite scores in TD children also differed by characteristics of children and their mothers (Supplemental Table S1), with lower MSEL and VABS composite scores for male children, and lower MSEL scores for children whose mothers were younger, less educated, had a higher pre-pregnancy BMI or were obese, and whose parents did not own their home. There were differences in VABS scores across maternal age group and pre-pregnancy BMI.

Vitamin D Quality Control Results

Over the 3 plates, none of the 4 NIST sera 25OHD calibrants varied by >15% compared with the reference value, see Supplemental Table S2. There was also no variation in mean participant concentrations between plates with 25(OH)D mean ± SD of 80.2 ± 37.9 nM for plate 1; 75.5 ± 39.2 for plate 2 and 83.4 ± 38.1 for plate 3.

Neonatal Vitamin D by Participant Characteristics

Children were all under 400 hours (17 days) old at the time of blood spot collection (the exclusion criteria). Across the entire study population, the mean concentration of 25(OH)D from newborn dried blood spots in this Northern California population was 79.6 (SD, 38.1) nmol/L; 23% (n=166) were deficient and another 27% (n=197) met criteria for insufficiency, leaving 50% (n=363) that were sufficient.

Within the TD children, neonatal vitamin D was significantly lower and vitamin D deficiency was more common in children who were Hispanic or of non-white race compared to non-Hispanic white children, children with higher gestational age, and in children whose mothers had higher pre-pregnancy BMI (Table 2). Neonatal vitamin D was also significantly lower in children whose mothers had a high school education or less compared to those whose mothers had bachelor degrees (Table 2). In contrast, neonatal vitamin D was significantly higher in children born in the summer, and whose family owned their home (Table 2).

Covariates Included as Confounders

Five covariates (child sex, race and ethnicity, and gestational age at delivery and maternal education and pre-pregnancy body mass index were broadly associated with both outcome (either ASD or DD) and exposure (see Table 1). Of them, only maternal education and pre-pregnancy body mass index produced a beta change of 10%, both in unadjusted and after adjusting for the effect of the other and were retained in the final model for outcome. Four covariates (child sex, race and ethnicity and maternal education and pre-pregnancy body mass index were broadly associated with both MSEL scores and exposure (see Table 1). Of them, only child race ethnicity, maternal education and pre-pregnancy body mass index produced a beta change of 10% in unadjusted analyses, and only maternal education and pre-pregnancy body mass index met that criteria after adjusting for the effect of the other

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and were retained in the final model for MSEL. Three covariates (child gestational age at delivery, maternal education and pre-pregnancy body mass index were broadly associated with both Vineland scores and exposure (see Table 1). Of them only maternal education and pre-pregnancy body mass index produced a beta change of 10%, both in unadjusted and after adjusting for the effect of the other and were retained in the final model for outcome.

Neonatal Vitamin D by Neurodevelopmental Outcomes

The distribution of 25(OH)D presented some right skewness in all outcome groups (Supplemental Figure S2). Transformations using natural log did not improve the normality and empirical logit plots suggested the assumption of linearity of the relationship between the log odds and the exposure was met for the original variable, we used non-transformed 25(OH)D for our primary analysis results; results for the ln-transformed 25(OH)D are presented in the supplement (Supplemental Tables S3) for comparisons with other studies that made this transformation.

Unadjusted mean (SD) 25(OH)D was significantly lower for DD [73.0 (37.2)] than for TD [82.7 (39.3); P=0.02] and borderline significantly lower than for ASD [79.9 (36.9); P=0.07]; the difference in means between ASD and TD was not significant. No association between a 25 nmol/L increase in 25(OH)D and ASD was observed before or after adjustment (odds ratio, OR=0.96, 95% confidence interval, CI: 0.86, 1.06 and OR=0.97, CI: 0.87, 1.08, respectively) (Table 3). The lack of an association with ASD remained when we examined categories of deficient and insufficient vitamin D (Table 3) as well as with severe vitamin D deficiency in post-hoc analyses (Supplemental Table S4).

A 25 nmol/L increase in 25(OH)D was significantly associated with reduced DD prior to (OR=0.84, 95% CI: 0.73, 0.98) but not after adjustment for maternal pre-pregnancy body mass index, and education (OR=0.90, 95% CI: 0.78, 1.06) (Table 3). Similarly, there was a significant association with 25(OH)D in the deficient category before, but not after adjustment for these factors (Table 3). However, in post-hoc analyses, an association between severely deficient vitamin D and increased odds of DD remained significant after adjustment (Supplemental Table S4).

There was a significant association between a 25 nmol/L increase in neonatal 25(OH)D and MSEL composite score (Table 4) and a marginally significant association with VABS composite score (Table 4) before adjustment, but these associations were attenuated after adjustment for maternal education and pre-pregnancy BMI and the association with MSEL composite score became only marginally significant. The risk estimates between neonatal 25(OH)D and the MSEL and VABS composite scores were further attenuated and were non-significant when we additionally adjusted for diagnostic outcome (Table 4).

Effect Modification by Child Sex, Race/Ethnicity, and Case Status—There was a significant interaction effect by sex (p=0.03), with a significant association between a 25 nmol/L increase in 25(OH)D and ASD in females only (adjusted OR=0.74; 95% CI: 0.55, 0.99) (Table 5). There were also non-significant differences across race/ethnicity where there was an association between a 25 nmol/L increase in 25(OH)D and DD only in non-Hispanic white children that remained significant after adjustment (OR=0.79; 95% CI: 0.63, 0.98)

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(Table 5). Similarly, the associations between neonatal vitamin D and both MSEL and VABS composite scores were limited to females, and were strongest in non-Hispanic white children, though interaction terms were only significant for VABS across sex (Table 6). The association between neonatal 25(OH)D and MSEL composite score differed by case status in the crude models with stronger positive associations for children with ASD and DD, but the difference across groups was attenuated and not statistically significant in the adjusted models (Table 6). The association between neonatal 25(OH)D and VABS composite scores was non-significant when stratified by case status, and did not differ significantly across diagnostic outcome (Table 6). When ASD diagnosis was separated into those with and without intellectual disability, 25(OH)D was non-significantly associated with reduced ASD only when combined with intellectual disability (Table 7). When we examined subsets of children with DD based on whether they had a Down Syndrome, another genetic or metabolic syndrome, or no syndrome, the association between 25(OH)D and DD due to Down Syndrome was the strongest protective association that was significant before but not after adjustment (OR=0.79; 95% CI: 0.58–1.06; Table 8).

DISCUSSION

Mean neonatal vitamin D concentrations from dried blood spots in this Northern California population, at 80 nmol/L, were on average higher than mean concentrations previously reported in studies using DBS with similar methods in other populations, including other predominantly Caucasian populations that ranged from 29 to 53 nmol/L (McGrath et al. 2010; Tornhammar et al. 2014; Fernell et al. 2015; Nielsen et al. 2017; Smith et al. 2017) (Supplemental Table S5). Additionally, the percentage that met criteria for vitamin D deficiency (23%) or insufficiency (27%) were much lower than in other populations where the majority met these criteria (Supplemental Table S5). The higher mean 25(OH)D could be due to higher ultraviolet indexes and/or exposure in this California population or fortification of vitamin D in several foods within the U.S.; previous studies have shown similarly higher umbilical cord blood serum 25(OH)D3 (59 – 67 nmol/L) in U.S. Caucasians (from Ohio and Pennsylvania) (Bodnar et al. 2007; Seto et al. 2016) compared to Europeans (17 – 67 nmol/L) (Delvin et al. 1986; Pludowski et al. 2014; Wierzejska et al. 2018).

Neonatal vitamin D concentrations were associated with maternal and child characteristics in an expected manner, with lower vitamin D for children with races/ethnicities associated with darker skin pigment who have a less efficient ultraviolet light conversion of 7-dehydrocholesterol to the vitamin D pre-hormone (Jablonski 2004; Armas et al. 2007; Rockell et al. 2008; Hall et al. 2010; Nessvi et al. 2011; Libon et al. 2013), and mothers with higher BMI who are prone to vitamin D deficiencies (Lagunova et al. 2009). Only one mother reported taking vitamin D-specific supplements and prenatal vitamins, which typically contain 400 IU vitamin D and were the most common source of supplemental vitamin D in this population, were not significantly associated with 25(OH)D.

Our findings for a lack of an association between neonatal vitamin D and ASD is counter to findings of a previous small sibling-pair study using neonatal dried blood spots that found that children with ASD had lower average neonatal 25(OH)D that was not explained by birth season (Fernell et al. 2015). A much larger (n=4,229) cohort study showed an association

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between vitamin D deficiency measured at mid-gestation and in cord blood and autism-related traits (Vinkhuyzen et al. 2018), but the association with cord blood vitamin D deficiency appeared to be driven primarily by those who had deficiencies at both time points. Similarly, in the same cohort (n=4,334), vitamin D deficiency in cord blood was not associated with ASD diagnosis, while low maternal mid-gestation 25(OH)D was associated (Vinkhuyzen et al. 2017). Taken together, these studies and a study that found low maternal first trimester 25(OH)D was associated with higher risk for ASD diagnosis (Chen et al. 2016), suggest that there may be a critical period for gestational vitamin D and ASD earlier in pregnancy, that was not well-measured in this study. Further, given that concentrations in our overall study population were high and our proportion with vitamin D deficiency were low relative to other study populations, it is possible our study was not powered to detect associations at very low 25(OH)D concentrations. The indication in our study for a weak association with ASD when combined with ID (but not without) suggests that associations with ASD could be driven by the overlap with DD and that careful clinical classification of ASD and DD separately is important for understanding specificity of any associations.

We also observed a significant interaction effect by child sex with a significant association between higher neonatal vitamin D and reduced ASD only in females. To our knowledge, this effect modification by sex has not been previously reported for the association between vitamin D and ASD but could have biologically plausible explanations. Evidence supports synergistic effects of 17-β estradiol and 25(OH) D3, with vitamin D3 stimulating 17-β estradiol synthesis and conversion of androgen to estrogen in glial cells, and 17-β estradiol promoting vitamin D3 receptor expression and its function in the central nervous system (Spach and Hayes 2005; Nashold et al. 2009), and functional synergy for their immunomodulatory effects (Correale et al. 2013; Al-Daghri et al. 2014). Vitamin D deficiency during pregnancy results in sex-specific dysregulation of placental inflammation in mice and rats (Ali et al. 2018; Liu et al. 2018). Gender differences in the metabolism of vitamin D3 and its interaction with estrogen production (Bertone-Johnson et al. 2010; Lee et al. 2012; Correale et al. 2013) are congruent with sex-specific health effects induced by vitamin D deficiency (Stadlmayr et al. 2015; Verdoia et al. 2015) including attentional deficits (Groves and Burne 2016) and sex-specific effects of vitamin D supplementation (Al-Daghri et al. 2014) and stronger protective effects in females against other conditions (Fereidan-Esfahani et al. 2013).

We observed a crude inverse association between neonatal vitamin D and DD that was attenuated and non-significant after adjustment for factors that were associated with both neurodevelopmental outcomes and vitamin D status, including maternal BMI and education. This could indicate that the crude association between vitamin D and DD resulted from confounding by these factors; alternatively, these findings could suggest that vitamin D is one potential mediator of the associations between these factors and DD. Though there was no statistically significant association between severely deficient vitamin D and increased odds of DD before or after adjustment for confounding factors, the ORs were greatest in this group, which could suggest the non-significant association with DD was driven by those with 25(OH)D below 25 nmol/L, concentrations previously defined as deficient based on negative implications for calcium absorption and bone health (2010; Gallagher and Sai

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2010; Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium 2011).

We found a significant association between higher 25(OH)D and decreased risk for DD in non-Hispanic whites that remained after adjustment. This suggested evidence for effect modification by race is interesting given the biologic role for pigmentation and melanin in vitamin D production. Other studies that have found a significant association between neonatal vitamin D and ASD or traits were conducted in primarily Caucasian populations (Fernell et al. 2015; Vinkhuyzen et al. 2017).

The inverse association between 25(OH)D and DD appeared to be driven primarily by those with Down Syndrome (trisomy 21). This could be due to reverse causation given that individuals with Down Syndrome have been shown to have lower 25(OH)D concentrations (Stagi et al. 2015), which could result from increased susceptibility to genetically altered vitamin D metabolism and utilization or a greater need for vitamin D's immunomodulating effects (Guillot et al. 2010) given increased neuroinflammation observed in Down Syndrome (Wilcock and Griffin 2013). There is a lack of seasonality in Down Syndrome (Stolwijk et al. 1997), which might be expected if this were a causative association. On the other hand, vitamin D has been shown to have a role in DNA repair (Kinney et al. 2010; Fleet et al. 2012), vitamin D treatment has been shown to significantly reduce chromosomal aberrations (Chatterjee 2001), and supplementation with other nutrients near conception has been associated with decreased Down Syndrome occurrence (Czeizel and Puho 2005; Hollis et al. 2013), so if the higher vitamin D observed in the newborn reflects higher vitamin D at the time of conception, there could be biologic plausibility for higher vitamin D to lower risk for nondisjunction.

This study provides some evidence that neonatal vitamin D status could be associated with ASD in females and with DD in non-Hispanic white children. Additional, larger studies are needed to further investigate subgroups and timing of vitamin D depletion and to provide insight into potential mechanisms and to inform guidelines regarding maternal or infant vitamin D supplementation to provide the greatest public health impact.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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LIST OF ABBREVIATIONS USED

25(OH)D	total 25-hydroxyvitamin D
ADI–R	Autism Diagnostic Interview–Revised
ADOS	Autism Diagnostic Observation Schedule–Generic
ASD	autism spectrum disorder
BMI	body mass index
CHARGE	Childhood Autism Risks from Genetics and the Environment
DBS	dried blood spot
DD	developmental delay
DNA	deoxyribonucleic acid
MSEL	Mullen Scales of Early Learning
VABS	Vineland Adaptive Behavior Scales
SCQ	Social Communication Questionnaire
SD	standard deviation
TD	typical development
OR	odds ratio
CI	95% confidence intervals

REFERENCES

(2010). Dietary Reference Intakes for Calcium and Vitamin D. Washington DC, USA, National Academies Press.

Al-Daghri NM, Al-Attas OS, et al. (2014). “Whole serum 3D LC-nESI-FTMS quantitative proteomics reveals sexual dimorphism in the milieu interieur of overweight and obese adults.” J Proteome Res 13(11): 5094–5105. [PubMed: 25072778]

Ali A, Cui X, et al. (2018). “The placental immune response is dysregulated developmentally vitamin D deficient rats: Relevance to autism.” J Steroid Biochem Mol Biol 180: 73–80. [PubMed: 29408533]

Armas LA, Dowell S, et al. (2007). “Ultraviolet-B radiation increases serum 25-hydroxyvitamin D levels: the effect of UVB dose and skin color.” J Am Acad Dermatol 57(4): 588–593. [PubMed: 17637484]

Bertone-Johnson ER, Chocano-Bedoya PO, et al. (2010). “Dietary vitamin D intake, 25-hydroxyvitamin D3 levels and premenstrual syndrome in a college-aged population.” J Steroid Biochem Mol Biol 121(1–2): 434–437. [PubMed: 20398756]

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Bodnar LM, Simhan HN, et al. (2007). “High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates.” J Nutr 137(2): 447–452. [PubMed: 17237325]

Bonilla C, Ness AR, et al. (2014). “Skin pigmentation, sun exposure and vitamin D levels in children of the Avon Longitudinal Study of Parents and Children.” BMC Public Health 14: 597. [PubMed: 24924479]

Cannell JJ (2008). “Autism and vitamin D.” Med Hypotheses 70(4): 750–759. [PubMed: 17920208]

Chatterjee M (2001). “Vitamin D and genomic stability.” Mutat Res 475(1–2): 69–87. [PubMed: 11295155]

Chen J, Xin K, et al. (2016). “Lower maternal serum 25(OH) D in first trimester associated with higher autism risk in Chinese offspring.” J Psychosom Res 89: 98–101. [PubMed: 27663117]

Correale J, Balbuena Aguirre ME, et al. (2013). “Sex-specific environmental influences affecting MS development.” Clin Immunol 149(2): 176–181. [PubMed: 23498776]

Czeizel AE and Puho E (2005). “Maternal use of nutritional supplements during the first month of pregnancy and decreased risk of Down’s syndrome: case-control study.” Nutrition 21(6): 698–704; discussion 774. [PubMed: 15925294]

Delvin EE, Salle BL, et al. (1986). “Vitamin D supplementation during pregnancy: effect on neonatal calcium homeostasis.” J Pediatr 109(2): 328–334. [PubMed: 3488384]

Eyles D, Anderson C, et al. (2009). “A sensitive LC/MS/MS assay of 25OH vitamin D3 and 25OH vitamin D2 in dried blood spots.” Clin Chim Acta 403(1–2): 145–151. [PubMed: 19232332]

Eyles DW, Burne TH, et al. (2012). “Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease.” Front Neuroendocrinol.

Eyles DW, Feron F, et al. (2009). “Developmental vitamin D deficiency causes abnormal brain development.” Psychoneuroendocrinology.

Eyles DW, Morley R, et al. (2010). “The utility of neonatal dried blood spots for the assessment of neonatal vitamin D status.” Paediatr Perinat Epidemiol 24(3): 303–308. [PubMed: 20415760]

Fereidan-Esfahani M, Ramagopalan SV, et al. (2013). “Vitamin d: shining a light on clinical and sex specific effects in multiple sclerosis?” Int J Prev Med 4(5): 499–500. [PubMed: 23930158]

Fernell E, Bejerot S, et al. (2015). “Autism spectrum disorder and low vitamin D at birth: a sibling control study.” Mol Autism 6: 3. [PubMed: 25874075]

Fleet JC, DeSmet M, et al. (2012). “Vitamin D and cancer: a review of molecular mechanisms.” Biochem J 441(1): 61–76. [PubMed: 22168439]

Gallagher JC and Sai AJ (2010). “Vitamin D insufficiency, deficiency, and bone health.” J Clin Endocrinol Metab 95(6): 2630–2633. [PubMed: 20525913]

Grecksch G, Ruthrich H, et al. (2009). “Transient prenatal vitamin D deficiency is associated with changes of synaptic plasticity in the dentate gyrus in adult rats.” Psychoneuroendocrinology.

Greenland S, Pearl J, et al. (1999). “Causal diagrams for epidemiologic research.” Epidemiology 10(1): 37–48. [PubMed: 9888278]

Groves NJ and Burne TH (2016). “Sex-specific attentional deficits in adult vitamin D deficient BALB/c mice.” Physiol Behav 157: 94–101. [PubMed: 26836278]

Guillot X, Semerano L, et al. (2010). “Vitamin D and inflammation.” Joint Bone Spine 77(6): 552–557. [PubMed: 21067953]

Hall LM, Kimlin MG, et al. (2010). “Vitamin D intake needed to maintain target serum 25-hydroxyvitamin D concentrations in participants with low sun exposure and dark skin pigmentation is substantially higher than current recommendations.” J Nutr 140(3): 542–550. [PubMed: 20053937]

Heath AK, Williamson EJ, et al. (2014). “Measurements of 25-hydroxyvitamin D concentrations in archived dried blood spots are reliable and accurately reflect those in plasma.” J Clin Endocrinol Metab 99(9): 3319–3324. [PubMed: 24885629]

Hernan MA, Hernandez-Diaz S, et al. (2002). “Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology.” Am J Epidemiol 155(2): 176–184. [PubMed: 11790682]

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Hertz-Picciotto I, Croen LA, et al. (2006). “The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism.” Environ Health Perspect 114(7): 1119–1125. [PubMed: 16835068]

Hollis ND, Allen EG, et al. (2013). “Preconception folic acid supplementation and risk for chromosome 21 nondisjunction: a report from the National Down Syndrome Project.” Am J Med Genet A 161A(3): 438–444. [PubMed: 23401135]

Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium (2011). Dietary Reference Intakes for Calcium and Vitamin D. Ross AC, Taylor CL, Yaktine AL and Del Valle HB. Washington DC, National Academies Press (US).

Jablonski NG (2004). “The Evolution of Human Skin and Skin Color.” Annual Review of Anthropology 33: 585–623.

Jablonski NG (2004). “The Evolution of Human Skin and Skin Color.” Annual Review of Anthropology 33(1): 585–623.

Kinney DK, Barch DH, et al. (2010). “Environmental risk factors for autism: do they help cause de novo genetic mutations that contribute to the disorder?” Med Hypotheses 74(1): 102–106. [PubMed: 19699591]

Kvaskoff D, Heath AK, et al. (2016). “Minimizing Matrix Effects for the Accurate Quantification of 25-Hydroxyvitamin D Metabolites in Dried Blood Spots by LC-MS/MS.” Clin Chem 62(4): 639–646. [PubMed: 26888893]

Lagunova Z, Porojnicu AC, et al. (2009). “The dependency of vitamin D status on body mass index, gender, age and season.” Anticancer Res 29(9): 3713–3720. [PubMed: 19667169]

Lee DM, Tajar A, et al. (2012). “Association of hypogonadism with vitamin D status: the European Male Ageing Study.” Eur J Endocrinol 166(1): 77–85. [PubMed: 22048968]

Levenson CW and Figueiroa SM (2008). “Gestational vitamin D deficiency: long-term effects on the brain.” Nutr Rev 66(12): 726–729. [PubMed: 19019042]

Libon F, Cavalier E, et al. (2013). “Skin color is relevant to vitamin D synthesis.” Dermatology 227(3): 250–254. [PubMed: 24134867]

Liu NQ, Larner DP, et al. (2018). “Vitamin D-deficiency and sex-specific dysregulation of placental inflammation.” J Steroid Biochem Mol Biol 177: 223–230. [PubMed: 28676458]

Lord C, Risi S, et al. (2000). “The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism.” J Autism Dev Disord 30(3): 205–223. [PubMed: 11055457]

Lord C, Rutter M, et al. (1994). “Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders.” J Autism Dev Disord 24(5): 659–685. [PubMed: 7814313]

Magnusson C, Lundberg M, et al. (2016). “Maternal vitamin D deficiency and the risk of autism spectrum disorders: population-based study.” BJPsych Open 2(2): 170–172. [PubMed: 27703770]

McGrath JJ, Eyles DW, et al. (2010). “Neonatal vitamin D status and risk of schizophrenia: a population-based case-control study.” Arch Gen Psychiatry 67(9): 889–894. [PubMed: 20819982]

Mullen EM (1995). Scales of Early Learning. Circle Pines, MN, American Guidance Services Inc.

Nashold FE, Spach KM, et al. (2009). “Estrogen controls vitamin D3-mediated resistance to experimental autoimmune encephalomyelitis by controlling vitamin D3 metabolism and receptor expression.” J Immunol 183(6): 3672–3681. [PubMed: 19710457]

Nessvi S, Johansson L, et al. (2011). “Association of 25-hydroxyvitamin D3)levels in adult New Zealanders with ethnicity, skin color and self-reported skin sensitivity to sun exposure.” Photochem Photobiol 87(5): 1173–1178. [PubMed: 21679191]

Nielsen NM, Munger KL, et al. (2017). “Neonatal vitamin D status and risk of multiple sclerosis: A population-based case-control study.” Neurology 88(1): 44–51. [PubMed: 27903815]

Pludowski P, Grant WB, et al. (2014). “Vitamin d status in central europe.” Int J Endocrinol 2014: 589587. [PubMed: 24790600]

Rockell JE, Skeaff CM, et al. (2008). “Association between quantitative measures of skin color and plasma 25-hydroxyvitamin D.” Osteoporos Int 19(11): 1639–1642. [PubMed: 18408879]

Rubin DB (1987). Multiple Imputation for Nonresponse in Surveys. New York, John Wiley & Sons.

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Rutter M, Bailey A, et al. (2003). Social Communication Questionnaire. Los Angeles, CA, Western Psychological Services.

SAS Institute Inc. (2002–2012). SAS/STAT Version 9.4. Cary, NC.

Schmidt RJ, Tancredi DJ, et al. (2012). "Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study." Am J Clin Nutr 96(1): 80–89. [PubMed: 22648721]

Seto TL, Tabangin ME, et al. (2016). "Racial disparities in cord blood vitamin D levels and its association with small-for-gestational-age infants." J Perinatol 36(8): 623–628. [PubMed: 27101387]

Smith CA, Sun C, et al. (2017). "Determinants of Neonatal Vitamin D Levels as Measured on Neonatal Dried Blood Spot Samples." Neonatology 111(2): 153–161. [PubMed: 27756067]

Spach KM and Hayes CE (2005). "Vitamin D3 confers protection from autoimmune encephalomyelitis only in female mice." J Immunol 175(6): 4119–4126. [PubMed: 16148162]

Sparrow SS, Balla DA, et al. (1984). Vineland Adaptive Behavior Scales Interview Edition Expanded Form Manual. Circle Pines, MN, American Guidance Services, Inc.

Stadlmayr A, Aigner E, et al. (2015). "Relations of vitamin D status, gender and type 2 diabetes in middle-aged Caucasians." Acta Diabetol 52(1): 39–46. [PubMed: 24849007]

Stagi S, Lapi E, et al. (2015). "Determinants of vitamin d levels in children and adolescents with down syndrome." Int J Endocrinol 2015: 896758. [PubMed: 25685147]

Stolwijk AM, Jongbloet PH, et al. (1997). "Seasonal variation in the prevalence of Down syndrome at birth: a review." J Epidemiol Community Health 51(4): 350–353. [PubMed: 9328537]

Tonnesen R, Hovind PH, et al. (2016). "Determinants of vitamin D status in young adults: influence of lifestyle, sociodemographic and anthropometric factors." BMC Public Health 16: 385. [PubMed: 27170258]

Tornhammar P, Ueda P, et al. (2014). "Season of birth, neonatal vitamin D status, and cardiovascular disease risk at 35 y of age: a cohort study from Sweden." Am J Clin Nutr 99(3): 472–478. [PubMed: 24401716]

Verdoia M, Schaffer A, et al. (2015). "Impact of gender difference on vitamin D status and its relationship with the extent of coronary artery disease." Nutr Metab Cardiovasc Dis 25(5): 464–470. [PubMed: 25791862]

Vinkhuyzen AAE, Eyles DW, et al. (2018). "Gestational vitamin D deficiency and autism-related traits: the Generation R Study." Mol Psychiatry 23(2): 240–246. [PubMed: 27895322]

Vinkhuyzen AAE, Eyles DW, et al. (2017). "Gestational vitamin D deficiency and autism spectrum disorder." BJPsych Open 3(2): 85–90. [PubMed: 28446959]

Wierzejska R, Jarosz M, et al. (2018). "Maternal and Cord Blood Vitamin D Status and Anthropometric Measurements in Term Newborns at Birth." Front Endocrinol (Lausanne) 9: 9. [PubMed: 29472892]

Wilcock DM and Griffin WS (2013). "Down's syndrome, neuroinflammation, and Alzheimer neuropathogenesis." J Neuroinflammation 10: 84. [PubMed: 23866266]

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Table 1.

Demographic and clinical characteristics of children and their mothers in the CHARGE study

Variable	TD (*n* = 234)	ASD (*n* = 357)	DD (*n* = 134)	*P*-value
Child Sex, *n (%)*				< 0.001
Female	43 (18.4%)	47 (13.2%)	40 (29.9%)	
Male	191 (81.6%)	310 (86.8%)	94 (70.1%)	
Child Race-Ethnicity[1], *n (%)*				0.03
White Non-Hispanic	125 (53.4%)	191 (53.5%)	59 (44.7%)	
Hispanic	63 (26.9%)	104 (29.1%)	56 (42.4%)	
Other	46 (19.7%)	62 (17.4%)	17 (12.9%)	
Child Age at DBS Collection (hours)[2], *mean (SD)*	37.5 (30.0)	36.7 (23.2)	43.6 (30.0)	0.06
Child Age at Diagnosis (months), *mean (SD)*	42.3 (9.8)	43.6 (9.6)	45.9 (8.9)	0.004
Child Birth Year, *n (%)*				< 0.001
1998–2001	38 (16.2%)	142 (39.8%)	22 (16.4%)	
2002	57 (24.4%)	62 (17.4%)	31 (23.1%)	
2003	68 (29.1%)	51 (14.3%)	37 (27.6%)	
2004	55 (23.5%)	56 (15.7%)	31 (23.1%)	
2005–2006	16 (6.8%)	46 (12.9%)	13 (9.7%)	
Child Birth Season, *n (%)*				0.98
Spring (Mar, Apr, May)	58 (24.8%)	89 (24.9%)	30 (22.4%)	
Summer (Jun, Jul, Aug)	61 (26.1%)	87 (24.4%)	37 (27.6%)	
Fall (Sep, Oct, Nov)	56 (23.9%)	95 (26.6%)	34 (25.4%)	
Winter (Dec, Jan, Feb)	59 (25.2%)	86 (24.1%)	33 (24.6%)	
Gestation Age (Weeks)[3], *mean (SD)*	39.2 (1.8)	39.2 (2.2)	38.7 (3.0)	0.06
Maternal Age (Years), *mean (SD)*	30.7 (5.7)	30.5 (5.3)	29.8 (6.7)	0.36
Maternal Age Group, *n* (%)				0.02
<25	41 (17.5%)	51 (14.3%)	33 (24.6%)	
25 to <30	47 (20.1%)	96 (26.9%)	35 (26.1%)	
30 to 35	106 (45.3%)	148 (41.5%)	38 (28.4%)	
35+	40 (17.1%)	62 (17.4%)	28 (20.9%)	
Maternal Education[4], *n (%)*				< 0.001
Bachelor degree or more	127 (54.3%)	167 (46.9%)	41 (30.6%)	
Some college/Vocational degree	70 (29.9%)	138 (38.8%)	48 (35.8%)	
High school or less	37 (15.8%)	51 (14.3%)	45 (33.6%)	
Maternal Pre-Pregnancy BMI (kg/m^2)[5], *mean (SD)*	24.9 (4.4)	25.8 (5.8)	27.6 (6.5)	< 0.001
Maternal Pre-Pregnancy BMI[5], *n (%)*				< 0.001
Underweight	6 (2.6%)	13 (3.8%)	4 (3.2%)	
Normal	127 (55.2%)	179 (52.2%)	39 (31.5%)	
Overweight	68 (29.6%)	78 (22.7%)	45 (36.3%)	
Obese	29 (12.6%)	73 (21.3%)	36 (29.0%)	

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Variable	TD (*n* = 234)	ASD (*n* = 357)	DD (*n* = 134)	*P*-value
Periconceptional Prenatal Vitamin Use?[6], *n (%)*				0.008
Yes	139 (61.8%)	169 (51.5%)	55 (45.5%)	
No	86 (38.2%)	159 (48.5%)	66 (54.5%)	
Folic Acid Intake (μg)[7], *mean (SD)*	580 (423)	502 (418)	475 (489)	0.07
Paternal Age (Years)[8], *mean (SD)*	32.9 (6.7)	33.1 (6.4)	32.3 (7.8)	0.57
Home Ownership[9], *n (%)*				< 0.001
Own Home	182 (79.8%)	228 (69.5%)	75 (61.0%)	
Do Not Own Home	46 (20.2%)	100 (30.5%)	48 (39.0%)	

Abbreviations: TD, typical development; ASD, autism spectrum disorders; DD, development delay; DBS = dried blood spot; BMI = body mass index; SD = standard deviation.

P-values are from multinomial logistic regression models.

[1]Missing for 2 children in DD group;

[2]Missing for 19 children in TD group, 27 in ASD, and 9 in DD;

[3]Missing for 3 children in TD group and 4 in ASD;

[4]Missing for 1 child in ASD group;

[5]Missing for 4 children in TD, 14 in ASD, and 10 in DD;

[6]Any in first pregnancy month or 3 months before; missing for 9 children in TD, 29 in ASD, and 13 in DD.

[7]Continuous, summed from all vitamins/supplements taken in the first pregnancy month as previously described (Schmidt et al. 2012); missing for 37 children in TD group, 71 in ASD, and 22 in DD;

[8]Missing for 4 children in ASD group and 3 in DD;

[9]Missing for 6 children in TD group, 29 in ASD and 11 in DD.

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Table 2.

Associations of demographic and clinical characteristics of typically developing children (n = 234) and their mothers with categorical and continuous dried blood spot 25-hydroxyvitamin D

	Categorical				*Continuous*	
Variable	**Deficient (<50 nmol/L) (n = 49)**	**Insufficient (50-<75 nmol/L) (n = 62)**	**Sufficient (≥75 nmol/L) (n = 123)**	***P*-value**	**Estimate (*SE*)**	***P*-value**
Child Sex, *n (%)*				0.35		
Female	7 (14.3%)	11 (17.7%)	25 (20.3%)		reference	–
Male	42 (85.7%)	51 (82.3%)	98 (79.7%)		−10.79 (6.34)	0.09
Child Race-Ethnicity, *n (%)*				< 0.001		
White Non-Hispanic	18 (36.7%)	26 (41.9%)	81 (65.9%)		reference	–
Hispanic	14 (28.6%)	25 (40.3%)	24 (19.5%)		−18.21 (5.60)	0.011
Other	17 (34.7%)	11 (17.7%)	18 (14.6%)		−21.88 (6.25)	< 0.001
Child Age at DBS Collection (hours)[1], *mean (SD)*	35.1 (23.3)	35.3 (19.9)	39.5 (36.0)	0.49	0.14 (0.08)	0.11
Child Age at Diagnosis (months), *mean (SD)*	43.5 (9.9)	42.0 (8.8)	42.0 (10.4)	0.43	−0.27 (0.25)	0.28
Child Birth Year, *n (%)*				0.42		
1998–2001	4 (8.2%)	11 (17.7%)	23 (18.7%)		7.80 (7.65)	0.31
2002	11 (22.4%)	17 (27.4%)	29 (23.6%)		1.85 (6.78)	0.79
2003	23 (46.9%)	12 (19.4%)	33 (26.8%)		reference	–
2004	6 (12.2%)	19 (30.6%)	30 (24.4%)		8.83 (6.85)	0.20
2005–2006	5 (10.2%)	3 (4.8%)	8 (6.5%)		−2.51 (10.49)	0.81
Child Birth Season, *n (%)*				0.56		
Spring (Mar, Apr, May)	17 (34.7%)	18 (29.0%)	23 (18.7%)		−12.85 (6.88)	0.06
Summer (Jun, Jul, Aug)	7 (14.3%)	11 (17.7%)	43 (35.0%)		13.65 (6.79)	0.04
Fall (Sep, Oct, Nov)	10 (20.4%)	13 (21.0%)	33 (26.8%)		reference	–
Winter (Dec, Jan, Feb)	15 (30.6%)	20 (32.3%)	24 (19.5%)		−10.42 (6.85)	0.13
Gestation Age (weeks)[2], *mean (SD)*	39.6 (1.3)	39.7 (1.3)	38.8 (2.1)	0.04	−3.85 (1.33)	0.004
Maternal age (years), *mean (SD)*	31.0 (5.8)	29.4 (5.2)	31.1 (5.8)	0.50	0.49 (0.43)	0.26
Maternal age group, *n (%)*				0.34		
<25	10 (20.4%)	11 (17.7%)	20 (16.3%)		−8.00 (6.92)	0.25
25 to <30	6 (12.2%)	20 (32.3%)	21 (17.1%)		−1.93 (6.59)	0.77

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	Categorical				*Continuous*	
Variable	**Deficient (<50 nmol/L) (*n* = 49)**	**Insufficient (50-<75 nmol/L) (*n* = 62)**	**Sufficient (≥75 nmol/L) (*n* = 123)**	***P*-value**	**Estimate (*SE*)**	***P*-value**
30 to 35	25 (51.0%)	25 (40.3%)	56 (45.5%)		reference	–
35+	8 (16.3%)	6 (9.7%)	26 (21.1%)		4.26 (6.98)	0.54
Maternal Education, *n (%)*				0.17		
Bachelor degree or more	27 (55.1%)	30 (48.4%)	70 (56.9%)		reference	–
Some College/Vocational Degree	12 (24.5%)	17 (27.4%)	41 (33.3%)		0.24 (5.55)	0.97
High school or less	10 (20.4%)	15 (24.2%)	12 (9.8%)		−16.09 (6.96)	0.02
Maternal Pre-Pregnancy BMI (kg/m^2)[3], *mean (SD)*	25.8 (5.0)	25.8 (5.1)	24.1 (3.7)	0.03	−1.24 (0.55)	0.02
Maternal Pre-Pregnancy BMI[3], *n (%)*				0.001		
Underweight	1 (2.1%)	1 (1.6%)	4 (3.3%)		reference	–
Normal	21 (44.7%)	31 (50.8%)	75 (61.5%)		−15.05 (15.46)	0.33
Overweight	16 (34.0%)	19 (31.1%)	33 (27.0%)		−21.42 (15.76)	0.17
Obese	9 (19.1%)	10 (16.4%)	10 (8.2%)		−30.02 (16.59)	0.07
Periconceptional Prenatal Vitamin Use?[4], *n (%)*						
Yes	29 (64.4%)	29 (48.3%)	81 (67.5%)	0.33	6.67 (5.09)	0.19
No	16 (35.6%)	31 (51.7%)	39 (32.5%)		reference	–
Folic Acid Intake (µg)[5], *mean (SD)*	521 (406)	524 (485)	629 (392)	0.06	0.01 (0.006)	0.06
Paternal age (years), *mean (SD)*	34.0 (6.6)	31.2 (6.2)	33.2 (6.9)	0.93	0.24 (0.37)	0.52
Home Ownership[6], *n (%)*						
Own Home	33 (73.3%)	50 (80.6%)	99 (81.8%)	0.26	12.17 (6.06)	0.04
Do Not Own Home	12 (26.7%)	12 (19.4%)	22 (18.2%)		reference	–

Abbreviations: DBS, Dried blood spot; BMI, body mass index; SD, standard deviation; SE, standard error. Associations with categorical dried blood spot 25-hydroxyvitamin D were assessed using Mantel-Haenszel Chi-Square test for categorical variables and Spearman correlation for continuous variables. Associations with continuous dried blood spot 25-hydroxyvitamin D were assessed using robust regression.

[1]Missing for 3 children in the Deficient group, 6 in Insufficient, 10 in Sufficient.

[2]Missing for 2 children in the Deficient group and 1 in Sufficient.

[3]Missing for 2 children in the Deficient group, 1 in Insufficient, and 1 in Sufficient.

[4]In first pregnancy month or 3 months before; missing for 4 children in the Deficient group, 2 in Insufficient, and 3 in Sufficient.

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[5] In first pregnancy month; missing for 12 children in the Deficient group, 8 in Insufficient, and 17 in Sufficient.

[6] Missing for 4 children in the Deficient group, 2 in Sufficient.

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Table 3.

Adjusted and unadjusted associations of diagnosis with neonatal dried blood spot total 25-hydroxyvitamin D

				ASD vs. TD		DD vs. TD	
Variable	**TD (*n* = 234)**	**ASD (*n* = 357)**	**DD (*n* = 134)**	**OR (95% CI)**	***P*-Value**	**OR (95% CI)**	***P*-Value**
Dried blood spot Vitamin D (nmol/L)							
		Mean (SD)					
Crude model	82.7 (39.3)	80.1 (37.4)	73.2 (37.6)	0.96 (0.86–1.06)	0.41	0.84 (0.73–0.98)	0.02
Adjusted model[1]				0.97 (0.87–1.08)	0.58	0.90 (0.78–1.06)	0.22
Dried blood spot Vitamin D category							
		Frequency (%)					
Crude model							
Deficient (< 50 nmol/L)	49 (20.9%)	77 (21.6%)	39 (29.1%)	1.05 (0.68–1.60)	0.84	1.78 (1.05–3.02)	0.03
Insufficient (50 - <75 nmol/L)	62 (26.5%)	95 (26.6%)	40 (29.9%)	1.02 (0.69–1.51)	0.93	1.44 (0.87–2.40)	0.16
Sufficient (≥ 75 nmol/L)	123 (52.6%)	185 (51.8%)	55 (41.0%)	reference	–	reference	–
Adjusted model[1]							
Deficient (<50 nmol/L)				0.98 (0.63–1.51)	0.91	1.36 (0.78–2.36)	0.27
Insufficient (50 - <75 nmol/L)				0.98 (0.66–1.46)	0.94	1.25 (0.74–2.11)	0.41
Sufficient (≥ 75 nmol/L)				reference	–	reference	–

Note: OR = odds ratio; OR are reported for 25 nmol/L increase in total 25-hydroxyvitamin D.

[1]Adjusted for maternal education and pre-pregnancy body mass index; adjusted models were performed using multiple imputations ($n = 10$), to account for the fact that some covariates were missing.

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Table 4.

Adjusted and unadjusted associations of children's assessment scores with neonatal dried blood spot total 25-hydroxyvitamin D

	Mullen Early Learning Composite DQ[1]		Vineland Composite Standard Score	
	Estimate (*SE*)[2]	*P*-value	Estimate (*SE*)[2]	*P*-value
Crude model	2.01 (0.76)	0.008	0.96 (0.56)	0.09
Adjusted model 1[3]	1.37 (0.76)	0.07	0.68 (0.60)	0.26
Adjusted model 2[3,4]	0.72 (0.49)	0.14	0.06 (0.32)	0.84

Abbreviations: DQ, Developmental Quotient; SE, standard error.

[1] Missing = 1;

[2] Reported for 25 nmol/L increase in dried blood spot 25(OH)D;

[3] Adjusted for maternal education and pre-pregnancy body mass index;

[4] The final models are missing data on some covariates for 59 missing for the Mullen model and for 29 for the Vineland model;

[5] Adjusted for child's diagnostic group in addition to maternal education and pre-pregnancy body mass index.

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Table 5.

Stratified adjusted[1] and unadjusted associations of diagnosis with neonatal total 25-hydroxyvitamin D

	Vitamin D, *Mean* (*SD*)			ASD vs. TD		DD vs. TD	
Variable	**TD (*n* = 234)**	**ASD (*n* = 357)**	**DD (*n* = 134)**	**OR (95% CI)**	**Interaction *P*-Value**	**OR (95% CI)**	**Interaction *P*-Value**
Child Gender							
Male	191	310	94				
Female	43	47	40				
Male (crude)	80.4 (37.6)	81.1 (38.1)	72.8 (36.8)	1.01 (0.90–1.14)		0.86 (0.72–1.03)#	
Female (crude)	92.7 (45.1)	73.0 (31.8)	74.3 (40.0)	0.73 (0.55–0.96)*	**0.04**	0.75 (0.56–0.99)*	0.52
Male (adjusted)				1.03 (0.91–1.17)		0.93 (0.78–1.12)	
Female (adjusted)				0.74 (0.55–0.99)*	**0.03**	0.79 (0.59–1.08)	0.39
Child Race/Ethnicity[2]							
White Non-Hispanic	125	191	59				
Hispanic	63	104	56				
Other	46	62	17				
White Non-Hispanic (crude)	93.0 (42.8)	86.8 (35.1)	78.5 (35.2)	0.90 (0.78–1.04)		0.77 (0.62–0.95)*	
Hispanic (crude)	72.6 (30.4)	70.7 (33.9)	71.5 (32.8)	0.96 (0.75–1.21)	0.66	0.95 (0.72–1.25)	0.22
Other (crude)	68.6 (32.2)	74.9 (45.5)	64.4 (56.9)	1.09 (0.87–1.37)	0.16	0.93 (0.65–1.34)	0.37
White Non-Hispanic (adjusted)				0.91 (0.79–1.06)		0.79 (0.63–0.98)*	
Hispanic (adjusted)				0.98 (0.77–1.26)	0.64	1.14 (0.85–1.54)	0.11
Other (adjusted)				1.05 (0.83–1.34)	0.17	0.96 (0.66–1.38)	0.31

Note: OR = odds ratio; OR are reported for 25 nmol/L increase in 25(OH)D.

*$p < .05$;

#$p < .10$.

[1]Adjusted for maternal education and pre-pregnancy body mass index; adjusted models were performed using multiple imputations ($n = 10$), to account for the fact that some covariates were missing;

[2]Missing for 2 DD children.

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Table 6.

Stratified adjusted and unadjusted associations of child assessment scores and neonatal dried blood spot total 25-hydroxyvitamin D

		Mullen Early Learning Composite DQ		Vineland Composite Standard Score	
Variable	***n***	**Estimate[1] (*SE*)**	**Interaction *P*-Value**	**Estimate[1] (*SE*)**	**Interaction *P*-Value**
Child Gender					
Male (crude)	595	1.33 (0.78)#		0.41 (0.61)	
Female (crude)	130	3.53 (1.69)*	0.37	3.11 (1.42)*	**0.0498**
Male (adjusted[2])	595	1.59 (0.84)		0.15 (0.65)	
Female (adjusted[2])	130	3.68 (1.79)*	0.26	3.00 (1.61)#	**0.03**
Child White Non-Hispanic					
White Non-Hispanic (crude)	375	1.92 (1.05)#		1.72 (0.83)*	
Hispanic (crude)	223	1.82 (1.54)		−0.01 (1.19)	0.25
Other (crude)[3]	124	0.79 (1.76)	0.91	−0.31 (1.42)	0.20
White Non-Hispanic (adjusted)	375	1.59 (1.05)	0.56	1.54 (0.84)#	
Hispanic (adjusted)	223	0.76 (1.56)	0.68	−0.54 (1.20)*	0.22
Other (adjusted)[3]	124	0.74 (1.80)	0.55	−0.24 (1.45)	0.19
Child Diagnosis[2]					
ASD (crude)[3]	356	1.65 (0.82)*	0.051	0.002 (0.40)	0.86
DD (crude)	234	1.25 (1.12)	0.39	0.58 (0.67)	0.67
TD (crude)	134	0.04 (0.63)		−0.07 (0.65)	
ASD (adjusted[2])[3]	356	1.55 (0.83)#	0.12	0.12 (0.39)	0.64
DD (adjusted[2])	234	1.53 (1.15)	0.45	0.78 (0.70)	0.41
TD (adjusted[2])	134	−0.41 (0.62)		−0.17 (0.70)	

SE = standard error;

*$p < .05$;

#$p < .10$.

[1]Reported for 25 nmol/L increase in neonatal dried blood spot 25(OH)D;

[2]Adjusted for maternal education and pre-pregnancy body mass index

[3]Missing 1 observation

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Table 7.

Unadjusted and adjusted associations between ASD children with and without intellectual disability (ID) and neonatal dried blood spot total 25-hydroxyvitamin D [25(OH)D] compared to TD children.

	TD (*n* = 234)	ASD with ID (*n* = 243)	ASD without ID (*n* = 113)	ASD with ID vs. TD OR (95% CI)	ASD without ID vs. TD OR (95% CI)
Crude model	82.7 (39.3)	77.5 (36.2)	85.9 (39.3)	0.91 (0.81–1.03)	1.05 (0.91–1.21)
Adjusted Model[1]				0.93 (0.82–1.04)	1.06 (0.92–1.23)

Note: OR = odds ratio; CI = confidence interval; OR are reported for 25 nmol/L increase in 25(OH)D.

[*] $p < .05$. This analysis is restricted to the ASD and TD groups.

[1] Adjusted for maternal education and pre-pregnancy body mass index; in the adjusted model, data on some covariates are missing on 27 children.

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Table 8.

Unadjusted and adjusted associations between genetic syndrome and dried blood spot total 25-hydroxyvitamin D [25(OH)D] in syndromic subgroups of children with DD compared to TD children.

	TD (*n* = 234)	Down Syndrome (*n* = 29)	Other Syndrome (*n* = 18)	No syndrome (*n* = 86)	Down Syndrome vs. TD OR (95% CI)	Other Syndrome vs. TD OR (95% CI)	No syndrome vs. TD OR (95% CI)
Dried blood spot 25(OH)D (nmol/L)							
			Mean (*SD*)				
Crude model	82.7 (39.3)	67.0 (41.9)	76.0 (25.3)	75.1 (38.5)	0.74 (0.56–0.99) *	0.89 (0.65–1.23)	0.88 (0.74–1.04)
Adjusted Model [1]					0.79 (0.58–1.06)	0.99 (0.71–1.38)	0.97 (0.81–1.15)

Note: OR = odds ratio; CI = confidence interval; OR are reported for 25 units increase in 25(OH)D.

* $p < .05$. This analysis is restricted to the DD and TD groups.

[1] Adjusted for maternal education, pre-pregnancy body mass index; in the adjusted model, data on some covariates are missing on 27 children.