Supplemental Information

METHODS

Dietary Sugar Intake

Children were classified as low, medium, or high sugar consumers depending on the reported weekly frequency of consumption of a number of sweets, including chocolate, lollies, sweet biscuits, soft drinks, ice cream, and sweet spreads. The options for responses included 0, 1, 2, 3, 4, 5, or ≥6 times a week. The response to each of the sweet items were combined to give an estimate of the number of times per week in total. Children having sweets <10 times per week were considered to have a low sugar intake, 10 to 20 times, a medium sugar intake, and ≥20 times, a high sugar intake.

Vitamin D Levels at Birth

Children's 25-hydroxyvitamin D levels at birth were determined from serum and plasma from cord blood by using the LIAISON 25-OH Vitamin D TOTAL kit (DiaSorin Australia Pty Ltd) at the Monash Medical Centre Pathology Department in Clayton, Australia.

To address possible batching effects, sera from 10 children with birth vitamin D levels measured in 2011 were measured again (from another sample of stored frozen cord blood serum) in 2017. A further 10 participants had vitamin D levels measured from both serum and plasma samples.

After data checking and comparison of the distributions of the 3 batches (batch 1: serum [2011]; batch 2: serum [2017]; batch 3: plasma [2017]), 2 regression analyses in which both linear and Deming regression were used were performed to compare, firstly, the relationship between batches 1 and 2 and, secondly, the relationship between batches 2 and 3.[-31] Estimated regression coefficients were then used to adjust the vitamin D levels obtained in batch 1 and batch 3 to equate to the levels obtained in batch 2 by treating values predicted from the models as observed data.

Batch 1 comprised 103 individuals (both twins in 27 pairs and only 1 twin in 49 pairs) who had vitamin D levels measured from serum in 2011. The mean vitamin D level was 62.18 nmol/L (SD 21.78; variance = 474.48; range: 12.9–138).

Batch 2 (2017) comprised 103 individuals (both twins in 27 pairs and only 1 twin in 49 pairs) who had vitamin D levels measured from serum in 2017. This batch included 11 individuals who were measured to check batching effects with levels from 2011. The mean vitamin D level was 59.22 nmol/L (SD 24.83; variance = 616.70; range: 15.9–132).

Batch 3 comprised 40 individuals (both twins in 4 pairs and only 1 twin in 32 pairs) who had vitamin D levels measured from serum in 2017. This batch included 11 individuals who were measured to check batching effects with levels from 2011. The mean vitamin D level was 66.92 nmol/L (SD 25·01; variance = 625.56; range: 20.1–148).

The relationship between measurements in 2011 and 2017 (Supplemental Table 3) was evaluated graphically (Supplemental Fig 3) and by using *t* tests and variance ratio tests, to check differences in mean and variance, and linear and Deming regression (Supplemental Table 4). The mean difference between measurements in 2017 and 2011 was 1.25 (95% CI −6.65 to 9.16; P = 0.73), and all outcomes were consistent, with no difference between batches. To ensure comparability with measurements from serum in 2017, birth vitamin D levels measured in 2011 were adjusted according to the following formula:

$$\text{Adjusted vitamin D level} = 9.19 + 0.84{*}x$$

(in which x = the vitamin D level measured in 2011).

The relationship between measurements from serum and plasma (all in 2017) (Supplemental Table 5) were evaluated graphically (Supplemental Fig 4) and using *t* tests and variance ratio tests, to check differences in mean and variance, and linear and Deming regression (Supplemental Table 4). The mean difference between measurements from serum and plasma was −5.71 (95% CI −11.56 to 0.14; P = .06), and all outcomes were consistent, with no difference between batches. To ensure comparability with measurements from serum in 2017, all birth vitamin D levels measured from plasma (2017) were adjusted

according to the following formula:

$$\text{Adjusted vitamin D level} = 5.48 + 0.84{*}x$$

(in which x = the vitamin D level measured from plasma).

Dental Examinations

Dental examinations were performed on-site at Murdoch Children's Research Institute or, for participants unable to travel, at home. The standard cross-infection protocol was followed for all examinations, which were performed by 2 trained and calibrated oral health professionals (M.J.S. and P.L.). In Supplemental Tables 6 and 7, the inter- and intraexaminer reliability for MIH and/or HSPMs and ICDAS are detailed. On-site dental examinations were performed, with the patient reclined on a clinical examination bed, by using an overhead light. During home visits (n = 66), dental examinations were performed, with children supine (on couches or beds as available at the location), with a headlight. Teeth were cleaned with cotton rolls but not air dried before examination. Dental caries was recorded by using the ICDAS, which allows for quantification of carious lesions, from early, noncavitation white-spot lesions to large cavities with significant destruction of tooth structure. From mid-2015, the presence, presentation, and extent of HSPMs were routinely recorded for the buccal, lingual, and occlusal surfaces of the second primary molars per standardized criteria.[12] The presentation of HSPMs included demarcated white opacities, demarcated yellow or brown opacities, posteruptive breakdown, atypical restorations, atypical caries, and extractions due to HSPMs. However, an HSPM-specific examination was not part of the protocol for ~158 children who completed dental examinations before mid-2015, with a broader examination for all developmental defects of enamel. Therefore, for these children, already collected data regarding dental caries, opacities, and hypoplasia were reviewed to identify children potentially with HSPMs. Children whose second primary molars were all present, who had no caries experience (ICDAS caries codes of 2 or below and restoration and/or sealant codes of 2 and below), who had no signs of opacities, and who had no hypoplasia were deemed to be unaffected by HSPMs. All other children (n = 52) were reexamined in July 2016 by using standardized HSPM criteria.

Data Analysis

Outcome Variable

Children with ≥1 teeth with any carious lesions (including early, non-cavitated white-spot lesions [ICDAS caries codes 2–6]), restorations, or extractions due to caries were categorized as affected by any caries. Children with ≥1 teeth with signs of more advanced carious lesions (ICDAS codes 4–6), restorations, or extractions due to caries were categorized as affected by advanced caries. Because many children had either lost primary incisors because of natural exfoliation or did not yet have permanent teeth erupted, to ensure consistency across the cohort, only the primary canines and molars were included in the outcome variable.

Estimating Differences in Concordance Between Monozygotic and Dizygotic Twin Pairs, Adjusting for Known Risk Factors

To estimate similarities for monozygotic and dizygotic twins after adjusting for known risk factors, a multiple logistic regression model (fitted by using a GEE approach to account for correlation within pairs) was fitted to estimate the OR of being affected for a twin with twin who was affected after adjustment for covariates.[32,33] This model avoids the assumption that an underlying continuous liability exists and is easy to fit and interpret compared with alternatives such as generalized linear mixed models. Variance components models were to be fitted if differences in concordances were found to determine the contribution of additive genetic, common environmental, and unique environmental effects to variation in risk of any caries and advanced caries, respectively.

Environmental Risk Factors

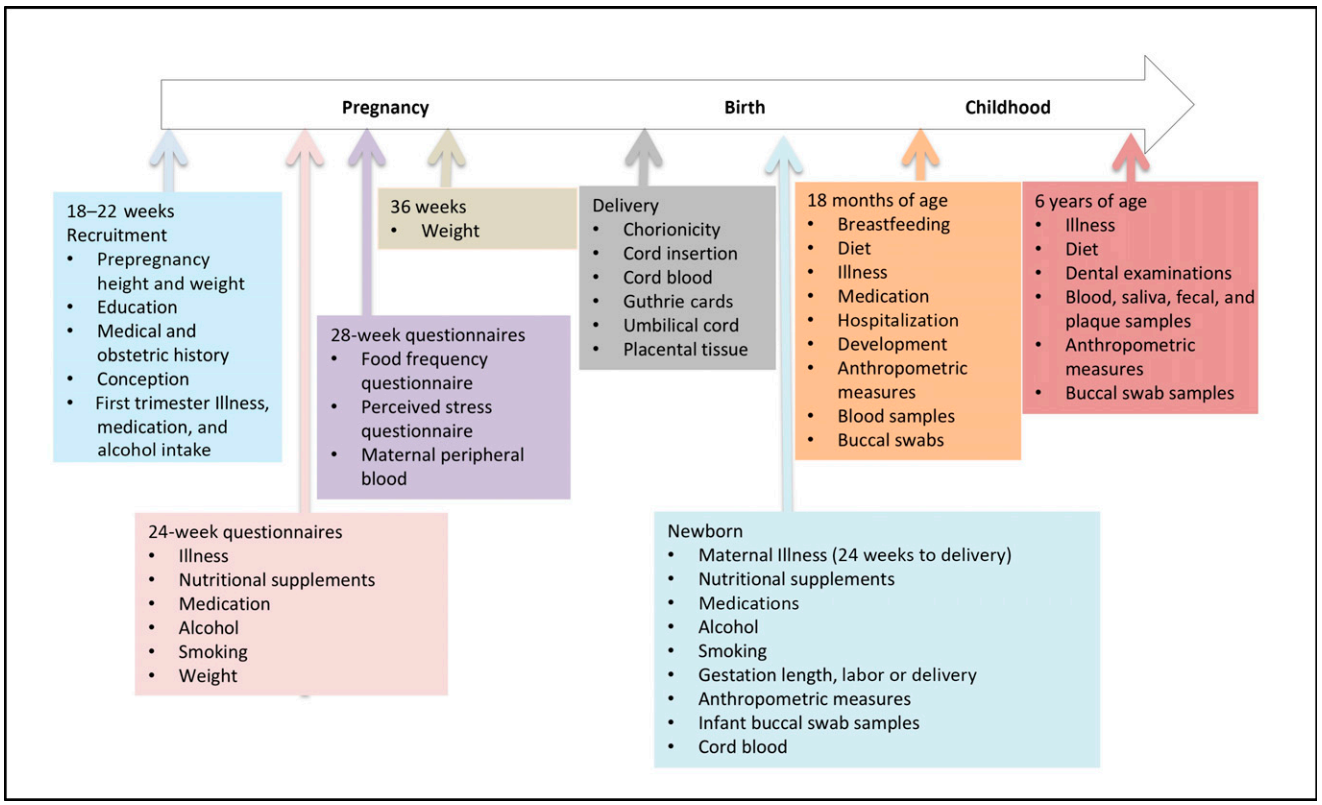
Linearity was checked for all continuous variables (age, SEIFA, maternal age at delivery, maternal BMI, maternal stress, BW, gestational age, and maternal and newborn vitamin D levels), and if disproven, a categorical variable was used instead. Exposure variables with $P <$ .1 in the simple regression models were combined in the final multiple regression models to adjust for confounding. Each of the 2 models (any and advanced caries) were then further refined by gradually eliminating all covariates with $P > .1$ in the multiple regression model until all remaining factors had $P < 0.1$ (Supplemental Table 9). As an exploratory study, an inclusive approach was adopted for model building because we aimed to identify potential factors rather than exclude factors. Goodness of fit of models with the same number of observations were compared by using the Bayesian information criterion. Postestimation diagnostics were performed by checking the Pearson residuals, deviance residuals, leverages, and DFBETAs to explore influential observations, and none were found to significantly influence the estimated regression coefficients.

RESULTS: DENTAL CARIES CONCORDANCE AFTER ADJUSTING FOR KNOWN RISK FACTORS

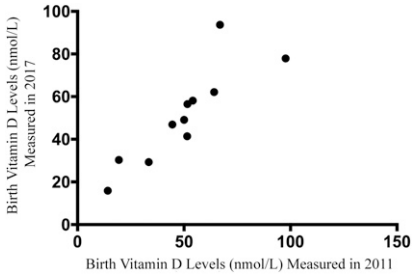
After adjusting for known risk factors for any caries (HSPMs, nonfluoridated town water, and dichorionicity), the OR of an individual with an affected

twin having any caries experience was 13.97 (95% CI 5.70 to 34.29; $P <$ .001). In addition, the OR of a dizygotic twin having an affected twin was 12.64 (95% CI 4.25 to 37.59; $P <$ .001) compared with 16.76 (95% CI 3.51 to 80.11; $P <$ .001) for monozygotic twins. However, there was little evidence for a difference between these (P = .77), consistent with no additive genetic influences on any caries experience after adjusting for known risk factors.

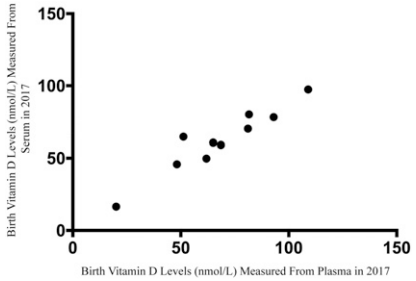
After adjusting for known risk factors for advanced caries (HSPMs, nonfluoridated town water, and maternal obesity), the OR of an individual with an affected twin having advanced caries experience was 8.24 (95% CI 3.24 to 20.97; $P <$ .001). In addition, the OR of a dizygotic twin having an affected twin was 8.89 (95% CI 2.61 to 30.25; $P <$ .001) compared with 7.47 (95% CI 1.79 to 31.16, P = .01) for monozygotic twins. However, there was little evidence for a difference between these (P = .86), consistent with no additive genetic influences on advanced caries experience after adjusting for known risk factors.



SUPPLEMENTAL FIGURE 2
Data collected in PETS cohort. The PETS cohort participated in 3 major phases of data collection: (1) pregnancy and birth, (2) 18 months of age, and (3) 6 years of age.



SUPPLEMENTAL FIGURE 3
Relationship between birth vitamin D level measurement in 2011 and 2017.



SUPPLEMENTAL FIGURE 4
Relationship between birth vitamin D level measurement from plasma in 2017 and serum in 2017.

SUPPLEMENTAL TABLE 3 Birth Vitamin D Levels Measured in 2011 and 2017

Participant	Birth Vitamin D Levels (nmol/L) Measured in 2011	Birth Vitamin D Levels (nmol/L) Measured in 2017
1	44.5	46.9
2	64.1	62.1
3	51.6	56.5
4	33.4	29.3
5	14.2	15.9
6	66.9	93.7
7	50.0	49.1
8	97.7	77.9
9	54.1	58.1
10	19.4	30.3
11	51.5	41.4

All measurements were obtained from serum samples from cord blood collected at birth.

SUPPLEMENTAL TABLE 5 Birth Vitamin D Levels Measured From Serum and Plasma (All 2017)

Participant	Birth Vitamin D Levels (nmol/L) Measured From Serum in 2017	Birth Vitamin D Levels (nmol/L) Measured From Plasma in 2017
1	16.5	20.1
2	49.8	61.9
3	80.1	81.6
4	78.2	93.0
5	70.3	81.1
6	64.7	51.2
7	60.7	65.0
8	45.8	48.2
9	97.4	109.0
10	59.1	68.6

SUPPLEMENTAL TABLE 6 Inter- and Intraexaminer Reliability for Diagnosis of MIH and HSPMs

	Mihiri J. Silva, DCD	Gold Standard	Pamela Leong, PhD
MIH and HSPMs: clinical presentation			
M.J.S.	93.4	95	82
P.L.	—	89	83
MIH and HSPMs: extent of lesion			
M.J.S.	96	90.6	80.1
P.L.	—	81.2	84.3

κ values for inter- and intraexaminer reliability for clinical presentation and extent of MIH and HSPMs. M.J.S., Mihiri J. Silva, DCD; P.L., Pam Leong, PhD; —, not applicable.

SUPPLEMENTAL TABLE 7 Inter- and Intraexaminer Reliability for Diagnosis of Dental Caries

	Mihiri J. Silva, DCD	Gold Standard	Pamela Leong, PhD
M.J.S.	94.2	91.3	84
P.L.	—	85.2	90.1

κ values for inter- and intraexaminer reliability for caries detection as scored by ICDAS. M.J.S., Mihiri J. Silva, DCD; P.L., Pam Leong, PhD; —, not applicable.

SUPPLEMENTAL TABLE 4 Summary of Batching Effects

	Mean (SD)	Difference in Means (95% CI), *P* (*t* Test)	*P*, Variance Ratio Test	Linear Regression: Slope (95% CI), *P*	Linear Regression: Intercept (95% CI), *P*	Deming Regression: Slope (95% CI), *P*	Deming Regression: Intercept (95% CI), *P*
Batching effect 1 (n = 11)							
Serum (2017)	51.02 (22.35)	1.25 (−6.65 to 9.16), .73	.93	0.84 (0.47 to 1.20), .001	9.20 (−10.70 to 29.10), .32	0.97 (0.19 to 1.74), .02	2.95 (−27.31 to 33.22), .83
Serum (2011)	49.76 (23.03)	—	—	—	—	—	—
Batching effect 2 (n = 10)							
Serum (2017)	62.26 (22.14)	−5.71 (−11.56 to 0.14), .06	.71	0.84 (0.61 to 1.06), .001	5.48 (−11.02 to 21.99), .47	0.88 (0.68 to 1.07), <.001	2.77 (−13.54 to 19.07), .71
Plasma (2017)	67.97 (25.12)	—	—	—	—	—	—

—, not applicable.

SUPPLEMENTAL TABLE 8 Comparison of Shared Exposures Between Subgroups Participating in Dental Examinations and Lost to Dropout

Variable	Dental Study *n* (%)	Dropouts *n* (%)	*P*
Zygosity, *n* (%)			
Monozygotic	71 (41.0)	29 (37.2)	.29
Dizygotic	102 (59.0)	48 (61.5)	
Unknown	0 (0)	1 (1.3)	
SEIFA, mean (SD)	1014.4 (57.9)	1007.6 (56.1)	.39
Missing, *n* (%)	1 (0.6)	1 (1.3)	
Maternal obesity (maternal BMI >30), *n* (%)			
Yes	22 (12.8)	9 (11.5)	.95
No	132 (76.3)	61 (78.2)	
Missing BMI	19 (11.0)	8 (10.3)	
Maternal stress (per unit), mean (SD)	22.6 (7.7)	20.8 (7.1)	.09
Missing values, *n* (%)	8 (4.6)	5 (6.4)	
Maternal infection during pregnancy, *n* (%)			.14
Yes	103 (59.5)	39 (50.0)	
No	70 (40.5)	38 (48.7)	
Missing	0 (0)	1 (1.3)	
Maternal antibiotic use during pregnancy, *n* (%)			.05
Yes	33 (19.1)	7 (9.0)	
No	140 (80.9)	70 (89.7)	
Missing	0 (0)	1 (1.3)	
Maternal vitamin D level at 28 wk (20 nmol/L), mean (SD)	56·3 (20.8)	53.3 (21.1)	.31
Missing, *n* (%)	8 (4.6)	2 (2.5)	
Maternal smoking in second or third trimester, *n* (%)			.27
Yes	23 (13.3)	8 (10.3)	
No	150 (86.7)	69 (88.5)	
Missing	0 (0)	1 (1.3)	
Maternal alcohol intake during pregnancy, *n* (%)			.12
Yes	100 (57.8)	37 (47.4)	
No	73 (42.2)	40 (51.3)	
Missing	0 (0)	1 (1.3)	
Gestational age, mean (SD), wk	35.8 (2.4)	35.9 (2.1)	.76
Missing values, *n* (%)	0 (0)	1 (1.3)	
Chorionicity, *n* (%)			.64
Monochorionic	48 (27.8)	19 (24.4)	
Dichorionic	109 (63.0)	49 (62.8)	
Missing	16 (9.3)	10 (12.8)	
Age of child at dental visit, mean (SD), y	6.7 (0.64)	—	—
Missing, *n* (%)	0 (0)		
Location of dental examination, *n* (%)		—	—
Home visit	142 (82.1)	—	—
Research facility	31 (17.9)	—	—
Nonfluoridated town water, *n* (%)	13 (7.5)	—	—
Fluoridated town water, *n* (%)	153 (88.4)	—	—
Fluoridation status unknown, *n* (%)	7 (4.1)	—	—

P values are from the χ^2 test (categorical variables) or *t* test (continuous variables). —, not applicable.

SUPPLEMENTAL TABLE 9 Comparison of Nonshared Exposures Collected at Birth Between Subgroups Participating in Dental Examinations and Lost to Dropout

Variable	Dental Study *n* (%)	Dropouts *n* (%)	*P*
Sex, *n* (%)			
Male	160 (46.4)	84 (53.5)	.21
Female	185 (53.6)	71 (45.2)	
Missing	0 (0)	2 (1.3)	
Mode of delivery, *n* (%)			.88
Vaginal delivery	119 (34.5)	54 (34.4)	
Cesarean delivery	226 (65.5)	99 (63.1)	
Missing	0 (0)	4 (2.6)	
Cord attachment, *n* (%)			
Central	141 (40.9)	58 (36.9)	Reference
Peripheral	122 (35.4)	58 (36.9)	.48
Velamentous	37 (10.7)	10 (6.4)	.31
Missing	45 (13.1)	31 (19.8)	
Birth wt, mean (SD), g	2483.2 (541.8)	2437.3 (535.1)	.23
Missing, *n* (%)	0 (0)	2 (1.3)	
Admission to NICU or SCN, *n* (%)			
Yes	146 (42.3)	59 (37.6)	.64
No	195 (56.5)	93 (59.2)	
Missing	4 (1.6)	5 (3.2)	
Child vitamin D level at birth (adjusted for batching effects), mean (SD), nmol/L	60.4 (21.4)	63.5 (18.7)	—
Missing, *n* (%)	104 (30.1)	96 (61.1)	

P values are from logistic regression by using GEEs to predict dropout at 6 y from exposure, adjusting for twin correlation. —, not applicable; SCN, special care nursery.

SUPPLEMENTAL TABLE 10 Comparison of Nonshared Exposures Collected at 18 Months and 6 Years of Age Between Subgroups Participating in Dental Examinations and Lost to Dropout

Variable	Dental Study *n* (%)	Dropouts *n* (%)	*P*
Breastfeeding, ever			.07
Yes	301 (87.3)	96 (61.2)	
No	29 (8.4)	22 (14.0)	
Missing	15 (4.4)	39 (24.8)	
Hospitalization in first 18 mo			.28
Yes	67 (19.4)	18 (11.5)	
No	264 (76.5)	99 (63.1)	
Missing	14 (4.1)	40 (25.5)	
Infection in first 18 mo			.24
Yes	171 (49.6)	51 (32.5)	
No	160 (46.4)	66 (42.0)	
Missing	14 (4.1)	40 (25.5)	
Antibiotics in first 18 mo			.10
Yes	75 (21.8)	17 (10.8)	
No	256 (74.2)	102 (65.0)	
Missing	14 (4.1)	38 (24.2)	
Asthma (18 mo to 6 y)			
Yes	55 (15.9)	—	—
No	282 (81.7)	—	—
Missing	8 (2.3)	—	—
High sugar intake	38 (11.0)	—	—
Medium sugar intake	214 (62.0)	—	—
Low sugar intake	83 (24.1)	—	—
Missing	10 (2.9)	—	—
Brushing frequency	—	—	—
Twice daily	204 (59.1)	—	—
Once a day	106 (30.7)	—	—
Once every 2–4 d	21 (6.1)	—	—
Missing	14 (4.1)	—	—
HSPMs	—	—	—
Yes	68 (19.7)	—	—
No	275 (79.7)	—	—
Missing	2 (0.6)	—	—

P values are from logistic regression by using GEEs to predict dropout at 6 y from exposure, adjusting for twin correlation. —, not applicable.

SUPPLEMENTAL TABLE 11 Multiple Regression Models for Any Caries

Factor	Model 1 With All Factors (n = 268)		Model 2 With $P < .1$ (n = 268)	
	Adjusted OR (95% CI)	P	Adjusted OR (95% CI)	P
HSPMs (n = 52)	2.63 (1.16 to 5.95)	.02	2.16 (1.04 to 4 > 47)	.04
Nonfluoridated water (n = 22)	5.51 (1.77 to 17.19)	.003	5.98 (1.59 to 22.55)	.01
Home visit (n = 45)	—	—		
Age				
Q1 (n = 64)				
Q2 (n = 51)	1.15 (0.39 to 3.38)	.80		
Q3 (n = 48)	1.31 (0.49 to 3.53)	.59		
Q4 (n = 58)	0.92 (0.30 to 2.83)	.88		
Q5 (n = 47)	1.74 (0.51 to 5.97)	.38		
Placenta, monochorionic (n = 89)	0.27 (0.11 to 0.63)	.003	0.37 (0.17 to 0.78)	.01
Maternal stress score				
Q1 (n = 58)				
Q2 (n = 64)	0.32 (0.11 to 0.94)	.01		
Q3 (n = 47)	0.20 (0.06 to 0.63)	.01		
Q4 (n = 66)	0.28 (0.10 to 0.78)	.02		
Q5 (n = 33)	1.18 (0.35 to 4.04)	.60		
Cord attachment				
Central cord (n = 124)				
Peripheral cord (n = 110)	1.88 (0.97 to 3.65)	.06		
Velamentous cord (n = 34)	1.29 (0.54 to 3.08)	.56		
Smoked after 12 wk (n = 34)	1.38 (0.46 to 4.14)	.57		

Model 1 includes all factors with $P < .1$ in the unadjusted regression analysis. Model 2 (final model) refines model 1, excluding factors with $P > .1$ in the multiple regression model. Although both models identify the same risk factors, the difference of 35.39 in Bayesian information criterion provides strong support for model 2 over model 1. —, not applicable; Q, quartile.

SUPPLEMENTAL TABLE 12 Multiple Regression Models for Advanced Caries

Factor	Model 1 With All Factors (n = 202)		Model 2 With $P < .1$ (n = 202)		Model 3 Excluding Birth Vitamin D, Including Maternal Vitamin D (n = 265)		Model 4 Excluding Birth Vitamin D, Including Maternal Vitamin D, $P <$ .1 (n = 265)	
	Adjusted OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Female sex	0.72 (0.33 to 1.56)	.41	—	—	0.77 (0.42 to 1.42)	.40	—	—
Age								
Q1	Reference	—	—	—	—	—	—	—
Q2	0.76 (0.19 to 3.03)	.70	—	—	0.65 (0.19 to 2.25)	.50	—	—
Q3	2.58 (0.74 to 8.97)	.14	—	—	1.71 (0.52 to 5.61)	.38	—	—
Q4	0.79 (0.25 to 2.46)	.68	—	—	0.47 (0.14 to 1.53)	.21	—	—
Q5	2.02 (0.53 to 7.67)	.30	—	—	1.44 (0.46 to 4.47)	.53	—	—
Nonfluoridated water	5.20 (1.40 to 18.49)	.01	5.85 (1. 45 to 23.70)	.01	5.20 (1.77 to 15.29)	.003	6.26 (1.74 to 22.53)	.01
Smoked after 12 wk	0.55 (0.13 to 2.39)	.42	—	—	1.64 (0.59 to 4.55)	.34	—	—
Birth vitamin D (20 nmol)	1.41 (0.90 to 2.21)	.13	—	—	—	—	—	—
Maternal vitamin D (20 nmol)	—	—	—	—	1.49 (0.90 to 2.45)	.12	—	—
Maternal obesity	5.45 (1.80 to 16.47)	.003	4.44 (1.73 to 11.37)	.002	3.25 (1.32 to 8.02)	.01	2.68 (1.19 to 6.08)	.02
HSPMs	2.62 (0.99 to 6.92)	.05	2.31 (0.94 to 5.67)	.07	2.08 (0.92 to 4.67)	.08	2.43 (1.11 to 5.36)	.03
Monochorionic	0.33 (0.12 to 0.93)	.04	0.39 (0.15 to 0.97)	.04	0.44 (0.19 to 1.05)	.06	—	—

Model 1 includes all factors with $P < .1$ in the unadjusted regression analysis; Because of collinearity with maternal vitamin D, only newborn vitamin D is included in this model. Model 2 refines model 1, excluding factors with $P > .1$ in the multiple regression model. Model 3 includes all factors with $P < .1$ in the unadjusted regression analysis (including maternal vitamin D instead of newborn vitamin D), resulting in a larger number of observations (n = 265). Model 4 (final model) refines model 3, excluding factors with $P > .1$ in the multiple regression model. Although both models identify similar risk factors, the difference of 28.03 in Bayesian information criterion provides strong support for model 4 over model 3. Q, quartile; —, not applicable.

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