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ORIGINAL ARTICLE

Vitamin D deficiency associated with neurodevelopmental problems in 2-year-old Japanese boys

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Abstract

Aim: While associations between vitamin D deficiency and neurodevelopmental disorders have been found, large studies on child vitamin D, neurodevelopment, and sex differences among the general population are lacking. This study aimed to investigate the association between child serum 25-hydroxyvitamin D (25(OH)D)) levels and neurodevelopmental problems (NDPs).

Methods: Serum 25(OH)D and NDPs were measured at age two among the subcohort study of the Japan Environment and Children's Study. NDPs were assessed with the Kyoto Scale of Psychological Development 2001 (Kyoto scale). Adjusted odds ratios (aORs) for the Kyoto-scale developmental quotient scores <70 were calculated, for postural–motor, cognitive–adaptive, and language–social domains and overall scores, adjusted for test month, latitude, small for gestational age, maternal age, and daycare attendance.

Results: Among 2363 boys and 2290 girls, boys had higher 25(OH)D levels, but scored lower in the Kyoto scale. For boys in the vitamin D deficiency (<20 ng/mL) group, aORs of scoring the Kyoto-scale DQs <70 were 2.33 ($p = 0.006$) for overall DQs, 1.91 ($p = 0.037$) for cognitive–adaptive, and 1.69 ($p = 0.024$) for language–social domains. For girls, results were inconclusive.

Conclusion: Only boys showed a clear and cross-modal association between vitamin D deficiency and NDPs.

KEYWORDS
Japan Environment and Children's Study, Kyoto Scale of Psychological Development 2001, neurodevelopmental problems, sex differences, vitamin D deficiency

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ALSPAC, Avon Longitudinal Study of Parents and Children; aOR, adjusted odds ratio; ASD, Autism spectrum disorder; DQ, developmental quotients; JECS, Japan Environment and Children's Study; Kyoto scale, Kyoto Scale of Psychological Development 2001; NDP, neurodevelopmental problem; OR, odds ratio.

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1 | INTRODUCTION

Vitamin D is known to be an essential nutrient for calcium regulation and phosphate metabolism.[1,2] Inadequate levels of vitamin D have been associated with increased risks of rickets, osteoporosis, osteomalacia, and fragility fractures. The most common barometer for individual's vitamin D status is serum 25-hydroxyvitamin D [25(OH)D], a metabolite with approximately 2–3 weeks of half-life.[3] Low levels of 25(OH)D have also been reported to be associated with immune-related diseases and disorders, including respiratory infection, allergies, rheumatoid arthritis, type 1 diabetes, psoriasis, hypothyroidism, tuberculosis, sepsis, and, more recently, COVID-19.[2,4]

Furthermore, vitamin D has been suggested as a neurosteroid, playing a crucial role in neurodevelopment from as early as the foetal and neonatal periods.[5–7] Some studies have associated low vitamin D status with low cognitive function, impaired social–emotional development, and autism spectrum disorder (ASD).[1,5,8,9] The ubiquitous expression of vitamin D receptors indicates that vitamin D regulates various mechanisms, including in the central nervous system.[1,8] Vitamin D supplementation has also been examined for alleviating neurodevelopmental problems (NDPs) including ASD.[10,11] However, not all published results have been consistent regarding the association between low vitamin D and neurodevelopment or regarding the efficacy of vitamin D supplementation for neurodevelopment, and the mechanisms in humans also remain to be studied further.[5–8,12]

The main source of vitamin D in humans is from sun exposure, which leads to the synthesis of vitamin D_3, that is cholecalciferol, consisting of 80%–90% of the total body vitamin D.[1] The remaining 10%–20% can be provided by diet: animal-derived vitamin D_3, such as that found in fish, cod liver oil, and egg yolks; and plant-derived vitamin D_2, that is ergocalciferol, including sun-dried mushrooms.[9] Although the importance of vitamin D in overall health has been recognised globally, the problem of vitamin D deficiency or insufficiency has "re-emerged" among developed countries. Vitamin D deficiency has become a public health concern, mainly due to lifestyle changes, such as increased indoor sedentary time, increased numbers of individuals with obesity, and a lack of available fortified food or supplementation.[1] However, no international consensus has been reached regarding what might be considered sufficient levels of vitamin D. Very few studies on young children's vitamin D levels and neurodevelopment have been conducted at a general population level.

Large-scale studies about vitamin D deficiency and its possible association with health problems are particularly critical in Japan. Unlike some other developed countries such as the United States, Canada, Sweden, Finland, and Norway, the Japanese government does not systematically recommend fortifying food with vitamin D nor has it encouraged supplementation.[13,14] The aim of the present study was to investigate the association between child serum 25(OH)D levels [25(OH)D3 + 25(OH)D2], and children's neurodevelopment at age two, by using data from 4653 participants of a subcohort study of the Japan Environment and Children's Study (JECS), one of the world's largest ongoing birth cohort studies.[13,15–17]

Key notes

- This study is the first large-scale general population study on young children's vitamin D levels and neurodevelopment, stratified by child sex.
- For boys, there was a clear association between low vitamin D levels and NDPs, in the cognitive–adaptive and language–social domains.
- No association was found either in boys or girls between low vitamin D and gross motor problems.

2 | METHODS

2.1 | Study design and data collection

This vitamin D neurodevelopment study used data from the subcohort of the JECS Main Study, covering approximately 5% of the main study cohort. The details of the main study and the subcohort study have been described elsewhere.[15–17] Briefly, 104 062 foetal records were registered nationwide during the recruitment between January 2011 and March 2014, and 100 303 live births were recorded by December 2014. The major aim was to investigate possible associations between environmental factors and children's health. The children of mothers registered in the subcohort study were randomly selected from all 15 JECS Regional Centres with the following inclusion criteria: children born on 1 April 2013 through 31 December 2014, and no questionnaires, medical records, or biospecimens were missing, except for umbilical cord blood in some cases.[15] Of the 10 302 children selected, 5017 participated in the subcohort study with written consent. The baseline profiles of parents and children, such as maternal age at delivery, marital status, gestational age at birth, and birthweight, were similar to those of the remaining main study participants.[15] To date, thorough follow-up studies, including face-to face neurodevelopmental tests, medical examinations, and blood and urine sample collection, have been conducted at the ages of 2, 4, 6, and 8 years. In addition, home visits have been performed to collect environmental samples at 1.5 and 3 years of age. The JECS protocol was reviewed and approved by the Japan Ministry of the Environment's Institutional Review Board on Epidemiological Studies (no. 100910001) and all participating institutions.[15] All the participating mothers and fathers provided written informed consent.[15–17]

The present study utilised a part of the JECS data collected until children turned 3 years of age (the jecs-ta-20190930 dataset released in October 2019) and supplementary dataset (ageof03_comparisontable001_ver003), specifically the 2-year-old data on 25(OH)

D levels and the Kyoto Scale of Psychological Development (Kyoto scale), a developmental screening tool as explained below.

2.2 | Study participants

In the present study, 4653 of the 5017 participants of the JECS subcohort study (92.7% of the whole subcohort study sample) met the inclusion criteria (Figure S1). There were 2363 boys and 2290 girls. Mean test age of the children was 24 months for blood and Kyoto-scale testing, with >95% taking both tests within 3 months of their 2-year-old birthday month.

2.3 | Serum 25(OH)D3 and 25(OH)D2 concentration measurements

The blood samples were analysed using liquid chromatography tandem mass spectrometry (LC–MS/MS) to measure serum 25(OH)D3 and 25(OH)D2 concentrations, calibrated with 6PLUS1 Multilevel Serum Calibrator Set 25-OH-Vitamin D3/D2.[13] Values for 25(OH)D3 and 25(OH)D2 concentrations below 4 ng/mL were truncated as 4 ng/mL.[13] The sum of the 25(OH)D3 and 25(OH)D2 concentrations was defined as the total serum 25(OH)D level. In addition to the exact values of the total 25(OH)D concentrations, the participants were divided into three groups: deficiency (<20 ng/mL); insufficiency (≥20 and <30 ng/mL); and sufficiency (≥30 ng/mL) according to the cut-offs for vitamin D concentrations used in previous international studies.[13]

2.4 | The Kyoto Scale of Psychological Development

The Kyoto scale yields four Developmental Quotient (DQ) scores: overall, postural–motor, cognitive–adaptive, and language–social. DQ scores <70, the most commonly used cut-off to determine eligibility for public service access and in medical/research settings,[18] were applied in the current study. All JECS assessors at the 15 Regional Centres, including clinical psychologists and nurses, had been trained and certified by the JECS Core Centre to guarantee consistency of test results across Japan.[15]

2.5 | Statistical analysis

As both exposure (serum 25(OH)D2 and 25(OH)D3) and outcome (the Kyoto-scale DQ) variables showed significant differences between boys and girls, all the analyses were stratified by child sex. The 25(OH)D concentration levels and the Kyoto-scale DQ results were treated both as continuous and categorical variables to investigate their relationship using four combinations: continuous–continuous, continuous–categorical, categorical–continuous, and categorical–categorical. For the first combination, correlations between continuous 25(OH)D concentration levels and the Kyoto-scale DQ scores of each of the three domains and overall were examined with Spearman's correlation coefficient. Test month, the month that the two-year-old follow-up was conducted, and geographical latitudes of the cities of the Regional Centres as proxy for participant residences (between 26°N in Okinawa and 43°N in Hokkaido) were also included in the analysis as they are known to be associated with UV levels and accordingly with 25(OH)D3 levels. Second, differences in the mean and median of 25(OH)D concentration levels between the two groups – the Kyoto-scale DQs ≥70 and <70 – were assessed using Wilcoxon, Kolmogorov–Smirnov, and independent samples *t*-tests. Third, the Kruskal–Wallis test was conducted to assess differences between the continuous Kyoto-scale DQ results in the three different levels of 25(OH)D groups (<20, ≥20 and <30, and ≥30 ng/mL). Finally, logistic regression was conducted to calculate crude odds ratio (OR) and to assess Kyoto-scale DQ scores in each of the 25(OH)D-level groups.

After multicollinearity (variance inflation factor 1.14) and interactions had been ruled out, test month, latitude, and major known medical risk factors for neurodevelopmental delay – small for gestational age and maternal age – as well as a common social factor – daycare attendance at the age two – were added in the final analysis to calculate adjusted odds ratio (aOR). Maternal education was not included in the final model, as no association was found with the Kyoto-scale DQ scores in the bivariate analysis. The significance level was set at $p < 0.05$ with a 95% confidence interval (CI). All of the data analyses were performed by using Stata/MP version 17.0 (StataCorp LP).

3 | RESULTS

3.1 | Serum 25(OH)D concentration

When analysing 25(OH)D3 and 25(OH)D2 levels separately, 25(OH)D3 levels fluctuated by test month, that is highest in August and lowest in February, with boys' means consistently higher than those of girls (Figure 1). The number of boys and girls in each test month was not significantly different ($p = 0.994$). Similarly, no significant sex difference was found regarding parent-reported time spent outside per day (mean approximately 2.6 h in summer with $p = 0.229$, and 2.2 h in winter with $p = 0.211$), nor for daycare attendance (48.8% of 2363 boys and 48.2% of 2290 girls, $p = 0.664$). Regarding 25(OH)D3, mean discrepancies between boys and girls were widest in summer months, and narrowest in winter months. In contrast, 25(OH)D2 levels were 4 ng/mL – the truncated value – for all but three children whose levels were 5.5 ng/mL (boy), 5.4 ng/mL (girl), and 10.3 ng/mL (girl), respectively, indicating that almost all 25(OH)D2 levels were ≤4.0 ng/mL. Accordingly, the trend of the total 25(OH)D levels reflected those of 25(OH)D3 and was significantly higher in boys than in girls, with means of 25.6 ng/mL (95% CI 25.4–26.0) versus 24.6 ng/mL (95% CI 24.3–24.8) ($p < 0.001$). This significant difference by child sex remained after categorising them into the three groups by total 25(OH)D level – deficient, insufficient, and sufficient

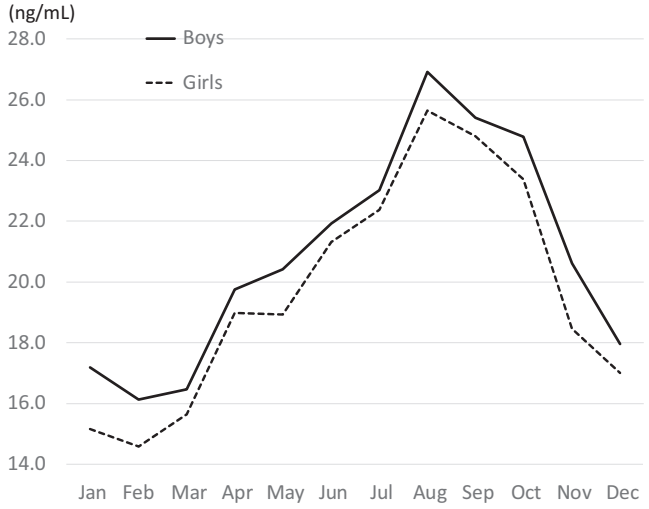

FIGURE 1 Mean 25(OH)D3 by month by child sex.

levels – with a higher proportion of boys in the sufficient group and a higher proportion of girls in the deficient group (Table S1a). The 25(OH)D concentrations also differed significantly depending on test month, latitude, and daycare attendance (Table S1a).

3.2 | Kyoto-scale DQ scores

When the participants were dichotomised according to the Kyoto-scale DQ cut-off, 2045 boys (86.5%) and 2083 girls (91.0%) passed the cut-off (DQs ≥70) in all the three subdomains and the overall DQs ($p < 0.001$). For those with DQs <70, boys outnumbered girls in every subdomain and overall DQs, but this difference was significant only for the language–social domain (Table S1b). Associations were found between small for gestational age and DQs (all except the language–social subdomain) as well as between daycare attendance and the language–social and overall DQs (Table S1b). Mean and median DQs of every domain (postural–motor, cognitive–adaptive, and language–social) and the overall DQs were significantly higher for girls, with the largest discrepancy for the language–social domain and the narrowest for postural–motor domain (Table S2a). Regarding failure overlaps among the three domains, language–social domain only was most common for boys ($n = 113$), followed by postural–motor domain only ($n = 76$) and all three domains ($n = 59$), whereas for girls, postural–motor domain only was most common ($n = 73$), followed by all three domains ($n = 41$). Among those who failed in the overall DQs (108 boys and 80 girls, $p = 0.062$), the majority (59 boys and 41 girls) failed in all the three domains (Table S2b).

3.3 | Association between serum 25(OH)D levels and Kyoto-scale DQ results

When treated as continuous variables, serum 25(OH)D concentration showed a weak but statistically significant positive correlation with Kyoto-scale DQ in the language–social domain for both boys and girls, meaning that the higher the vitamin D levels, the higher their language–social DQs became, with boys showing a stronger correlation. Boys also showed a weak but significant positive correlation between 25(OH)D and the Kyoto-scale overall DQs (Table S3a,b).

When categorised by the three different levels of 25(OH)D concentration, there were significant differences in both boys' and girls' language–social DQ (both with mean and median) in a dose-dependent manner. The vitamin D "sufficient" group scored highest and the language–social DQ gradually decreased in the "insufficient" groups, and it became lowest in the "deficient" group (Table S4).

When dichotomised at the Kyoto-scale DQ <70, only boys' 25(OH)D concentrations were consistently lower (both mean and median) among those scoring <70 with statistical significance in all but the postural–motor domain (Table S5). For girls, there were no statistically significant differences between the two groups (DQs <70 and ≥70), nor was there the same tendency of those scoring <70 having lower 25(OH)D concentration (Table S5). Only boys' OR and aOR of 25(OH)D concentrations were <1.00, indicating that the higher 25(OH)D concentration, the less likely they scored below the cut-off, with statistical significance for all the Kyoto-scale domains but postural–motor. Adjusted OR was 0.96 ($p = 0.015$, 95% CI 0.93, 0.99) for cognitive–adaptive, 0.97 ($p = 0.012$, 95% CI 0.95, 0.99) for language–social, and 0.95 ($p = 0.005$, 95% CI 0.93, 0.99) for overall DQs (Table S6).

A similar tendency was observed when 25(OH)D concentration was categorised into three groups and Kyoto-scale DQ was dichotomised at 70. Only boys showed clear differences among the three different vitamin D-level groups with $p < 0.05$: the lower the vitamin D-level group boys belonged to, the more likely they scored below the cut-off in all the Kyoto-scale domains except postural–motor (Table 1). With the vitamin D sufficient group (≥30 ng/mL) as reference, adjusted ORs showed that boys with vitamin D deficiency (<20 ng/mL) were more likely to score below the cut-off for all the domains but postural–motor: 1.99 for cognitive–adaptive, 1.72 for language–social, and 2.39 for overall DQs (Table 2). Only the girls' insufficient group (≥20 and <30 ng/mL) showed a significant aOR 1.97 for cognitive–adaptive and 1.95 for overall DQs (Table 2).

4 | DISCUSSION

The major finding in this study of a Japanese general child population was that only boys showed a clear association between vitamin D deficiency and delayed cognitive and communication development, with an indication of a dose-dependent association. Future investigations are necessary for girls, because insufficient vitamin D levels showed an association with low cognitive function only when both vitamin D and Kyoto-scale scores were treated as categorical variables. However, no clear patterns were observed throughout other analyses among girls. Since 25(OH)D2 was ≤4 ng/mL for all but three children and it was 25(OH)D3 that differed among the subcohort, it is highly likely that 25(OH)D3, not 25(OH)D2, contributed to our findings.

TABLE 1 25(OH)D concentrations in three categories and KSPD DQ (<70).

25(OH)D concentration		KSPD DQ									
		P-M < 70		p	C-A < 70		p	L-S < 70		p	Overall <70		p
(Boys)				0.476			0.030			0.029			0.007
<20 ng/mL	(n = 541, 22.9%)	38	(7.0)		37	(6.8)		62	(11.5)		38	(7.0)	
≥20 & <30 ng/mL	(n = 1196, 50.6%)	91	(7.6)		53	(4.4)		100	(8.4)		48	(4.0)	
≥30 ng/mL	(n = 626, 26.5%)	38	(6.1)		23	(3.7)		45	(7.2)		22	(3.5)	
Total	(n = 2363)	167	(7.1)		113	(4.8)		207	(8.8)		108	(4.6)	
(Girls)				0.567			0.150			0.853			0.250
<20 ng/mL	(n = 607, 26.5%)	39	(6.4)		21	(3.5)		24	(4.0)		20	(3.3)	
≥20 & <30 ng/mL	(n = 1189, 51.9%)	75	(6.3)		56	(4.7)		21	(3.2)		48	(4.0)	
≥30 ng/mL	(n = 494, 21.6%)	25	(5.1)		14	(2.8)		22	(4.5)		12	(2.4)	
Total	(n = 2290)	139	(6.1)		91	(4.0)		92	(4.0)		80	(3.5)	

Abbreviations: C-A, Cognitive–Adaptive; DQ, Developmental quotient; KSPD, Kyoto Scale of Psychological Development; L-S, Language–Social; P-A, Postural–Motor.

The truncated values of 25(OH)D2 (≤4 ng/mL) for all children except three follow the current understanding that vitamin D_2 consists of only 10%–20% of total vitamin D. It also reflects the current situation in Japan that no systemic fortification nor supplementation is conducted and that few children take plant-derived vitamin D_2.

According to parental reports, there was no difference between boys and girls in terms of time spent outside. However, the most likely reason for boys having higher vitamin D levels is that they probably do spend more time outside compared with girls. It is possible that the parental report in this respect was not sufficiently detailed to pick such a difference up.

In previous studies on the association between vitamin D and neurodevelopment in a general population, maternal blood during pregnancy or cord blood, rather than the child's blood, has been measured. Even when child serum 25(OH)D was measured, like using neonatal blood spot, the outcome was usually dichotomised, that is *with or without* neurodevelopmental *disorders*, such as ASD, with no detailed assessment of neurodevelopment of the control groups.[5,6,12] The only study that we have been able to find with similar design to that of our study was a subcohort of the Avon Longitudinal Study of Parents and Children (ALSPAC). The ALSPAC results were interpreted as showing no association between vitamin D deficiency and neurodevelopment, but sex differences were not analysed.[19] Other possible reasons for detecting no association in the ALSPAC sample might have been due to a smaller sample size (approximately 2500 compared to 4653 in our study), the different country, child age of assessment (mean age 11.7 years compared to 2 years), and timing of blood test (mean age almost 10 in the ALSPAC compared to 2 years in the JECS). Furthermore, the ALSPAC participants with previous behavioural problems had been excluded in the above-mentioned subcohort analysis.

Although boys' vitamin D deficiency and cognitive/social development problems showed a clear association in this study, we could not elucidate any causal relationship. Vitamin D deficiency could be a risk factor for some NDPs for some individuals, and/or some children with neurodevelopmental challenges could have abnormalities in their steroid metabolism, including vitamin D, which has been suggested as a neurosteroid.[7,20] Both directions of causality may coexist as do various types of NDPs and varied aetiologies of even very similar symptoms/neurodevelopmental diagnoses. Accordingly, several possible mechanisms underlying the connection between lower vitamin D and NDPs have been suggested. Studies from Sweden and Minnesota have demonstrated that children with Somali origin have increased rates of autism.[21,22] The Somali mothers, regardless of ASD diagnosis of their children, had much lower vitamin D levels compared to the Swedish mothers. The mean vitamin D levels of both Somali and Swedish mothers with children with ASD were lower than their respective control groups although these differences were not statistically significant, most likely due to the sample size.[21] Another Swedish study in 58 sibling pairs showed that children with ASD had significantly lower vitamin D than their non-ASD sibling just after birth, indicating that vitamin D deficiency could be an early marker for future NDPs.[23] In experimental studies, low

TABLE 2 Association between 25(OH)D and KSPD DQ.

	P-M < 70			C-A < 70			L-S < 70			Overall < 70		
Boys												
25(OH)D (ng/mL)	**OR**	***p***	**(95% CI)**	**OR**	***p***	**(95% CI)**	**OR**	***p***	**(95% CI)**	**OR**	***p***	**(95% CI)**
<20	1.17	0.511	(0.73, 1.86)	1.92	0.016	(1.13, 3.28)	1.67	0.012	(1.12, 2.50)	2.07	0.008	(1.21, 3.55)
≥20 and <30	1.27	0.225	(0.86, 1.89)	1.22	0.443	(0.74, 2.00)	1.18	0.380	(0.82, 1.70)	1.15	0.599	(0.69, 1.92)
25(OH) (ng/mL)	**aOR**	***p***	**(95% CI)**	**aOR**	***p***	**(95% CI)**	**aOR**	***p***	**(95% CI)**	**aOR**	***p***	**(95% CI)**
<20	1.47	0.150	(0.87, 2.50)	1.91	0.037	(1.04, 3.50)	1.69	0.024	(1.07, 2.67)	2.33	0.006	(1.27, 4.29)
≥20 and <30	1.41	0.106	(0.93, 2.13)	1.25	0.393	(0.75, 2.10)	1.15	0.463	(0.79, 1.69)	1.23	0.445	(0.72, 2.09)
Girls												
25(OH)D (ng/mL)	**OR**	***p***	**(95% CI)**	**OR**	***p***	**(95% CI)**	**OR**	***p***	**(95% CI)**	**OR**	***p***	**(95% CI)**
<20	1.29	0.337	(0.77, 2.16)	1.23	0.557	(0.62, 2.44)	0.88	0.680	(0.49, 1.60)	1.37	0.397	(0.66, 2.83)
≥20 and <30	1.26	0.325	(0.79, 2.01)	1.69	0.082	(0.93, 3.07)	0.86	0.579	(0.51, 1.45)	1.69	0.109	(0.89, 3.21)
25(OH) (ng/mL)	**aOR**	***p***	**(95% CI)**	**aOR**	***p***	**(95% CI)**	**aOR**	***p***	**(95% CI)**	**aOR**	***p***	**(95% CI)**
<20	1.53	0.155	(0.85, 2.74)	1.79	0.133	(0.84, 3.80)	0.92	0.815	(0.46, 1.83)	1.75	0.175	(0.78, 3.92)
≥20 and <30	1.32	0.261	(0.81, 2.14)	1.97	0.031	(1.07, 3.63)	0.88	0.659	(0.51, 1.53)	1.95	0.048	(1.01, 3.77)

Note: Adjusted for test month, latitude, small for gestational age, maternal age and daycare attendance. Reference: 25(OH)D concentration ≥ 30 ng/mL.
Abbreviations: aOR, adjusted odds ratio; OR, odds ratio.

vitamin D has been found to impact adversely on brain development, including alteration in the dopaminergic turnover in the forebrain.[7,24] Experimental studies have also shown associations between developmental vitamin D deficiency and elevated testosterone levels directly in the foetal brain. The results indicate that increased foetal exposure to testosterone, leading to increased androgenisation of the foetal brain, may be a possible pathogenetic process for the pronounced sex bias in autism.[7,25] The finding in our study, that boys with deficient vitamin D levels had poorer outcomes on cognitive and social developmental tests, could be considered in the context of these findings, indicating boys' possible specific vulnerability to vitamin D deficiency.

No association between vitamin D and the Kyoto-scale postural–motor domain was found in any of our analyses. The results could be due to participants with *gross* motor development delay spending similar amounts of time being outside (and hence get "sufficient" vitamin D levels from being exposed to sunlight) regardless of their gross motor development delay. Another possibility is that low vitamin D levels do not affect gross motor development as much as they do cognitive and communication development. Finally, vitamin D metabolism may not differentiate children with and without gross motor development problems from each other. Further studies are needed to confirm the role of vitamin D in different domains of child neurodevelopment, as comorbidities between motor and cognitive/social developmental delays are common.

Vitamin D has been studied from both prevention and treatment perspectives. Although previous study results on the efficacy of supplementation have been inconsistent, many studies found that inadequate levels of vitamin D in early life negatively affect neurodevelopment, and vitamin D supplementation may alleviate ASD symptoms.[23,26–29] The fact that fewer than a quarter of the participating children in this study had "sufficient" levels of 25(OH)D and a quarter were overtly deficient might be seen to be alarming from a public health perspective. Unlike some other developed countries such as the United States, Canada, Sweden, Finland, and Norway, Japan does not systematically fortify food with vitamin D.[13,14] It could be worthwhile to reconsider an appropriate amount of sun exposure and vitamin D supplementation/fortification in Japan to benefit Japanese children's overall health.

The particular strengths of the present study are the relatively large number of both boys and girls from a general population, that blood samples obtained from children themselves, and the use of prospectively collected data within a nationwide birth cohort. Taken together, these strengths enabled us to identify sex differences in the association between child vitamin D deficiency and NDPs.[15] Another strength is the use of liquid chromatography tandem mass spectrometry (LC–MS/MS), a widely used method to measure serum 25(OH)D concentrations on its excellent analytical specificity and sensitivity.[30] The final strength is the use of the Kyoto scale, as it provided a dimensional and in-depth measure, rather than just a "yes" or "no" to having a particular diagnosis. The fact that the Kyoto scale has been widely used in Japan, and that all the testers in this study were trained and certified by the JECS Core Centre, guarantees the quality of the data as well.

The study has four major limitations. First, we could only assess the association between serum 25(OH)D and child development at age two and were not able to document any causal relationship. Second, serum 25(OH)D concentrations were measured only around the time of child's birthday; therefore, no individual longer-term serum 25(OH)D information was available, nor could we distinguish between seasonal fluctuation and long-term vitamin D status. Third, the detailed analysis on 25(OH)D2 was not possible because the levels <4 ng/mL were truncated. Finally, even though the size of the JECS subcohort was large compared to previous studies, it was not large enough for more granular analysis, particularly when we stratified the data by child sex and adjusted for test month and latitude.

5 | CONCLUSION

Boys showed a clear association between vitamin D deficiency (<20 ng/mL) and delays in neurodevelopment as measured by the Kyoto scale in all the domains but the postural–motor domain, and most likely the significance derived from low 25(OH)D3 levels. Because serum 25(OH)D concentrations were measured only once around the second birthday, further studies are necessary to replicate the study results and to acquire longitudinal data before firm conclusions can be drawn about the role of vitamin D in early child development.

AUTHOR CONTRIBUTIONS

Kahoko Yasumitsu-Lovell: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; visualization; writing – original draft; writing – review and editing. **Lucy Thompson:** Investigation; methodology; supervision; writing – review and editing. **Elisabeth Fernell:** Investigation; methodology; supervision; writing – review and editing. **Masamitsu Eitoku:** Data curation; resources; writing – review and editing. **Narufumi Suganuma:** Data curation; funding acquisition; resources; supervision; writing – review and editing. **Christopher Gillberg:** Conceptualization; investigation; methodology; supervision; visualization; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX

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