HHS Public Access

Author manuscript

Autism Res. Author manuscript; available in PMC 2020 June 01.

Published in final edited form as:

Autism Res. 2019 June ; 12(6): 989–998. doi:10.1002/aur.2092.

Newborn Vitamin D Levels in Relation to Autism Spectrum Disorders and Intellectual Disability: a Case-Control Study in California

Gayle C. Windham[1], **Michelle Pearl**[1], **Meredith C. Anderson**[2], **Victor Poon**[2], **Darryl Eyles**[3], **Karen L. Jones**[4], **Kristen Lyall**[5], **Martin Kharrazi**[1], and **Lisa A. Croen**[6]

[1]California Department of Public Health, Environmental Health Investigations Branch, Richmond, CA

[2]Sequoia Foundation, La Jolla, CA

[3]Queensland Brain Institute, University of Queensland, Brisbane, Australia

[4]Division of Rheumatology, Allergy and Clinical Immunology, University of California, Davis Davis, CA

[5]A.J. Drexel Autism Institute, Drexel University, Philadelphia, PA

[6]Kaiser Permanente Division of Research, Oakland, CA

Abstract

Vitamin D deficiency has been increasing concurrently with prevalence of autism spectrum disorders (ASD), and emerging evidence suggests vitamin D is involved in brain development. Most prior studies of ASD examined vitamin D levels in children already diagnosed, but a few examined levels during perinatal development, the more likely susceptibility period. Therefore, we examined newborn vitamin D levels in a case-control study conducted among births in 2000–2003 in southern California. Children with ASD (N=563) or Intellectual Disability (ID) (N=190) were identified from the Department of Developmental Services and compared to population controls (N=436) identified from birth certificates. 25-hydroxyvitamin D (25(OH)D) was measured in archived newborn dried blood spots by a sensitive assay, and corrected to sera equivalents. We categorized 25(OH)D levels as deficient (<50nmol/L), insufficient (50–74 nmol/L), and sufficient (≥75 nmol/L), and also examined continuous levels, using logistic regression. The adjusted odds ratios (AOR) and 95% confidence intervals (CI) for ASD were 0.96 (0.64–1.4) for 25(OH)D deficiency (14% of newborns) and 1.2 (0.86–1.6) for insufficiency (26% of newborns). The AORs for continuous 25(OH)D (per 25nmol/L) were 1.0 (0.91–1.09) for ASD and 1.14 (1.0–1.30) for ID. Thus, in this relatively large study of measured newborn vitamin D levels, our results do not support the hypothesis of lower 25(OH)D being associated with higher risk of ASD (or ID), although we observed suggestion of interactions with sex and race/ethnicity. 25(OH)D levels were relatively high (median 84 nmol/L in controls) so results may differ in populations with higher prevalence of low vitamin D levels.

Corresponding author is Gayle C. Windham, Environmental Health Investigations Branch/CDPH, 850 Marina Bay Parkway, Richmond, CA 94804, gayle.windham@cdph.ca.gov.

Abstract

Lay Summary:

We studied whether vitamin D levels measured at birth were related to whether a child later developed autism (or low IQ). Our results did not show that children with autism, or low IQ, overall had lower vitamin D levels at birth than children without autism. Vitamin D levels were fairly high, on average, in these children born in Southern California.

Keywords

autism; ASD; vitamin D; intellectual disability; hydroxy-vitamin D

Introduction

Autism spectrum disorders (ASD) are serious developmental disabilities with lifelong consequences. Despite a steady increase in prevalence to rates of 1–2% of school-aged children in the U.S., the etiology is complex and not well-elucidated (Christensen et al. 2016; Lyall et al. 2017a). Recent research indicates environmental factors play a role in ASD, potentially interacting with genetic susceptibilities (Grandjean and Landrigan 2014; Lyall et al. 2017a). Further, maternal immune system aberrations have increasingly been associated with ASD, including chronic inflammation, elevated pro-inflammatory cytokine profiles during mid-gestation, the presence of fetal brain-reactive maternal autoantibodies, and changes in immune cell function in affected children and mothers (Gesundheit et al. 2013; Lyall et al. 2014; Jones et al. 2017; Meltzer and Van de Water 2017).

Vitamin D deficiency or insufficiency has re-emerged as a serious public health concern in the last 20 years because of documentation of more widespread occurrence (Centers for Disease Control and Prevention 2012). Various definitions of deficiency are used, so prevalence is difficult to compare. U.S. data (NHANES 2003–2006) showed that 11% of women of childbearing age were deficient in vitamin D, while another 26% were insufficient (Zhao et al. 2012), using a conservative definition (<12 and 12-<20 ng/ml, respectively, which combined are defined as deficient in other studies, equivalent to 50 nmol/L). Higher rates of deficiency have been reported in other studies including of pregnant women (Hamilton et al. 2010; Marshall et al. 2016; Kiely et al. 2017). “Vitamin D” refers to a group of steroid molecules, with formation of vitamin D_3, the major circulating form, in the body dependent on sunlight (UVB) exposure. Thus lower levels are typically found in individuals that have darker-pigmented skin, who live at higher latitudes, avoid sun exposure (via use of sunscreen, clothing, or indoor activities), or when measured in the winter (Holick 2007; Humble et al. 2010). Levels may also vary by age, parity and obesity (Bodnar et al. 2007; Dror et al. 2011). Other sources of vitamin D include supplements and a few foods, such as fatty fish, mushrooms and eggs.

There is increasing evidence of associations of vitamin D deficiency with a wide range of maternal and child health conditions in addition to the well-known effect on calcium metabolism and bone mineralization (Holick 2007; Dror et al. 2011; Basit 2013; Marshall et al. 2016). Vitamin D has been shown to play a role in brain development, leading to the

suggestion of a possible association with ASD a decade ago (Cannell 2008). Further, vitamin D plays a well-documented role in mediating immune function (Hayes et al. 2003; Cantorna et al. 2004; Hewison 2012). Maternal vitamin D levels during pregnancy are of particular concern because fetal and neonatal vitamin D status depends on them, with neonatal levels typically measured in cord blood at 60–80% of maternal (Hollis et al. 2011; Marshall et al. 2016; Kiely et al. 2017). Yet the optimal level of vitamin D to prevent adverse pregnancy outcomes is not clear and separate standards of intake for pregnant women are not established, although there is movement towards supplementation of pregnant women (ACOG Committee on Obstetric Practice 2011; Hollis et al. 2011; Urrutia and Thorp 2012).

Several studies examining the association between vitamin D and ASD were published following Cannell's hypothesis, however, many examined surrogates of vitamin D status, such as latitude or season, or measured vitamin D levels after ASD diagnosis, so are likely to reflect current lifestyle (Fernell et al. 2010; Humble et al. 2010; Meguid et al. 2010; Molloy et al. 2010; Mostafa and Al-Ayadhi 2012; Du et al. 2015). Only recently have vitamin D levels (typically as total 25-hydroxyvitamin D, e.g. 25(OH)D) been measured pre- or peri-natally, a critical period for brain development; two small studies found lower 25(OH)D levels among ASD cases compared with controls, but did not adjust for many other factors (Fernell et al. 2015; Chen et al. 2016), as did a larger study from China (Wu et al. 2018) that also reported increased ASD risk with neonatal vitamin D deficiency in a thoroughly matched analysis. Other studies with measured perinatal 25(OH)D levels have examined related neuro-behavioral metrics, with several finding adverse outcomes associated with lower vitamin D levels (Morales et al. 2012; Whitehouse et al. 2012; Hanieh et al. 2014; Darling et al. 2017; Vinkhuyzen et al. 2018). However, not all studies, including the one U.S. study (Keim et al. 2014), observed associations of prenatal (maternal serum or cord blood) 25(OH)D levels with IQ or other neurodevelopmental outcomes, especially when assessments were done at older ages (Gale et al. 2008; Strom et al. 2014).

Therefore, our objective was to determine the association of newborn vitamin D levels and ASD in an existing population-based study in the U.S. To evaluate the specificity of any such associations, we also examined the association with intellectual disability (ID), and studied interactions by sex and race/ethnicity.

Methods

Study population

We used data from the Early Markers for Autism (EMA) study, details of which have been previously described (Croen et al. 2008). EMA is a population-based case control study designed to identify biologic markers of autism using archived prenatal and newborn blood samples from California state-wide genetic screening programs. The EMA study sample was drawn from the population of children born in three counties from Southern California (Orange, San Diego, and Imperial) in 2000–2003. Only mother-child pairs with live birth records linked to banked prenatal and newborn specimens were eligible for inclusion in EMA. Study activities were approved by the Institutional Review Board at Kaiser Permanente and the State Committee for the Protection of Human Subjects.

Case Ascertainment

Diagnostic information to identify cases in the defined study population was obtained from the California Department of Developmental Services (DDS), which provides services to individuals with substantial disabilities – including ASD and other developmental disabilities (http://www.dds.ca.gov). DDS data were linked to eligible live birth records for the study region and birth years to identify, based on DDS service codes: children with ASD or children with intellectual disability (ID) without autism (originally: "mental retardation of unknown etiology", thus excluding Down Syndrome for example). General population (GP) controls were identified by randomly sampling from eligible birth certificates, frequency matched to original ASD cases by sex, birth month and birth year. Children who died in the first year of life or became clients of DDS were excluded from the eligible control population. At the time of data linkage in 2007 and 2009, children would have been between 4.5 and 9 years old; the majority of ASD cases were between 4.5 and 7 years old when identified.

Final case status was determined by expert clinician review of diagnostic and clinical data abstracted from DDS records, using an approach based on national surveillance methodology (Yeargin-Allsopp et al. 2003; Christensen et al. 2016). A final diagnostic classification of ASD was made if DSM-IV-TR criteria were considered met. In our sample with vitamin D measurements, 427 children originally identified as ASD in DDS met these diagnostic criteria. Additionally, a total of 136 individuals originally identified as ID were reclassified as ASD following expert review. Final classification of ID (intellectual disability without ASD) was based on standardized test score results found in records (composite developmental/cognitive score <70) in the absence of meeting ASD criteria, yielding 190 children in this sample. ASD cases were further qualified by presence/absence of co-occurring ID. Case groups were compared to 436 GP controls.

Vitamin D measurements

After newborn screening assays were completed at the regional laboratory, residual newborn dried blood spot cards were routinely freezer archived, as has been done statewide since 1982. For this research, newborn blood spots were requested from the freezer archive in 2015, with 1,190 specimens located, retrieved and shipped frozen to the laboratory at the University of Queensland, Brisbane. An individual sample identification number was created such that the lab did not have access to confidential data or case status. One 3.2mm punch per individual was used for the vitamin D assay. On post hoc examination, there was good representation of cases and controls on each assay plate.

"Vitamin D" is a general term that actually describes a family of steroid compounds that are related structurally to cholesterol. The major circulating form, 25-hydroxyvitamin D (25(OH)D), also known as 25-hydroxycholecalciferol, is a reliable indicator of cumulative production and is therefore used clinically to measure vitamin D sufficiency. Total 25-Hydroxyvitamin D (sum of 25(OH)D2 and 25(OH)D3, hereinafter referred to as 25(OH)D) was measured by a sensitive isotope dilution liquid chromatography-tandem mass spectrometry method (LC/MS/MS) for dried blood spots as previously described (Eyles et al. 2009; Kvaskoff et al. 2016). One sample was mis-labelled so could not be linked; of the

remaining 1189, 25(OH)D3 was detectable in all newborn blood spots, of which 82 samples also had detectable 25(OH)D2, which is obtained from dietary sources or supplements. Method limit of reporting was 1 and 10 nmol/L for 25(OH)D3 and 25(OH)D2, respectively. 25(OH)D is completely excluded from erythrocytes and therefore requires a hematocrit correction to obtain sera equivalent values, e.g. “25(OH)D x (1/(1-Hct))”, where Hct=0.61, as a standard neonatal capillary hematocrit used by the lab. While there may be some small variation in individual haematocrit values, corrected dried blood spot 25(OH)D levels have been shown to be comparable to serum levels (Heath et al. 2014).

Covariate Data

Potential covariates were obtained from birth certificates, the CA Genetic Disease Screening Program, and DDS, including demographics, pregnancy-related characteristics, and newborn status. Potential covariates were selected on the basis of a priori associations with ASD and/or vitamin D. Birth characteristics included the original matching variables; child sex, birth year, and month categorized into seasons of Winter (Dec.-Feb.), Spring (March-May), Summer (June-August), and Fall (Sept.-Nov) as 25(OH)D levels vary by season. Maternal characteristics, all categorized, included race/ethnicity (non-Hispanic white; Hispanic white; and Black, Asian or other/unknown), age, education, parity, and preterm delivery (<37 weeks). We also examined hours from birth to heel stick as longer times may less likely reflect gestational 25(OH)D levels.

Statistical Analysis

These analyses included births with final case status and 25(OH)D measurements available. We categorized vitamin D levels as deficient (<50nmol/L), insufficient (50–74 nmol/L) and sufficient (≥75 nmol/L), based on IOM recommendations (Institute of Medicine 2011), and also examined continuous levels (reported per 25 nmol/L, as the distribution appeared normal). Covariates were compared between the the ASD, ID, and control groups by chi-square tests. Geometric mean concentrations of total 25(OH)D and their 95% confidence intervals (CI) were calculated and compared by case group and the potential confounders. The categorical distribution of total 25(OH)D levels was also examined by covariates among controls, and assessed by chi-square test.

Controls were originally matched to ASD cases, as noted, but a matched pair analysis was not conducted in order to include the ID reclassified to ASD cases. Logistic regression was used to calculate crude and multivariable adjusted odds ratios (AOR) and 95% confidence intervals (CI) for the association between 25(OH)D and ASD in comparison to GP controls. The original matching variables and those associated with ASD and/or 25(OH)D were included in models singly and together to determine the change in the effect estimates. None altered associations greatly but we retained most in fully adjusted models, including; child sex, birth year, and birth season, maternal age (five categories), education (four categories), race/ethnicity (three categories as above), parity (0, 1, ≥2), and hours until blood draw (continuous). Models with a reduced set of covariates produced similar results for all the primary analyses.

We examined whether odds of ASD differed according to presence of co-occurring intellectual disability by modeling ASD with and without ID separately. In parallel analyses, we examined odds of ID (without ASD) relative to GP controls. We explored interactions of 25(OH)D with child sex and with maternal race/ethnicity. Results for interaction analyses are presented for continuous 25(OH)D to maintain adequate sample size, with dichotomous terms (e.g. male vs. female, and non-Hispanic White vs. all other groups), and p-values <0.10 considered supportive of interactions.

We also conducted sensitivity analyses by limiting the sample to term singleton births (excludes 96 ASD, 50 ID, and 62 controls), examining a stricter definition of vitamin D deficiency (<37.5nmol/L), examining 25(OH)D3 only (25(OH)D2 is usually obtained from supplements), and examining non-linearity of continuous 25(OH)D using generalized additive models. As there was not evidence for a non-linear pattern of association with total 25(OH)D and the other sensitivity analysis results did not vary meaningfully from the primary analyses, those data are not shown.

Results

Table 1 provides characteristics of the study sample by final case status. Compared to controls, ASD cases were more likely to have mothers who were older, more highly educated, and nulliparous. Due to frequency matching, they were quite similar by child sex, birth year and season, and further, did not vary by maternal race/ethnicity (although cases were somewhat less likely to be Hispanic), preterm birth, or time to heel stick collection. Children with ID were more likely than controls to be female and to be born in the winter (as they were not matched), and to have mothers who were younger, less educated, of Hispanic ethnicity, and delivered preterm.

Among controls (Supplemental Table S1), newborns with mothers of Black, Asian or other race/ethnicity were most likely to be 25(OH)D-deficient (27%, compared to 7.5% among non-Hispanic White mothers), as were children born in winter months (28% deficient). Time to heel stick was weakly associated with 25(OH)D level, but not in a monotonic pattern. Other variables were not strongly associated with 25(OH)D levels, although females were slightly more likely to be deficient than males (21% vs. 14%, respectively, p=0.16). Total 25(OH)D levels did not vary by case status (Table 2), either categorized (14% of ASD were considered deficient compared to 12% of ID and 15% of controls), or by geometric mean levels (81.5, 84.6 and 80.1 nmol/L, respectively).

Adjusting for covariates did not change the pattern of association for ASD; odds were generally around the null for deficiency (AOR= 0.96, 95%CI 0.64–1.43) or insufficiency (AOR= 1.19, 95%CI 0.86–1.64), or for 25(OH)D levels on a continuous scale (AOR= 1.0, 95%CI 0.91–1.09, per 25 nmol/L increase) (Table 3). By presence of co-occurring ID, no real differences were seen for continuous 25(OH)D (Table 3) and although the direction of association varied for 25(OH)D-deficiency, the confidence intervals were wide.

In contrast to ASD, odds for ID tended to be lower with vitamin D deficiency, especially after adjustment for covariates (AOR=0.58, 95%CI 0.31–1.1), and odds for ID increased

with increasing levels of 25(OH)D on the continuous scale (AOR=1.14, 95%CI 1.002–1.3), per 25 nmol/L increase) (Table 3).

Examining interactions, results varied by child sex (p-value for interaction < 0.05 in fully adjusted model). Among females, odds of ASD increased as 25(OH)D levels increased (per 25 nmol/L; AOR 1.18, 95%CI 0.97–1.43), but males had slightly lower odds (AOR=0.95, 95%CI 0.86–1.05) (Table 4a). This was also reflected as girls who were vitamin D deficient at birth having statistically significantly lower odds of developing ASD than girls who had sufficient levels (AOR=0.33, 95% CI=0.12–0.91). For ID, there were also sex-specific differences in the same direction as for ASD (p-value for interaction = 0.08), e.g. with increased risk among females as 25(OH)D levels increased (AOR=1.31, 95%CI 1.07– 1.62, per 25 nmol/L), and no association among males (AOR=1.05, 95%CI 0.88 – 1.24, per 25 nmol/L).

There were suggestive differences by maternal race/ethnicity. For children whose mothers were non-Hispanic White, odds of ASD were slightly attenuated as 25(OH)D level increased (AOR=0.92, 95%CI 0.81–1.05, per 25 nmol/L), but odds were slightly increased among children of all other race/ethnic groups combined (AOR=1.07, 95%CI 0.95–1.22, per 25 nmol/L) (p-value for interaction=0.096) (Table 4b**)**. The overall increase in odds of ID (without ASD) with increasing 25(OH)D was apparent in the other race/ethnic group (AOR=1.25, 95%CI 1.05–1.48, per 25 nmol/L), but not in the non-Hispanic White group (AOR=1.03, 95%CI 0.84–1.27, per 25 nmol/L) (p-interaction=0.17, based on smaller sample sizes of ID than ASD).

Discussion

This study adds to the sparse literature on vitamin D levels measured during the perinatal period and risk of ASD or ID. Our results did not confirm the hypothesized protective effect of vitamin D overall, but suggested such an effect among non-Hispanic whites, who typically (and in this study) have higher mean levels of vitamin D than non-white race/ethnic groups. However, the range of vitamin D levels was similar in these two groups in our study. Surprisingly, our results showed an increasing risk of ID as vitamin D levels increased, despite adjusting for several variables. This may partly reflect the interaction with sex that we observed for both ASD and ID that has not previously been examined or reported. Among the ID group, there was a larger proportion of girls than in the group with ASD and their matched controls.

Comparing to prior literature, initial evidence of a link between vitamin D and ASD was somewhat circumstantial, based on reports indicating higher prevalence of ASD in dark-skinned immigrants to northern climates and with births or conceptions in winter or at high latitudes (Cannell 2008; Kocovska et al. 2012). Subsequent studies, some limited by small sample size, examined serum 25(OH)D or levels in children with ASD, or in their mothers, with several finding lower levels (Fernell et al. 2010; Meguid et al. 2010; Mostafa and Al-Ayadhi 2012; Kocovska et al. 2014; Du et al. 2015), but not all (Molloy et al. 2010; Hashemzadeh et al. 2015), compared to controls. However, all these measured 25(OH)D levels after ASD diagnosis, which may reflect differences in lifestyle (and thus sun

exposure) between children with and without ASD. Further, the prenatal period, or very early life, is considered to be the most likely period of susceptibility for development of ASD (Rodier 2000; Lyall et al. 2017a).

Relatively recently a few studies with measured neonatal, or prenatal (maternal), vitamin D status in relation to ASD were published, generally reporting an inverse association, including two small studies (<75 cases) based on newborn blood spots (Fernell et al. 2015) or first trimester maternal serum (Chen et al. 2016), that did not adjust for many other factors. A cohort study in the Netherlands with a similar small number of cases (n=68), but adjustment for multiple confounders, showed a twofold increased risk of ASD with maternal 25(OH)D deficiency (defined as <25nmol/L) mid-pregnancy (Vinkhuyzen et al. 2017). Interestingly there was no association with 25(OH)D deficiency in cord blood, despite a higher proportion of deficiency, or examining 25(OH)D continuously. A larger study from China (Wu et al. 2018) also reported increased risk of ASD with 25(OH)D3 deficiency, with some suggestion of a non-linear pattern with continuous levels. Median (or mean) 25(OH)D levels were lower in these studies compared to ours, perhaps contributing to differences in findings, e.g. 32–60 nmol/L (in control samples where specified) versus 84 nmol/L in our study.

Two studies examined ASD-like traits; an additional analysis from the Generation R study in the Netherlands (Vinkhuyzen et al. 2018), based on a subset of items from the Social Responsiveness Scale (SRS), showed higher scores (indicative of greater degree of autistic traits) among 6 year-old children with 25(OH)D deficiency (<25nmol/L) in mid-pregnancy or in cord blood. The authors also noted inverse associations with continuous 25(OH)D levels at either time point. The other study examined ASD-like traits in adults (Whitehouse et al. 2013), with results suggesting that those whose mothers had low 25(OH)D levels during early pregnancy (<49nmol/L) were at increased risk for attention deficits as measured by the Autism-Spectrum Quotient.

Relative to studies of ASD, a greater number of studies have examined associations with perinatal vitamin D levels (primarily maternal) and broader child development outcomes, producing somewhat inconsistent results. Some studies reported positive associations of increasing 25(OH)D level (or associations of adverse outcomes with 25(OH)D-deficiency) with higher scores on Bayley mental and psychomotor domains, and other measures of language and social development at various ages (Morales et al. 2012; Whitehouse et al. 2012; Hanieh et al. 2014; Darling et al. 2017). Although the recent study from the ALSPAC cohort in the UK found detrimental effects of maternal 25(OH)D deficiency on early developmental assessments there was no association at later assessments, nor with IQ at age 8y (Darling et al. 2017), consistent with an earlier study from the UK that found no association with IQ at age 9y (Gale et al. 2008). A study of cognitive outcomes in Danish adolescents in grade 9 also concluded that there was little evidence of beneficial effects of gestational 25(OH)D levels, with similar levels as our study, e.g. median of 76 nmol/L (Strom et al. 2014). An Australian study found no effects of gestational 25(OH)D levels on behavior/emotional problems measured by the Child Behavior Checklist at any of several child follow-ups up to age 17, despite some effects on language impairment at ages 5 and 10 (Whitehouse et al. 2012). The one study conducted in the U.S., from the Collaborative

Perinatal Project, concluded there was little or no association with maternal or cord blood 25(OH)D levels and a number of child development and behavior metrics assessed at ages 4 and 7, after adjustment (Keim et al. 2014). Thus, further work is needed to clarify whether potential associations with vitamin D levels may be specific to ASD-related outcomes or relate to neurodevelopment more broadly and whether this may vary by time point in development or specific vitamin D levels.

Our results indicated potential race/ethnic differences, with the hypothesized protective associations of higher 25(OH)D levels on ASD suggested only among non-Hispanic whites. Many of the studies of perinatal 25(OH)D were conducted in less racially diverse populations than California, primarily European or Australian, although some were conducted in Asian countries or included darker-skinned immigrants. Typically, non-white race/ethnic groups have lower vitamin D levels, in part due to more melanin in the skin reducing sunlight-induced production of the pre-vitamin D precursor. As far as we are aware, none of the epidemiologic studies examined an interaction of vitamin D with race/ethnicity, so further studies should pursue possible differences.

Our results also indicated an interaction of 25(OH)D with child sex, with lower risks of ASD at higher 25(OH)D levels suggested in males, but the opposite in females, not previously reported. A small study of Faorese aged 15–24 years (Kocovska et al. 2014) reported lower 25(OH)D_3 levels in males compared to females, but this was true in both ASD cases and unaffected siblings, so there was no interaction. In contrast, NHANES data from the U.S. (Centers for Disease Control and Prevention 2012) show slightly lower vitamin D levels in females than males, as did our data from neonates. Vitamin D metabolites are considered neurosteroid hormones, similar structurally to other hormones such as estrogen and testosterone. It has been reported that estrogen may protect against vitamin D deficiency and that immune-modulatory effects of vitamin D vary by sex, leading to the suggestion that males may be more susceptible to effects of vitamin D deficiency (Correale et al. 2010; Cannell 2017; Ali et al. 2018). An Australian study (Wilson et al. 2018) reported an interaction of fetal sex and gestational 25(OH)D on gestational diabetes mellitus, with female “pregnancies” showing an association in the unexpected direction, similar to our findings by sex. Other studies of environmental risk factors and ASD suggest sex-specific effects (Roberts et al. 2013; Schaafsma and Pfaff 2014; Kern et al. 2017; Lyall et al. 2017b), but small numbers of affected females often limit the ability to study sex differences in ASD etiology.

Strengths of our study include it being one of the few large studies to have documented autism diagnoses, another neurodevelopmental endpoint (ID), and vitamin D measured in an appropriate susceptibility window reflecting in utero development (vs. later during childhood). Another strength is the ability to control for a number of possible confounders and to examine interactions. Potential limitations include having higher average vitamin D levels than some other studies, and a smaller proportion considered vitamin D deficient, perhaps limiting our ability to detect an effect, especially if driven by severe deficiency. The California studies, or others with similarly high 25(OH)D levels, may help discern the upper limits of observable effects. There may also be residual confounding from factors that we did not have the ability to adjust for, such as other dietary factors, medications or maternal

BMI. Further, genetic susceptibilities, i.e. variation in genes involved in vitamin D metabolism or related to ASD, may play a role and therefore should be examined in the future.

In conclusion, given continued reports of widespread vitamin D deficiency and insufficiency and potential benefits of vitamin D supplementation, it is important to determine corresponding health effects, especially for pregnant women. While our findings on vitamin D measured in newborns did not support the prevailing hypothesis of a protective effect of higher levels on ASD, there is suggestion of such an effect for ASD among non-Hispanic whites only. Furthermore, an interaction with sex was found that indicates girls with higher vitamin D levels at birth are at higher risk of ASD or ID, which should be examined further in larger studies or by pooling data across studies. More studies on ASD are needed that measure vitamin D in the perinatal period, potentially at different time points to address specific susceptibility windows, and that are large enough to allow examination of sub-groups of ASD, including by sex and phenotype. Further, possible mechanisms could be addressed by examining genotypes and gestational immune or endocrine markers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

The work on this project is funded by the National Institute of Child Health and Human Development (NICHD) (***Grant Number*****:** *5R01HD079533)*. The parent study, Early Markers for Autism, was funded by NIH grant R01-ES016669. We thank the Department of Developmental Services (DDS) and the Genetic Disease Screening Program (GDSP SIS Biobank request number 502) for sharing their data and providing biospecimens, and Jasmine Carver, MPH, for intermediary data analyses.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the California Department of Public Health (CDPH).

References

ACOG Committee on Obstetric Practice (2011). ACOG Committee Opinion No. 495: Vitamin D: Screening and supplementation during pregnancy. Obstet Gynecol, 118, 197–198. doi:10.1097/AOG.0b013e318227f06b. [PubMed: 21691184]

Ali A, Cui X, Alexander S, & Eyles D (2018). The placental immune response is dysregulated developmentally vitamin D deficient rats: Relevance to autism. J Steroid Biochem Mol Biol, 180, 73–80. doi:10.1016/j.jsbmb.2018.01.015. [PubMed: 29408533]

Basit S (2013). Vitamin D in health and disease: a literature review. Br J Biomed Sci, 70, 161–172. [PubMed: 24400428]

Bodnar LM, Simhan HN, Power RW, Frank MP, Cooperstein E, & Roberts JM (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, 447–452. doi:10.1093/jn/137.2.447. [PubMed: 17237325]

Cannell JJ (2008). Autism and vitamin D. Med Hypotheses, 70, 750–759. doi:10.1016/j.mehy.2007.08.016. [PubMed: 17920208]

Cannell JJ (2017). Vitamin D and autism, what's new? Rev Endocr Metab Disord, 18, 183–193. doi:10.1007/s11154-017-9409-0. [PubMed: 28217829]

Cantorna MT, Zhu Y, Froicu M, & Wittke A (2004). Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr, 80(6 Suppl), 1717s–1720s. [PubMed: 15585793]

Centers for Disease Control and Prevention (2012). Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population (pp. 172–192): National Center for Environmental Health, Division of Laboratory Sciences.

Chen J, Xin K, Wei J, Zhang K, & Xiao H (2016). Lower maternal serum 25(OH) D in first trimester associated with higher autism risk in Chinese offspring. J Psychosom Res, 89, 98–101. doi:10.1016/j.jpsychores.2016.08.013. [PubMed: 27663117]

Christensen DL, Baio J, Van Naarden Braun K, Bilder D, Charles J, Constantino JN, et al. (2016). Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years--Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2012. MMWR Surveill Summ, 65, 1–23. doi:10.15585/mmwr.ss6503a1.

Correale J, Ysrraelit MC, & Gaitan MI (2010). Gender differences in 1,25 dihydroxyvitamin D3 immunomodulatory effects in multiple sclerosis patients and healthy subjects. J Immunol, 185, 4948–4958. doi:10.4049/jimmunol.1000588. [PubMed: 20855882]

Croen LA, Braunschweig D, Haapanen L, Yoshida CK, Fireman B, Grether JK, et al. (2008). Maternal mid-pregnancy autoantibodies to fetal brain protein: the early markers for autism study. Biol Psychiatry, 64, 583–588. doi:10.1016/j.biopsych.2008.05.006. [PubMed: 18571628]

Darling AL, Rayman MP, Steer CD, Golding J, Lanham-New SA, & Bath SC (2017). Association between maternal vitamin D status in pregnancy and neurodevelopmental outcomes in childhood: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). Br J Nutr, 117, 1682–1692. doi:10.1017/s0007114517001398. [PubMed: 28697816]

Dror DK, King JC, Durand DJ, & Allen LH (2011). Association of modifiable and nonmodifiable factors with vitamin D status in pregnant women and neonates in Oakland, CA. J Am Diet Assoc, 111, 111–116. doi:10.1016/j.jada.2010.10.002. [PubMed: 21185972]

Du L, Shan L, Wang B, Feng JY, Xu ZD, & Jia FY (2015). [Serum levels of 25-hydroxyvitamin D in children with autism spectrum disorders]. Zhongguo Dang Dai Er Ke Za Zhi, 17, 68–71. [PubMed: 25616297]

Eyles D, Anderson C, Ko P, Jones A, Thomas A, Burne T, 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. doi:10.1016/j.cca.2009.02.005. [PubMed: 19232332]

Fernell E, Barnevik-Olsson M, Bagenholm G, Gillberg C, Gustafsson S, & Saaf M (2010). Serum levels of 25-hydroxyvitamin D in mothers of Swedish and of Somali origin who have children with and without autism. Acta Paediatr, 99, 743–747. doi:10.1111/j.1651-2227.2010.01755.x. [PubMed: 20219032]

Fernell E, Bejerot S, Westerlund J, Miniscalco C, Simila H, Eyles D, et al. (2015). Autism spectrum disorder and low vitamin D at birth: a sibling control study. Mol Autism, 6, 3. doi:10.1186/2040-2392-6-3. [PubMed: 25874075]

Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, et al. (2008). Maternal vitamin D status during pregnancy and child outcomes. Eur J Clin Nutr, 62, 68–77. doi:10.1038/sj.ejcn.1602680. [PubMed: 17311057]

Gesundheit B, Rosenzweig JP, Naor D, Lerer B, Zachor DA, Prochazka V, et al. (2013). Immunological and autoimmune considerations of Autism Spectrum Disorders. J Autoimmun, 44, 1–7. doi:10.1016/j.jaut.2013.05.005. [PubMed: 23867105]

Grandjean P, & Landrigan PJ (2014). Neurobehavioural effects of developmental toxicity. LANCET NEUROL, 13(3), 330–338. doi:10.1016/s1474-4422(13)70278-3. [PubMed: 24556010]

Hamilton SA, McNeil R, Hollis BW, Davis DJ, Winkler J, Cook C, et al. (2010). Profound Vitamin D Deficiency in a Diverse Group of Women during Pregnancy Living in a Sun-Rich Environment at Latitude 32 degrees N. Int J Endocrinol, 2010, 917428. doi:10.1155/2010/917428. [PubMed: 21197089]

Hanieh S, Ha TT, Simpson JA, Thuy TT, Khuong NC, Thoang DD, et al. (2014). Maternal vitamin D status and infant outcomes in rural Vietnam: a prospective cohort study. PLoS ONE, 9, e99005. doi:10.1371/journal.pone.0099005. [PubMed: 24967813]

Hashemzadeh M, Moharreri F, & SOltanifar A (2015). Comparative study of vitamin D levels in children with Autism Spectrum Disorder and normal children: A case-control study. Journal of Fundamentals of Mental Health, 17, 197–201.

Hayes CE, Nashold FE, Spach KM, & Pedersen LB (2003). The immunological functions of the vitamin D endocrine system. Cell Mol Biol (Noisy-le-grand), 49, 277–300. [PubMed: 12887108]

Heath AK, Williamson EJ, Ebeling PR, Kvaskoff D, Eyles DW, & English DR (2014). Measurements of 25-hydroxyvitamin D concentrations in archived dried blood spots are reliable and accurately reflect those in plasma. J Clin Endocrinol Metab, jc20141269. doi:10.1210/jc.2014-1269.

Hewison M (2012). Vitamin D and immune function: an overview. Proc Nutr Soc, 71, 50–61. doi: 10.1017/s0029665111001650. [PubMed: 21849106]

Holick MF (2007). Vitamin D deficiency. N Engl J Med, 357, 266–281. doi:10.1056/NEJMra070553. [PubMed: 17634462]

Hollis BW, Johnson D, Hulsey TC, Ebeling M, & Wagner CL (2011). Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. J Bone Miner Res, 26, 2341–2357. doi:10.1002/jbmr.463. [PubMed: 21706518]

Humble MB, Gustafsson S, & Bejerot S (2010). Low serum levels of 25-hydroxyvitamin D (25-OHD) among psychiatric out-patients in Sweden: relations with season, age, ethnic origin and psychiatric diagnosis. J Steroid Biochem Mol Biol, 121(1–2), 467–470. doi:10.1016/j.jsbmb.2010.03.013. [PubMed: 20214992]

Institute of Medicine (2011). Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: The National Academies Press.

Jones KL, Croen LA, Yoshida CK, Heuer L, Hansen R, Zerbo O, et al. (2017). Autism with intellectual disability is associated with increased levels of maternal cytokines and chemokines during gestation. Mol Psychiatry, 22, 273–279. doi:10.1038/mp.2016.77. [PubMed: 27217154]

Keim SA, Bodnar LM, & Klebanoff MA (2014). Maternal and cord blood 25(OH)-vitamin D concentrations in relation to child development and behaviour. Paediatr Perinat Epidemiol, 28, 434–444. doi:10.1111/ppe.12135. [PubMed: 24938425]

Kern JK, Geier DA, Homme KG, King PG, Bjorklund G, Chirumbolo S, et al. (2017). Developmental neurotoxicants and the vulnerable male brain: a systematic review of suspected neurotoxicants that disproportionally affect males. Acta Neurobiol Exp (Wars), 77, 269–296. [PubMed: 29369294]

Kiely M, Hemmingway A, & O'Callaghan KM (2017). Vitamin D in pregnancy: current perspectives and future directions. Ther Adv Musculoskelet Dis, 9, 145–154. doi:10.1177/1759720×17706453. [PubMed: 28620423]

Kocovska E, Fernell E, Billstedt E, Minnis H, & Gillberg C (2012). Vitamin D and autism: clinical review. Res Dev Disabil, 33, 1541–1550. doi:10.1016/j.ridd.2012.02.015. [PubMed: 22522213]

Kocovska E, Andorsdottir G, Weihe P, Halling J, Fernell E, Stora T, et al. (2014). Vitamin d in the general population of young adults with autism in the faroe islands. J Autism Dev Disord, 44, 2996–3005. doi:10.1007/s10803-014-2155-1. [PubMed: 24927807]

Kvaskoff D, Heath AK, Simila HA, Ko P, English DR, & Eyles DW (2016). Minimizing Matrix Effects for the Accurate Quantification of 25-Hydroxyvitamin D Metabolites in Dried Blood Spots by LC-MS/MS. Clin Chem, 62, 639–646. doi:10.1373/clinchem.2015.251538. [PubMed: 26888893]

Lyall K, Ashwood P, Van de Water J, & Hertz-Picciotto I (2014). Maternal immune-mediated conditions, autism spectrum disorders, and developmental delay. J Autism Dev Disord, 44, 1546–1555. doi:10.1007/s10803-013-2017-2. [PubMed: 24337796]

Lyall K, Croen L, Daniels J, Fallin MD, Ladd-Acosta C, Lee BK, et al. (2017a). The Changing Epidemiology of Autism Spectrum Disorders. Annu Rev Public Health, 38, 81–102. doi:10.1146/annurev-publhealth-031816-044318. [PubMed: 28068486]

Lyall K, Croen LA, Weiss LA, Kharrazi M, Traglia M, Delorenze GN, et al. (2017b). Prenatal Serum Concentrations of Brominated Flame Retardants and Autism Spectrum Disorder and Intellectual Disability in the Early Markers of Autism Study: A Population-Based Case-Control Study in California. Environ Health Perspect, 125, 087023. doi:10.1289/ehp1079. [PubMed: 28895873]

Marshall I, Mehta R, Ayers C, Dhumal S, & Petrova A (2016). Prevalence and risk factors for vitamin D insufficiency and deficiency at birth and associated outcome. BMC Pediatr, 16, 208. doi: 10.1186/s12887-016-0741-4. [PubMed: 27931209]

Meguid NA, Hashish AF, Anwar M, & Sidhom G (2010). Reduced serum levels of 25-hydroxy and 1,25-dihydroxy vitamin D in Egyptian children with autism. J Altern Complement Med, 16, 641–645. doi:10.1089/acm.2009.0349. [PubMed: 20569030]

Meltzer A, & Van de Water J (2017). The Role of the Immune System in Autism Spectrum Disorder. Neuropsychopharmacology, 42, 284–298. doi:10.1038/npp.2016.158. [PubMed: 27534269]

Molloy CA, Kalkwarf HJ, Manning-Courtney P, Mills JL, & Hediger ML (2010). Plasma 25(OH)D concentration in children with autism spectrum disorder. Dev Med Child Neurol, 52, 969–971. doi: 10.1111/j.1469-8749.2010.03704.x. [PubMed: 20497452]

Morales E, Guxens M, Llop S, Rodriguez-Bernal CL, Tardon A, Riano I, et al. (2012). Circulating 25-hydroxyvitamin D3 in pregnancy and infant neuropsychological development. Pediatrics, 130, e913–920. doi:10.1542/peds.2011-3289. [PubMed: 22987876]

Mostafa GA, & Al-Ayadhi LY (2012). Reduced serum concentrations of 25-hydroxy vitamin D in children with autism: relation to autoimmunity. J Neuroinflammation, 9, 201. doi: 10.1186/1742-2094-9-201. [PubMed: 22898564]

Roberts AL, Lyall K, Hart JE, Laden F, Just AC, Bobb JF, et al. (2013). Perinatal air pollutant exposures and autism spectrum disorder in the children of Nurses' Health Study II participants. Environ Health Perspect, 121, 978–984. doi:10.1289/ehp.1206187. [PubMed: 23816781]

Rodier PM (2000). The early origins of autism. Scientific American, 282, 56–63.

Schaafsma SM, & Pfaff DW (2014). Etiologies underlying sex differences in Autism Spectrum Disorders. Front Neuroendocrinol, doi:10.1016/j.yfrne.2014.03.006.

Strom M, Halldorsson TI, Hansen S, Granstrom C, Maslova E, Petersen SB, et al. (2014). Vitamin D measured in maternal serum and offspring neurodevelopmental outcomes: a prospective study with long-term follow-up. Ann Nutr Metab, 64(3–4), 254–261. doi:10.1159/000365030. [PubMed: 25300268]

Urrutia RP, & Thorp JM (2012). Vitamin D in pregnancy: current concepts. Curr Opin Obstet Gynecol, 24, 57–64. doi:10.1097/GCO.0b013e3283505ab3. [PubMed: 22327734]

Vinkhuyzen AAE, Eyles DW, Burne THJ, Blanken LME, Kruithof CJ, Verhulst F, et al. (2017). Gestational vitamin D deficiency and autism spectrum disorder. BJPsych Open, 3, 85–90. doi: 10.1192/bjpo.bp.116.004077. [PubMed: 28446959]

Vinkhuyzen AAE, Eyles DW, Burne THJ, Blanken LME, Kruithof CJ, Verhulst F, et al. (2018). Gestational vitamin D deficiency and autism-related traits: the Generation R Study. Mol Psychiatry, 23, 240–246. doi:10.1038/mp.2016.213. [PubMed: 27895322]

Whitehouse AJ, Holt BJ, Serralha M, Holt PG, Kusel MM, & Hart PH (2012). Maternal serum vitamin D levels during pregnancy and offspring neurocognitive development. Pediatrics, 129, 485–493. doi:10.1542/peds.2011-2644. [PubMed: 22331333]

Whitehouse AJ, Holt BJ, Serralha M, Holt PG, Hart PH, & Kusel MM (2013). Maternal vitamin D levels and the autism phenotype among offspring. J Autism Dev Disord, 43, 1495–1504. doi: 10.1007/s10803-012-1676-8. [PubMed: 23070790]

Wilson RL, Leviton AJ, Leemaqz SY, Anderson PH, Grieger JA, Grzeskowiak LE, et al. (2018). Vitamin D levels in an Australian and New Zealand cohort and the association with pregnancy outcome. BMC Pregnancy Childbirth, 18, 251. doi:10.1186/s12884-018-1887-x. [PubMed: 29925344]

Wu DM, Wen X, Han XR, Wang S, Wang YJ, Shen M, et al. (2018). Relationship Between Neonatal Vitamin D at Birth and Risk of Autism Spectrum Disorders: the NBSIB Study. J Bone Miner Res, 33, 458–466. doi:10.1002/jbmr.3326. [PubMed: 29178513]

Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, & Murphy C (2003). Prevalence of autism in a US metropolitan area. JAMA, 289, 49–55. [PubMed: 12503976]

Zhao G, Ford ES, Tsai J, Li C, & Croft JB (2012). Factors Associated with Vitamin D Deficiency and Inadequacy among Women of Childbearing Age in the United States. ISRN Obstet Gynecol, 2012, 691486. doi:10.5402/2012/691486. [PubMed: 22523695]

Table 1.

Demographic Characteristics by Case Status in the Early Markers for Autism (EMA) Study, Vitamin D Analysis

Characteristic	ASD[§] (N=562)		ID[§] (N=190)		GP[§] Controls (N=436)			
	N	Percent (%)	N	Percent (%)	N	Percent (%)	P-value[†]	P-value[‡]
Birth Year								
2000	106	18.9	45	23.7	81	18.6	0.99	0.18
2001	149	26.5	56	29.5	113	25.9		
2002	221	39.3	69	36.3	176	40.4		
2003	86	15.3	20	10.5	66	15.1		
Birth Season								
Winter (Dec-Feb)	113	20.1	53	27.9	78	17.9	0.85	0.03
Spring (Mar-May)	168	29.9	52	27.4	135	30.9		
Summer (Jun-Aug)	149	26.5	41	21.6	119	27.3		
Fall (Sep-Nov)	132	23.5	44	23.2	104	23.9		
Child Gender								
Male	461	82.0	109	57.4	360	82.6	0.83	<0.0001
Female	101	18.0	81	42.6	76	17.4		
Maternal Race/ethnicity								
non-Hispanic white	196	34.9	36	19.0	146	33.5	0.12	<0.0001
White Hispanic	225	40.0	128	67.4	200	45.9		
Black, Asian, Other, unknown	141	25.1	26	13.7	90	20.6		
Maternal Age (years)								
< 20	17	3.0	26	13.7	24	5.5	0.03	0.0003
20–24	85	15.1	44	23.2	73	16.7		
25–29	152	27.0	52	27.4	132	30.3		
30–34	203	36.1	44	23.2	150	34.4		
≥ 35	105	18.7	24	12.6	57	12.1		
Maternal Education								
< High School	99	17.8	80	42.6	107	24.8	0.001	<0.0001
High School Grad	122	22.0	48	25.5	119	27.6		
College	230	41.4	49	26.1	147	34.0		
Postgraduate	104	18.7	11	5.9	59	13.7		
Parity								
0	262	46.6	66	34.7	169	38.8	<0.0001	0.55
1	217	38.6	60	31.6	137	31.4		
≥ 2	83	14.8	64	33.7	130	29.8		
Preterm Delivery								

Characteristic	ASD[§] (N=562)		ID[§] (N=190)		GP[§] Controls (N=436)			
Yes (<37 weeks)	90	16.0	49	25.8	59	13.5	0.28	0.0002
No (Term)	472	84.0	141	74.2	377	86.5		
Hours from birth to blood draw								
8–24	163	29.4	40	21.7	122	28.5	0.33	<0.0001
>24–36	208	37.5	58	31.5	164	38.3		
>36–48	83	15.0	25	13.6	78	18.2		
>48	101	18.2	61	33.2	64	15.0		

[†]P-value for the Chi-square test of Association for ASD vs GP controls; first 3 were matching variables.

[‡]P-value for the Chi-square test of Association for ID vs GP controls

[§]ASD=autism spectrum disorders, ID=intellectual disability, GP=general population

Table 2:

Distribution of Newborn Vitamin D Levels by Case Status, Early Markers for Autism (EMA) Study

Total 25(OH)D† Variable (nmol/L)	ASD Cases† (n=562)		ID† (n=190)		Controls(n=436)	
Continuous:						
GM† (95%CI†)	81.5	78.4–84.7	84.6	79.4–90.1	80.1	76.4–84.0
Median (IQR†)	85.1	47.6	88.9	53.3	84.3	54.0
Categorized‡:	N	Percent (%)	N	Percent (%)	N	Percent (%)
Deficient (<50)	77	13.7	22	11.6	66	15.1
Insufficient (50 to <75)	151	26.9	55	29.0	108	24.8
Sufficient (≥75)	334	59.4	113	59.5	262	60.1

†ASD=autism spectrum disorders, ID=intellectual disability, 25(OH)D=25hydroxyvitamin D, GM=geometric mean, CI=confidence interval, IQR=interquartile range

‡Chi-square p-values =0.67 for ASD and 0.35 for ID.

Table 3.

Risk of Autism Spectrum Disorder (ASD) or Intellectual Disability (ID) Compared to Controls, by Newborn Vitamin D

	Deficient 25OHD (<50 nmol/L)†		Insufficient 25OHD (50–<75 nmol/L)†		Total 25OHD (per 25 nmol/L)	
Model	**OR†**	**95% CI**	**OR†**	**95% CI**	**OR**	**95% CI**
ASD (N=562)						
Unadjusted	0.92	(0.63–1.32)	1.10	(0.82–1.47)	1.0	(0.93–1.09)
Adjusted‡	0.96	(0.65–1.43)	1.19	(0.86–1.64)	1.0	(0.91–1.09)
ASD w/ ID (N=292)						
Model						
Unadjusted	1.22	(0.81–1.86)	1.28	(0.91–1.81)	0.96	(0.88–1.05)
Adjusted‡	1.17	(0.74–1.86)	1.32	(0.91–1.93)	0.98	(0.88–1.09)
ASD w/o ID (N=232)						
Unadjusted	0.65	(0.39–1.08)	1.01	(0.69–1.46)	1.05	(0.95–1.16)
Adjusted‡	0.72	(0.42–1.26)	1.11	(0.73–1.68)	1.01	(0.90–1.14)
ID only (N=190)						
Unadjusted	0.77	(0.46–1.31)	1.18	(0.80–1.75)	1.05	(0.95–1.16)
Adjusted‡	0.58	(0.31–1.09)	1.03	(0.66–1.62)	1.14	(1.002–1.30)

†Compared to sufficient (25(OH)D ≥ 75 nmol/L)

‡Adjusted for birth year (2000=ref, 2001, 2002, 2003), birth season (Winter (Dec-Feb)=ref, Spring (Mar-May), Summer (Jun-Aug), Fall (Sep-Nov)), child sex (male=ref, female), maternal age (<20, 20–24, 25–29=ref, 30–34, ≥35) , maternal education (HS or less, HS=ref, some college, postgraduate), maternal race/ethnicity (Non-Hispanic White=ref; White Hispanic; Black, Asian, Other, and Unknown), parity (0=ref,1, ≥2), and hours until blood draw (continuous).

Table 4.

Interactions for Continuous Vitamin D (per 25 nmol/L) and Autism Spectrum Disorder (ASD) or Intellectual Disability (ID); with Child Sex or Maternal Race/ethnicity

4a. By Child Sex[†]

	Males		*Females*		
Model	**OR**	**95% CI**	**OR**	**95% CI**	**Interaction P-Value**
ASD					
Unadjusted	0.97	(0.89, 1.06)	1.16	(0.97, 1.38)	0.08
Adjusted[§]	0.95	(0.86, 1.05)	1.18	(0.97, 1.43)	0.049
ID					
Unadjusted	0.96	(0.83, 1.10)	1.23	(1.02, 1.48)	0.03
Adjusted[§]	1.05	(0.88, 1.24)	1.31	(1.07, 1.62)	0.08

4b. By Maternal Race/ethnicity[‡]

	non-Hispanic White		*Other race/ethnicity*		
Model	**OR**	**95% CI**	**OR**	**95% CI**	**Interaction P-Value**
ASD					
Unadjusted	0.92	(0.82, 1.04)	1.07	(0.95, 1.20)	0.08
Adjusted[§]	0.92	(0.81, 1.05)	1.07	(0.95, 1.22)	0.096
ID					
Unadjusted	1.03	(0.85, 1.24)	1.24	(1.07, 1.43)	0.13
Adjusted[§]	1.03	(0.84, 1.27)	1.25	(1.05, 1.48)	0.17

[†]N for males is 801 in adjusted ASD model, 453 in ID; for females is 171 in ASD and 153 in ID models.

[‡]N for non-Hispanic white is 337 in ASD and 178 in ID, adjusted models; for Others is 635 in ASD and 428 in ID, adjusted models. “Other race/ethnicity” includes Hispanic whites, Blacks, Asians, others or unknown

[§]Adjusted for birth year (2000=ref, 2001, 2002, 2003), birth season (Winter (Dec-Feb)=ref, Spring (Mar-May), Summer (Jun-Aug), Fall (Sep-Nov)), maternal age (<20, 20–24, 25–29=ref, 30–34, ≥35) , maternal education (HS or less, HS=ref, some college, postgraduate), parity (0=ref,1, ≥2), and hours until blood draw (continuous); as well as maternal race/ethnicity (Non-Hispanic White=ref; White Hispanic; Black, Asian, Other, Unknown) or child sex (male=ref, female) in 4a and 4b, respectively.