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Authors	McCarthy, Elaine K.;Malvisi, Lucio;Kiely, Mairead E.;Murray, Deirdre M.;Hourihane, Jonathan O'B.;Irvine, Alan D.;Kenny, Louise C.
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Antenatal vitamin D status is not associated with standard neurodevelopmental assessments at five years in a well-characterised prospective maternal-infant cohort

Elaine K McCarthy,[1,2] Deirdre M Murray,[2,3] Lucio Malvisi,[1,2] Louise C Kenny,[4] Jonathan O'B Hourihane,[2,3] Alan D Irvine,[2,5-7] Mairead E Kiely[1,2*]

[1]Cork Centre for Vitamin D and Nutrition Research, School of Food and Nutritional Sciences, University College Cork, Republic of Ireland

[2]The Irish Centre for Fetal and Neonatal Translational Research (INFANT), University College Cork, Republic of Ireland

[3]Department of Paediatrics and Child Health, University College Cork, Republic of Ireland

[4]Department of Women's and Children's Health, University of Liverpool, UK

[5]Department of Clinical Medicine, Trinity College, Dublin, Republic of Ireland

[6]Department of Paediatric Dermatology, Our Lady's Children's Hospital, Dublin, Republic of Ireland

[7]National Children's Research Centre, Dublin, Republic of Ireland

***Corresponding author** Prof. Mairead Kiely, Cork Centre for Vitamin D and Nutrition Research, Room 127, Food Science Building, University College Cork, Cork, Republic of Ireland. Email: m.kiely@ucc.ie Phone: +353214903394

Surnames McCarthy, Murray, Malvisi, Kenny, Hourihane, Irvine, Kiely

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Running title Antenatal 25(OH)D and childhood neurodevelopment

Clinical Trial Registration BASELINE Study NCT01498965 (www.clinicaltrials.gov); SCOPE Study ACTRN12607000551493 (http://www.anzctr.org.au)

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Abbreviations used BASELINE: Babies after SCOPE: Evaluating the Longitudinal Impact using Neurological and Nutritional Endpoints; CBCL: Child Behaviour Checklist; IQ: intelligence quotient; KBIT-2: Kaufman Brief Intelligence Test, 2nd Edition; SCOPE: Screening for Pregnancy Endpoints; 25(OH)D: 25-hydroxyvitamin D; 25(OH)D_3: 25-hydroxyvitamin D_3; 25(OH)D_2: 25-hydroxyvitamin D_2; 3-epi-25(OH)D_3: 3-epi-25-hydroxyvitamin D_3.

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ABSTRACT

Background Although animal studies show evidence for a role of vitamin D during brain development, data from human studies show conflicting signals.

Objective We aimed to explore associations between maternal and neonatal vitamin D status with childhood neurodevelopmental outcomes.

Methods Comprehensive clinical, demographic and lifestyle data were collected prospectively in 734 maternal-infant dyads from the Cork BASELINE Birth Cohort Study. Serum 25-hydroxyvitamin D (25(OH)D) concentrations were quantified at 15 weeks' gestation and in umbilical cord sera at birth using a CDC-accredited LC-MS/MS method. Children were assessed at five years using the Kaufman Brief Intelligence Test (2nd Edition, KBIT-2) and the Child Behaviour Checklist (CBCL). Linear regression was used to explore associations between 25(OH)D and neurodevelopmental outcomes.

Results 25(OH)D concentrations were <30nmol/L in 15% of maternal and 45% of umbilical cord sera and <50nmol/L in 42% of mothers and 80% of cords. At five years, the mean (SD) KBIT-2 IQ composite score was 104.6 (8.6); scores were 107.2 (10.0) in verbal and 99.8 (8.8) in non-verbal tasks. Developmental delay (scores <85) was seen in <3% of children across all domains. The mean (SD) CBCL total problem score was 21.3 (17.5); scores in the abnormal/clinical range for internal, external and total problem scales were present in 12%, 4% and 6% of participants. KBIT-2 and CBCL subscale scores at five years were not different between children exposed to low antenatal vitamin D status, either at 30 or 50nmol/L 25(OH)D thresholds. Neither maternal nor cord 25(OH)D (per 10nmol/L) were associated with KBIT-2 IQ composite scores (adjusted β (95% CI): maternal -0.01 (-0.03, 0.02); cord 0.01 (-0.03, 0.04)) or CBCL total problem scores (maternal 0.01 (-0.04, 0.05); cord 0.01 (-0.07, 0.09)).

Conclusions In this well-characterized prospective maternal-infant cohort, we found no evidence that antenatal 25(OH)D concentrations are associated with neurodevelopmental outcomes at five years.

KEYWORDS vitamin D, serum 25-hydroxyvitamin D, neurodevelopment, intelligence, antenatal.

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INTRODUCTION

Vitamin D deficiency is a public health concern, with pregnant women and their infants at particular risk (1, 2). A recent systematic review summarizing maternal and neonatal vitamin D status globally reported that over half of pregnant women and three-quarters of neonates have serum 25-hydroxyvitamin D (25(OH)D) concentrations <50 nmol/L (3). We have published similar findings in Ireland, indicating that 17% of mothers in their 2nd trimester and 46% of their neonates at birth have 25(OH)D concentrations <30 nmol/L (4, 5). This is concerning given that low 25(OH)D concentrations during pregnancy have been associated with an increased risk of pregnancy complications, including gestational diabetes, preeclampsia and small-for-gestational age infants (6). Additionally, as neonatal 25(OH)D concentrations are dependent on maternal concentrations, infants born to vitamin D deficient mothers are at an increased risk of neonatal deficiency and its associated consequences for infant and long-term health (7, 8).

One potential consequence of early life vitamin D deficiency for infant health is brain development and function. In vitro studies have provided compelling evidence for a potential role of vitamin D during fetal brain development. Both the vitamin D receptor and CYP27B1 are expressed in the human brain (9). Vitamin D metabolites have also been shown to cross the blood-brain barrier (10). Furthermore, animal models have illustrated that vitamin D influences the developing brain through the regulation of important processes, including the maintenance of calcium balance, enhancement of signal transmission and synaptic plasticity, neuroprotection and modulation of neuronal differentiation, maturation and growth (11, 12). These rodent models also suggest that vitamin D deficiency *in utero* can modify the expression of multiple genes and proteins in the brain resulting in altered brain structure and function (10). However, the translation of this evidence into humans is unclear.

To date, 10 observational studies in humans have investigated associations between 25(OH)D concentrations either in early/mid (13-15) or late pregnancy (16-19) and/or in umbilical cord blood at birth (20-22) and measures of childhood neurodevelopment. Findings have been mixed and

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inconclusive, due mainly to the substantial variability in study design, as summarised in **Table 1**. A number of these studies have also been restricted to historical data, while only one study has investigated the influence of 25(OH)D concentrations in both the fetal and early neonatal period (20). Given the high prevalence of vitamin D deficiency in pregnant women and their infants, its potential impact on childhood neurodevelopment is an important consideration. Therefore, the aim of the current study was to explore associations between maternal and neonatal serum 25(OH)D concentrations and neurodevelopmental outcomes in children aged five years in a prospective maternal-infant birth cohort in Ireland.

METHODS

Study design and participants

Participants were recruited to the Cork BASELINE (Babies after SCOPE: Evaluating the Longitudinal Impact using Neurological and Nutritional Endpoints) Birth Cohort Study (www.clinicaltrials.gov NCT01498965) between March 2008 and January 2011. The BASELINE Study is a follow-on to the SCOPE (Screening for Pregnancy Endpoints) Ireland pregnancy study (http://www.anzctr.org.au ACTRN12607000551493), where low risk, nulliparous women with a singleton pregnancy were recruited before 15 weeks' gestation from Cork University Maternity Hospital, as part of an international multicentre study aimed at investigating early indicators of pregnancy complications (23). At 15 weeks' gestation, research midwives collected information on maternal socioeconomic status, occupation, education, relationship status and a complete medical history. Information on nutritional supplement use, recreational activity, cigarette, drug and alcohol use were recorded for the three-month period prior to conception and during the first trimester. Maternal anthropometric and clinical measurements were also collected prospectively during pregnancy.

Women in the SCOPE Ireland study (n = 1537) provided written informed consent to the BASELINE Study for their infants at 20 weeks' gestation. Their infants were followed prospectively from birth, with assessments at day 2 and at 2, 6, 12 and 24 months. Assessments at five years of age were

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completed in December 2016. Detailed information on early life environment, diet, lifestyle, health, growth and development was gathered by interviewer-led questionnaires and clinical assessments performed by trained researchers in accordance with the Declaration of Helsinki, with further information on study design and procedures reported previously (24). Ethical approval for both SCOPE Ireland and the Cork BASELINE Birth Cohort Study was granted by the Clinical Research Ethics Committee of the Cork teaching hospitals (SCOPE: ECM 5(10) 05/02/2008, BASELINE: ECM 5(9) 01/07/2008).

Neurodevelopmental assessments

At the study's five year assessment, participants completed two neurodevelopmental assessments, 1) the Kaufman Brief Intelligence Test, 2nd Edition (KBIT-2) and 2) the Child Behaviour Checklist (CBCL).

The KBIT-2 is designed as a brief, individualised test to measure verbal and non-verbal intelligence in children and adults, from age 4-90 years (25). It is used to screen the intellectual abilities of an individual and identify those who may be at risk of academic problems. The assessment consists of three subtests, two of which are verbal (Verbal Knowledge and Riddles) and one non-verbal (Matrices). The subtests involve individually administered verbal and non-verbal tasks that do not require reading or spelling but consist of verbal questions, illustrations and visual stimuli. The verbal subtests assess verbal concept formation, word meaning and reasoning, while the non-verbal subtest assesses fluid reasoning, visual processing and problem solving. The assessment was administered by a research nurse trained in administration and interpretation of the test. After the examination was complete, the verbal and non-verbal scales were tallied, standardized for age and transformed into a composite IQ score. The standard score for each component has a mean of 100 and a standard deviation of 15, with scores less than 85 considered abnormal or represent developmental delay.

Emotional and behavioural problems were assessed by the CBCL for ages 1.5-5 years (26). The CBCL is a 99-item validated screener checklist completed by parents/caregivers, indicating the frequency of particular behaviours in their child over the past two months on a three-point scale (not

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104 true, sometimes true or very/often true), with increasing scores indicating increasing behavioural
105 issues/problems. The CBCL comprises of two broadband scales, Internal Problem Score and External
106 Problem Score. The Internal Problem Score is made up of scores from four individual syndrome
107 scales: Emotionally Reactive, Anxious/depressed, Somatic Complaints (physical complaints such as
108 nausea, headaches etc.) and Withdrawn. The External Problem Score is made up of scores from two
109 individual syndrome scales: Attention Problems and Aggressive Behaviour. Summing the Internal
110 Problem Score and the External Problem Score with two further individual scale scores: Sleep
111 Problems and Other Problems, provides a Total Problem Score. For all scales, scores ≥93rd percentile
112 were designated as borderline abnormal and scores ≥98th percentile as clinically abnormal. For this
113 analysis, all scores ≥93rd percentile were denoted as abnormal, indicating significant behavioural
114 problems.

115 **Biological samples and analytical methods**

116 Blood samples were collected from mothers at 15 weeks' gestation and from the umbilical cord at
117 birth and were processed to serum within three hours of collection and stored at -80°C until use.
118 Circulating 25-hydroxyvitamin D_3 (25(OH)D_3), 25-hydroxyvitamin D_2 (25(OH)D_2) and 3-epi-25-
119 hydroxyvitamin D_3 (3-epi-25(OH)D_3) concentrations were measured at the Cork Centre for Vitamin D
120 and Nutrition Research laboratory with the use of a liquid chromatography–tandem mass
121 spectrometry (LC-MS/MS) method that has been described in detail previously (4, 27). The
122 instrument used was a Waters Acquity UPLC system coupled to an Acquity Triple Quadrupole TQD
123 mass-spectrometer detector (Waters, Dublin 9, Ireland). Concentrations of 25(OH)D_3 and 25(OH)D_2
124 were quantified individually and their values were summed to generate total 25(OH)D.
125 Chromatographic separation and quantitation of 3-epi-25(OH)D_3 was also achieved. Four amounts of
126 serum-based National Institute of Standards and Technology (NIST)-certified quality-assurance
127 material (standard reference material 972) were used for method validation, while quality-control
128 materials that were assayed in parallel to all samples were purchased from Chromsystems (Germany).
129 NIST calibrators were used throughout the analysis (standard reference material 2972). The intra- and
130 inter-assay coefficients of variation were not greater than 6 and 5%, respectively, for all metabolites.

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The limit of detection for 25(OH)D_3, 3-epi-25(OH)D_3, and 25(OH)D_2 were 0.31, 0.20, and 0.44 nmol/L, respectively and the limit of quantitation was 1.03, 0.66, and 1.43 nmol/L, respectively. The quality and accuracy of the vitamin D metabolite analysis in our laboratory is assessed on an on-going basis by participation in the Vitamin D External Quality Assessment Scheme (DEQAS) (Charing Cross Hospital, London UK). We also participate in the CDC Vitamin D Standardization Certification program, which reports accuracy and bias for total 25(OH)D, 25(OH)D_3, 3-epi-25(OH)D_3 and 25(OH)D_2, since 2013.

Data analysis

Data were analysed using IBM SPSS® for Windows™ version 23 (IBM Corp., Armonk, NY, USA) and Statistical Analysis System (SAS) version 9.4 (SAS Institute Inc.). Descriptive statistics (mean, standard deviation (SD), median, quartiles (IQR), frequencies and percentages) were generated. Comparisons between categorical variables were made using Chi square (χ^2) tests, while independent t-tests or Mann-Whitney U tests were employed for continuous variables, depending on their distribution. Multiple linear regression was used to explore associations between maternal and neonatal 25(OH)D concentrations and neurodevelopmental outcomes at five years of age. Serum 25(OH)D concentrations were analysed firstly as continuous variables and secondly, to investigate a potential threshold effect, both maternal and neonatal 25(OH)D were divided into three categories (<30 nmol/L, 30-<50 nmol/L, ≥50 nmol/L). The categories were decided upon based on the thresholds for deficiency/sufficiency proposed by the US Institute of Medicine (28) and the vitamin D literature, given the lack of reference intervals for umbilical cord 25(OH)D concentrations in particular. Separate linear regression models (24 in total) were built for each predictor-outcome association with adjustment for covariates based on both statistical significance and clinical and theoretical knowledge. In each model, initial associations between serum 25(OH)D concentrations (and other potential confounders) with the outcomes (KBIT-2 and CBCL scores) were assessed by univariable linear regressions in which the significance level was set at alpha=0.25. Multivariable models that included serum 25(OH)D and other covariates that were significant in the univariable analysis were then built and assessed. At this stage, any non-significant covariates at alpha=0.05 were

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158 either kept in the model if clinically relevant or dropped. Linearity and constant error variance were
159 then evaluated visually, through scatter plots, and statistically, through the White test, for both the
160 outcome and each of the predictors in the model. Normality of distribution of residuals was also
161 assessed both visually, through histograms and normal probability plots, and statistically, through the
162 Shapiro-Wilk test. Final model selection between sets of potential covariates was also aided by
163 Mallows' Cp criterion and PRESS statistic. Associations were expressed as adjusted estimates with
164 95% confidence intervals (95% CI) and P <0.05 was considered significant in final models.

165 **RESULTS**

166 Of the 920 firstborn children that attended the study's five year assessment, 83% (n = 763) completed
167 both the KBIT-2 and the CBCL. Children that were born premature (<37 weeks' gestation, n = 29)
168 were excluded, providing a final sample size for this study of 734 (**Figure 1**). Principal
169 characteristics of the mothers and their infants are presented in **Table 2**. The median [IQR] age of
170 mothers at delivery was 31.0 [29.0, 33.0] years and most were Caucasian. Vitamin D supplements
171 (dose ranged from 2.5 to 10 µg/day) were taken by 42% of women at 15 weeks' gestation.

172 Serum 25(OH)D concentrations were measured in all 734 mothers at 15 weeks' gestation and in 547
173 umbilical cords. Mean (SD) serum 25(OH)D concentrations in mothers and infants were 58.3 (25.8)
174 nmol/L and 35.1 (18.2) nmol/L, respectively. Vitamin D deficiency (<30 nmol/L) was observed in
175 15% of mothers, while 42% had 25(OH)D concentrations <50 nmol/L. Almost half (45%) of infants
176 were born deficient (34% were <25 nmol/L) and 80% had concentrations <50 nmol/L. Both maternal
177 and neonatal mean (SD) 25(OH)D concentrations were higher in summer (maternal: 67.0 (23.7)
178 nmol/L, neonatal: 44.5 (17.7) nmol/L) than in winter (maternal: 52.0 (25.5) nmol/L, neonatal: 28.0
179 (15.1) nmol/L, both P <0.0001), with 63% of infants born deficient in winter compared to 22% in
180 summer (P <0.0001). Only two mothers, and no infants had 25(OH)D >125 nmol/L.

181 At five years, the mean (SD) IQ composite score was 104.6 (8.6), with higher scores reported in
182 verbal tasks (107.2 (10.0)) than non-verbal tasks (99.8 (8.8)). The prevalence of developmental delay,
183 indicated by scores <85 on the KBIT-2 was <3% across all domains. The mean (SD) CBCL total

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problem score for the study population was 21.3 (17.5), with scores in the clinical/abnormal range in the internal, external and total problem scales observed in 12%, 4% and 6% of participants, respectively.

KBIT-2 and CBCL subscales scores at five years did not differ between those with maternal or cord 25(OH)D concentrations above or below 30 nmol/L or 50 nmol/L. **Supplemental Figure 1** presents the distribution of maternal and cord serum 25(OH)D concentrations with neurodevelopmental outcomes. There was no evidence of an association between maternal serum 25(OH)D concentrations and intelligence or behavioural outcomes assessed by the KBIT-2 and CBCL, either in unadjusted or adjusted multivariable linear regression models (**Table 3**). When maternal 25(OH)D concentrations were categorised, using the lower threshold of <30 nmol/L as the reference group, no significant differences in KBIT-2 or CBCL subscale scores between 25(OH)D categories were observed (Table 3). Cord 25(OH)D at birth was not associated with intelligence or behavioural outcomes at five years and when cord 25(OH)D was divided into categories, there was also no evidence of an association with KBIT-2 or CBCL subscale scores (**Table 4**).

DISCUSSION

In this prospective maternal-infant birth cohort, with a high prevalence of low vitamin D status among pregnant women and new-borns, we found no evidence to suggest that antenatal 25(OH)D concentrations are associated with childhood neurodevelopmental outcomes at five years.

Our observation that maternal 25(OH)D concentrations at 15 weeks' gestation were not associated with childhood intelligence scores at five years was consistent with reports from two similar maternal-infant cohorts in the UK (16) and Denmark (18), although in both of those studies, maternal vitamin D status was measured in the 3rd trimester. The 2nd trimester has been suggested as a potentially important period of vulnerability to vitamin D deficiency during fetal brain development. In the Australian Raine cohort, using a quartile analysis, children born to women with 25(OH)D ≤46 nmol/L during their 2nd trimester had an almost twofold increased risk of language difficulties at five and 10 years of age compared to those whose mothers had concentrations >70 nmol/L (14). In a racially and

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socioeconomically diverse birth cohort in North America, Tylavsky and colleagues also reported a small, positive association with language development in two year olds (15). In contrast, Keim *et al.* observed no association between maternal 25(OH)D in the 2nd trimester and reading or spelling achievement (20), albeit within a different timeframe. With regard to motor development, modest associations with maternal 25(OH)D in preschool-age children have been observed in studies in Spain and the UK (13, 19), however this could be due to an effect of maternal 25(OH)D on fetal musculoskeletal development and/or brain development, resulting in altered motor function. Given these contrasting findings, the literature describing any influence of maternal vitamin D status during pregnancy on fetal brain development is immature and requires careful study.

Associations between cord 25(OH)D concentrations and childhood neurodevelopmental outcomes have been described previously in three studies (20-22), although ours is the first report from a European cohort. In contrast to these studies, we observed no significant association between cord 25(OH)D and intelligence at five years of age. Zhu and colleagues in China reported an inverted U-shaped relationship between cord 25(OH)D and mental and psychomotor development at 16-18 months (21), although these data should be interpreted with caution given the study's relatively small sample size and use of radioimmunoassay to measure cord 25(OH)D concentrations. In the secondary analysis of historical data from the US Collaborative Perinatal Project (1959-73) performed by Keim and colleagues, the modest, positive association observed with intelligence at four and seven years was inconsistent and attenuated following adjustment for confounders (20). In mother-child dyads recruited as part of an antenatal docosahexaenoic acid RCT, Gould *et al.* reported a small, positive association with language development at 18 months and four years, although a 10 nmol/L increase in cord 25(OH)D was only associated with a 0.60-0.67 increase in language scores (22). While these studies have observed relatively small associations between cord 25(OH)D and neurodevelopmental outcomes, the study designs were heterogeneous and importantly, the magnitude of the reported associations was very small.

Our finding of no association between either maternal or cord 25(OH)D with behavioural outcomes at five years is in accordance with previous reports. Parent-report assessments of behaviour similar to

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those used in the current study have been employed in three other studies, with all studies reporting no association with either maternal or cord 25(OH)D (14, 16, 19). The Strengths and Difficulties Questionnaire was used by Gale *et al.* (16) and Darling *et al.* (19) in the UK, while in the Raine cohort, no association between maternal 25(OH)D and behavioural outcomes, as assessed by the CBCL, were observed throughout childhood to the age of 17 years (14). Studies that have used more objective, psychologist administered assessments, such as the Bayley Scales of Infant and Toddler Development, have also reported no association with maternal or cord 25(OH)D concentrations (17, 20, 22). Altogether, there seems to be little evidence to suggest that either maternal or neonatal vitamin D status influences behavioural or emotional development.

While animal studies have provided a plausible biological basis indicating a role for vitamin D during fetal brain development, the evidence from human studies continues to show conflicting signals. Significant heterogeneity in study design, as summarised in Table 1, has contributed largely to the mixed findings, particularly in the timing and methods employed for both the exposure and outcome assessments and the statistical analysis applied with respect to the use of cut-offs for 25(OH)D concentrations and potential confounders. Therefore, the timing and duration, or indeed the presence of, a critical window of vulnerability and susceptibility to vitamin D deficiency or insufficiency during brain development is yet to be fully determined. Importantly, this critical window could be later in the postnatal period, as early infancy is another crucial period of rapid brain development. The plasticity of the young brain in the postnatal period and its ability for repair should also be acknowledged, as although almost half of our cohort had a 25(OH)D concentration <30 nmol/L at delivery, indicating a high risk of nutritional deficiency, fewer than 5% were <30 nmol/L at two and five years (29). Further consideration of these issues will enable more targeted and specific assessments of the developmental outcomes that are most likely to be affected by vitamin D deficiency. However, reliance on global developmental assessments in early childhood is a still a limitation of this research field as such assessments may not be sensitive enough to identify specific developmental processes that are affected by nutritional factors including vitamin D (30).

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Apart from the study by Keim *et al*. that utilised data from a 1950's US cohort (20), our study is the only other to report the effects of vitamin D status in both the fetal and early neonatal period on childhood neurodevelopmental outcomes. The prospective design of the Cork BASELINE Birth Cohort Study, with its multidisciplinary team and use of validated neurodevelopmental assessments are strengths of this study. The sample size, extensively characterised participants and use of the gold standard CDC-accredited method for measuring serum 25(OH)D concentrations are other advantages. The generalizability of our results may be limited, given the regional recruitment of the cohort and predominantly Caucasian sample; however, the findings are still generalizable to other healthy, Caucasian, low risk maternal-infant populations. Parental intelligence, considered an important determinant of child development was not measured directly in this study; however maternal educational attainment and household income were considered as proxy measures in the analysis. The overall normal developmental profile observed in our cohort is unsurprising and is reflective of the high-resource population studied.

To conclude, in this prospective maternal-infant birth cohort in Ireland, we found no evidence of an association between antenatal 25(OH)D concentrations and intelligence or behavioural outcomes in five-year-old children. Further research is required to identify and define the periods in brain development that vitamin D is critical for. Longitudinal studies with vitamin D status measured at multiple time-points throughout gestation and the early neonatal period, along with long-term follow-up of neurodevelopmental outcomes using appropriate validated assessments are required to ascertain this.

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Contributor statement E.K.M. and M.E.K. conducted the research, E.K.M. and L.M. analysed the data and E.K.M. and M.E.K. wrote the manuscript. M.E.K. had primary responsibility for the final content. D.M.M. is the overall PI of the Cork BASELINE Birth Cohort Study and J.O'B.H., L.C.K., A.D.I. and M.E.K. are co-PIs and specialist leads. L.C.K. is the PI of the SCOPE Ireland pregnancy cohort study. All PIs were responsible for design of the research project and all authors reviewed and approved the final manuscript.

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REFERENCES

1. Dawodu A, Wagner CL. Mother-child vitamin D deficiency: an international perspective. Arch Dis Child 2007;92(9):737-40.

2. Kiely M, Hemmingway A, O'Callaghan KM. Vitamin D in pregnancy: current perspectives and future directions. Ther Adv Musculoskelet Dis 2017;9(6):145-54.

3. Saraf R, Morton SM, Camargo CA, Jr., Grant CC. Global summary of maternal and newborn vitamin D status - a systematic review. Matern Child Nutr 2016;12(4):647-68.

4. Kiely ME, Zhang JY, Kinsella M, Khashan AS, Kenny LC. Vitamin D status is associated with uteroplacental dysfunction indicated by pre-eclampsia and small-for-gestational-age birth in a large prospective pregnancy cohort in Ireland with low vitamin D status. Am J Clin Nutr 2016;104(2):354-61.

5. Kiely M, O'Donovan SM, Kenny LC, Hourihane JO, Irvine AD, Murray DM. Vitamin D metabolite concentrations in umbilical cord blood serum and associations with clinical characteristics in a large prospective mother-infant cohort in Ireland. J Steroid Biochem Mol Biol 2017;167:162-8.

6. Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M, Rabi DM. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. BMJ 2013;346:f1169.

7. Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM. High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. J Nutr 2007;137(2):447-52.

8. Brannon PM, Picciano MF. Vitamin D in pregnancy and lactation in humans. Annu Rev Nutr 2011;31:89-115.

9. Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. J Chem Neuroanat 2005;29(1):21-30.

10. Eyles D, Burne T, McGrath J. Vitamin D in fetal brain development. Semin Cell Dev Biol 2011;22(6):629-36.

Accepted Manuscript

11. Groves NJ, McGrath JJ, Burne TH. Vitamin D as a neurosteroid affecting the developing and adult brain. Annu Rev Nutr 2014;34:117-41.

12. Tare M, Emmett SJ, Coleman HA, Skordilis C, Eyles DW, Morley R, Parkington HC. Vitamin D insufficiency is associated with impaired vascular endothelial and smooth muscle function and hypertension in young rats. J Physiol 2011;589(Pt 19):4777-86.

13. Morales E, Guxens M, Llop S, Rodriguez-Bernal CL, Tardon A, Riano I, Ibarluzea J, Lertxundi N, Espada M, Rodriguez A, et al. Circulating 25-hydroxyvitamin D3 in pregnancy and infant neuropsychological development. Pediatrics 2012;130(4):e913-20.

14. Whitehouse AJ, Holt BJ, Serralha M, Holt PG, Kusel MM, Hart PH. Maternal serum vitamin D levels during pregnancy and offspring neurocognitive development. Pediatrics 2012;129(3):485-93.

15. Tylavsky FA, Kocak M, Murphy LE, Graff JC, Palmer FB, Volgyi E, Diaz-Thomas AM, Ferry RJ, Jr. Gestational Vitamin 25(OH)D Status as a Risk Factor for Receptive Language Development: A 24-Month, Longitudinal, Observational Study. Nutrients 2015;7(12):9918-30.

16. Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, Godfrey KM, Cooper C. Maternal vitamin D status during pregnancy and child outcomes. Eur J Clin Nutr 2008;62(1):68-77.

17. Hanieh S, Ha TT, Simpson JA, Thuy TT, Khuong NC, Thoang DD, Tran TD, Tuan T, Fisher J, Biggs BA. Maternal vitamin D status and infant outcomes in rural Vietnam: a prospective cohort study. PLoS One 2014;9(6):e99005.

18. Strom M, Halldorsson TI, Hansen S, Granstrom C, Maslova E, Petersen SB, Cohen AS, Olsen SF. Vitamin D measured in maternal serum and offspring neurodevelopmental outcomes: a prospective study with long-term follow-up. Ann Nutr Metab 2014;64(3-4):254-61.

19. Darling AL, Rayman MP, Steer CD, Golding J, Lanham-New SA, Bath SC. 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 2017:1-11.

20. Keim SA, Bodnar LM, Klebanoff MA. Maternal and cord blood 25(OH)-vitamin D concentrations in relation to child development and behaviour. Paediatr Perinat Epidemiol 2014;28(5):434-44.

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21. Zhu P, Tong SL, Hao JH, Tao RX, Huang K, Hu WB, Zhou QF, Jiang XM, Tao FB. Cord blood vitamin D and neurocognitive development are nonlinearly related in toddlers. J Nutr 2015;145(6):1232-8.

22. Gould JF, Anderson AJ, Yelland LN, Smithers LG, Skeaff CM, Zhou SJ, Gibson RA, Makrides M. Association of cord blood vitamin D with early childhood growth and neurodevelopment. J Paediatr Child Health 2017;53(1):75-83.

23. Kenny LC, Black MA, Poston L, Taylor R, Myers JE, Baker PN, McCowan LM, Simpson NA, Dekker GA, Roberts CT, et al. Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers: the Screening for Pregnancy Endpoints (SCOPE) international cohort study. Hypertension 2014;64(3):644-52.

24. O'Donovan SM, Murray DM, Hourihane JO, Kenny LC, Irvine AD, Kiely M. Cohort profile: The Cork BASELINE Birth Cohort Study: Babies after SCOPE: Evaluating the Longitudinal Impact on Neurological and Nutritional Endpoints. Int J Epidemiol 2015;44(3):764-75.

25. Kaufman AS, Kaufman NL. Kaufman Brief Intelligence Test Second Edition Bloomington, MN: Pearson, Inc; 2004.

26. Achenbach RM, Rescorla LA. Manual for the ASEBA Preschool Forms & Profiles. Burlington, VT: University of Vermont, Research Center for Children, Youth & Families; 2000.

27. Cashman KD, Kiely M, Kinsella M, Durazo-Arvizu RA, Tian L, Zhang Y, Lucey A, Flynn A, Gibney MJ, Vesper HW, et al. Evaluation of Vitamin D Standardization Program protocols for standardizing serum 25-hydroxyvitamin D data: a case study of the program's potential for national nutrition and health surveys. Am J Clin Nutr 2013;97(6):1235-42.

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

29. Ni Chaoimh C, McCarthy EK, Hourihane JO, Kenny LC, Irvine AD, Murray DM, Kiely ME. Low vitamin D deficiency in Irish toddlers despite northerly latitude and a high prevalence of inadequate intakes. Eur J Nutr 2016, doi: 10.1007/s00394-016-1368-9.

30. Wainwright PE, Colombo J. Nutrition and the development of cognitive functions: interpretation of behavioral studies in animals and human infants. Am J Clin Nutr 2006;84(5):961-70.

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Table 1 Summary of observational studies exploring associations between antenatal 25-hydroxyvitamin D (25(OH)D) concentrations and childhood neurodevelopment outcomes

	Study type	No. of participants[1]	Sampling for 25(OH)D	25(OH)D analytical method	Neurodevelopmental assessment (age at assessment)
Morales *et al.*, 2012 [Spain] (13)	Prospective cohort Recruited: 2003-08	1820	13.5 weeks gestation	HPLC	BSID (14 months)
Whitehouse *et al.*, 2012 [Australia] (14)	Prospective cohort Recruited: 1989-91	743	18 weeks gestation	Enzyme immunoassay	CBCL (2, 5, 8, 10, 14, 17 years) Peabody Picture Vocabulary Test (5, 10 years)
Tylavsky *et al.*, 2015 [USA] (15)	Prospective cohort Recruited: 2006-11	1020	2nd trimester	Enzyme immunoassay	BSID (2 years)
Gale *et al.*, 2008 [UK] (16)	Prospective cohort Recruited: 1991-92	178	3rd trimester	Radioimmunoassay	Wechsler Intelligence Scale (9 years) Strengths and Difficulties (9 years)
Hanieh *et al.*, 2014 [Vietnam] (17)	Antenatal micronutrient RCT Recruited: 2010-12	960	32 weeks gestation	LC-MS/MS	BSID (6 months)
Strom *et al.*, 2014 [Denmark] (18)	Prospective cohort Recruited: 1988-89	798	30 weeks gestation	LC-MS/MS	Scholastic achievement results (15-16 years) obtained from national registry
Darling *et al.*, 2017 [UK] (19)	Prospective cohort Recruited: 1991-92	7065	30 weeks gestation	HPLC and LC-MS/MS	Parent-report tests (6, 18, 30, 42 months) Strengths and Difficulties (7 years) Wechsler Intelligence Scale (8 years) Neale Analysis of Reading Ability (9 years)

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Keim *et al.*, 2014 [USA] (20)	Prospective cohort Recruited: 1959-65	3896	≤26 weeks and umbilical cord	LC-MS/MS	BSID (8 months) Stanford-Binet Intelligence Scale (4, 7 years) Wechsler Intelligence Scale (4, 7 years) Wide Range Achievement Test (7 years) Psychologist assessed behaviour (4, 7 years)
Zhu *et al.*, 2015 [China] (21)	Prospective cohort Recruited: 2008	363	Umbilical cord	Radioimmunoassay	BSID (16-18 months)
Gould *et al.*, 2017 [Australia] (22)	Antenatal DHA RCT Recruited: 2005-08	337	Umbilical cord	LC-MS/MS	BSID (18 months) Differential Ability Scales (4 years) Clinical Evaluation of Language Fundamentals (4 years)

[1]Mother-child dyads with both exposure and outcome of interest measured. BSID, Bayley Scales of Infant and Toddler Development; CBCL, Child Behaviour Checklist; DHA, docosahexaenoic acid; HPLC, high performance liquid chromatography; LC, liquid chromatography; MS, mass spectroscopy; RCT, randomised controlled trial.

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Table 2 Maternal and infant characteristics of the study population[1]

Maternal	
Age at delivery (years)	31.0 [29.0, 33.0]
Caucasian	99 (728)
Attended university/third level education	89 (652)
Relationship status, single	5 (36)
Household income <€21,000 per annum	5 (34)
Pregnancy-related factors[2]	
Obesity (BMI >30 kg/m^2)	12 (91)
Smoking	7 (52)
Vitamin D supplement user	42 (306)
Serum 25(OH)D concentrations (nmol/L)	56.1 [38.1, 76.6]
Infant	
Gender, male	51 (377)
Birth weight (kg)	3.5 [3.2, 3.8]
Gestational age (weeks)	40.4 [39.6, 41.1]
Cord serum 25(OH)D concentrations (nmol/L)	32.1 [20.8, 46.3]
Infant feeding	
Breastfed at hospital discharge	75 (547)
Duration of breastfeeding (weeks)	16.0 [0.1, 99.0]
Age first weaned onto solids (weeks)	20.0 [17.0, 22.0]
Vitamin D supplement user (in first year)	60 (443)

[1]Values are medians [interquartile range] or frequencies (percentages), study population *n* = 734 (cord serum 25(OH)D measured in 547 infants only). BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D.

[2]Maternal data collected at 15 weeks' gestation unless otherwise stated.

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Table 3 Association between maternal serum 25(OH)D concentrations (continuous per 10 nmol/L and categorised) at 15 weeks' gestation and offspring neurodevelopmental outcomes at five years[1]

	Continuous measure (per 10 nmol/L increment)		**Categorical measure (reference category = 25(OH)D <30 nmol/L)**			
			25(OH)D 30-<50 nmol/L		**25(OH)D ≥50 nmol/L**	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Kaufman Brief Intelligence Test						
Verbal standard score	0.04 (-0.24, 0.32)	-0.01 (-0.03, 0.03)[2]	1.02 (-0.58, 2.61)	0.91 (-1.38, 3.20)[2]	-0.13 (-1.57, 1.30)	0.42 (-1.66, 2.49)[2]
Non-verbal standard score	0.01 (-0.24, 0.25)	0.01 (-0.02, 0.03)[3]	0.78 (-0.69, 2.25)	1.21 (-0.88, 3.30)[3]	0.50 (-0.82, 1.81)	1.29 (-0.60, 3.17)[3]
IQ composite score	0.02 (-0.23, 0.26)	-0.01 (-0.03, 0.02)[2]	1.18 (-0.22, 2.58)	1.39 (-0.58, 3.37)[2]	0.19 (-1.07, 1.44)	0.94 (-0.85, 2.72)[2]
Child Behaviour Checklist						
Internal problem score	0.04 (-0.13, 0.21)	0.01 (-0.01, 0.02)[4]	0.44 (-1.84, 0.95)	-0.30 (-1.73, 1.13)[4]	0.14 (-0.75, 1.03)	-0.01 (-1.29, 1.29)[4]
External problem score	0.01 (-0.18, 0.19)	-0.01 (-0.02, 0.02)[4]	-0.91 (-2.41, 0.59)	-0.73 (-2.24, 0.79)[4]	0.15 (-0.81, 1.10)	-0.40 (-1.77, 0.96)[4]
Total problem score	0.04 (-0.45, 0.53)	0.01 (-0.04, 0.05)[4]	-2.26 (-6.26, 1.73)	-1.75 (-5.77, 2.26)[4]	0.35 (-2.20, 2.90)	-0.71 (-4.34, 2.91)[4]

[1]Values are β coefficients (95% confidence interval), total n = 734.

[2]Model adjusted for infant sex, birth weight, maternal years of schooling (log) and maternal BMI at 15 weeks' gestation (log).

[3]Model adjusted for infant sex, birth weight, maternal years of schooling (log), maternal BMI at 15 weeks' gestation (log), maternal smoking at 15 weeks' gestation and duration of breastfeeding.

[4]Model adjusted for infant sex, marital status, maternal years of schooling (log), maternal BMI at 15 weeks' gestation (log), household income and age to weaning onto solids.

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Table 4 Association between cord serum 25(OH)D concentrations (continuous per 10 nmol/L and categorised) at birth and neurodevelopmental outcomes at five years[1]

	Continuous measure (per 10 nmol/L increment)		**Categorical measure (reference category = <30 nmol/L)**			
			25(OH)D 30-<50 nmol/L		**25(OH)D ≥50 nmol/L**	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Kaufman Brief Intelligence Test						
Verbal standard score	-0.10 (-0.56, 0.35)	-0.02 (-0.06, 0.03)[2]	-0.03 (-0.05, -0.01)	-0.01 (-1.87, 1.85)[2]	-0.03 (-0.05, -0.01)	-0.43 (-2.63, 1.78)[2]
Non-verbal standard score	0.28 (-0.11, 0.67)	0.02 (-0.02, 0.06)[3]	-0.03 (-0.05, -0.01)	0.14 (-1.50, 1.78)[3]	-0.03 (-0.05, -0.01)	0.95 (-1.02, 2.92)[3]
IQ composite score	0.11 (-0.27, 0.49)	0.01 (-0.03, 0.04)[4]	-0.04 (-0.06, -0.02)	0.56 (-0.97, 2.08)[4]	-0.04 (-0.06, -0.02)	0.52 (-1.29, 2.33)[4]
Child Behaviour Checklist						
Internal problem score	-0.09 (-0.37, 0.20)	-0.01 (-0.03, 0.03)[5]	-0.36 (-1.06, 0.35)	-0.67 (-1.82, 0.48)[5]	0.36 (-0.35, 1.07)	0.05 (-1.31, 1.41)[5]
External problem score	-0.08 (-0.39, 0.22)	0.01 (-0.02, 0.04)[6]	0.03 (-0.73, 0.79)	0.28 (-0.96, 1.52)[6]	-0.03 (-0.79, 0.73)	0.32 (-1.14, 1.78)[6]
Total problem score	-0.28 (-1.09, 0.53)	0.01 (-0.07, 0.09)[7]	-0.33 (-2.35, 1.69)	-0.41 (-3.70, 2.88)[7]	0.34 (-1.68, 2.36)	0.45 (-3.42, 4.32)[7]

[1]Values are β coefficients (95% confidence interval), total *n* = 547.

[2]Model adjusted for infant sex, birth weight, maternal years of schooling (log) and maternal BMI at 15 weeks' gestation (log).

[3]Model adjusted for infant sex, birth weight, maternal years of schooling (log), maternal BMI at 15 weeks' gestation (log), maternal smoking at 15 weeks' gestation and duration of breastfeeding.

[4]Model adjusted for infant sex, birth weight, marital status, maternal years of schooling (log) and maternal BMI at 15 weeks' gestation (log).

[5]Model adjusted for infant sex, maternal years of schooling (log), maternal BMI at 15 weeks' gestation (log) and age to weaning onto solids.

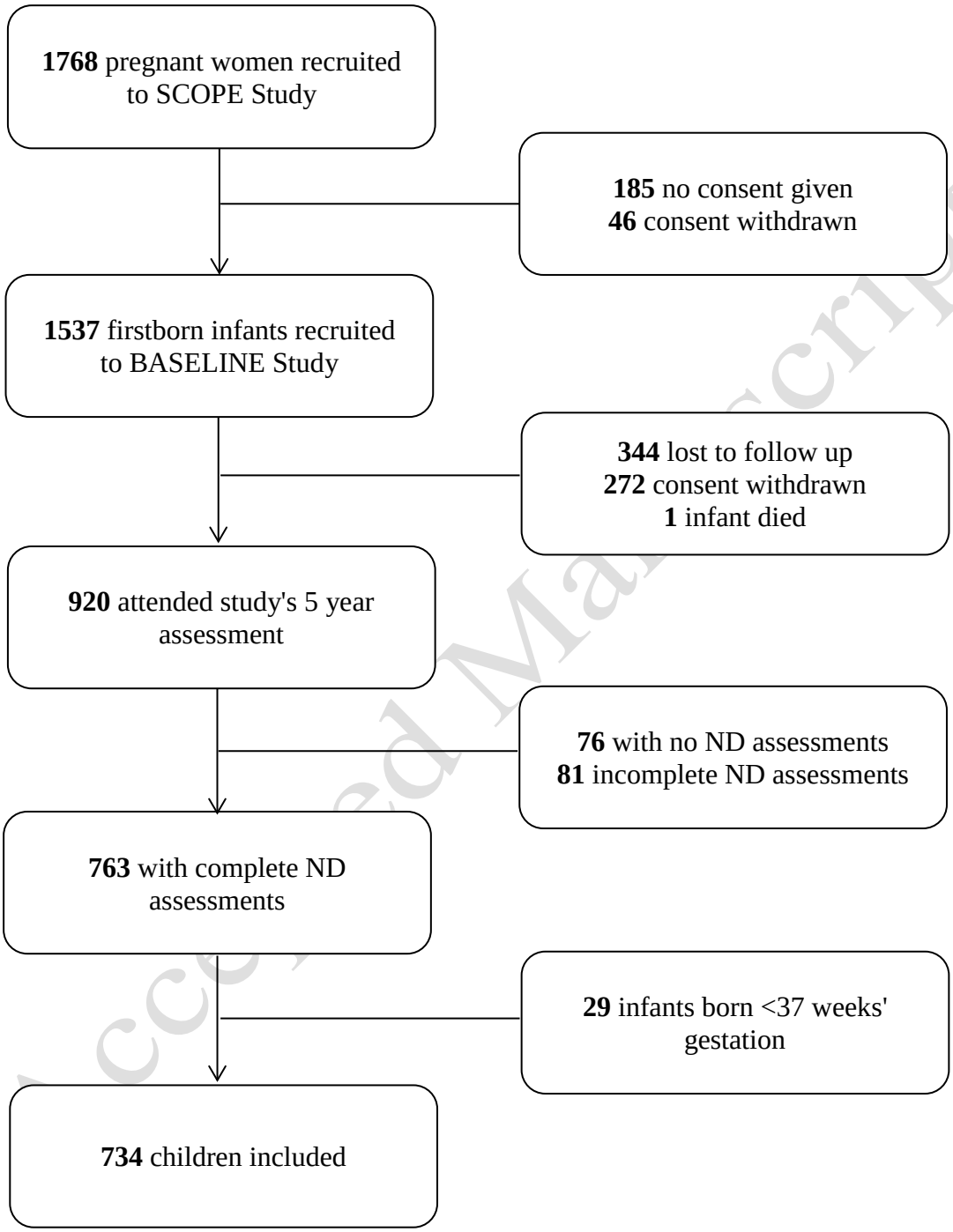
[6]Model adjusted for infant sex, marital status, maternal years of schooling (log), maternal BMI at 15 weeks' gestation (log), household income and age to weaning onto solids.

[7]Model adjusted for infant sex, maternal years of schooling (log), maternal BMI at 15 weeks' gestation (log), household income and age to weaning onto solids.

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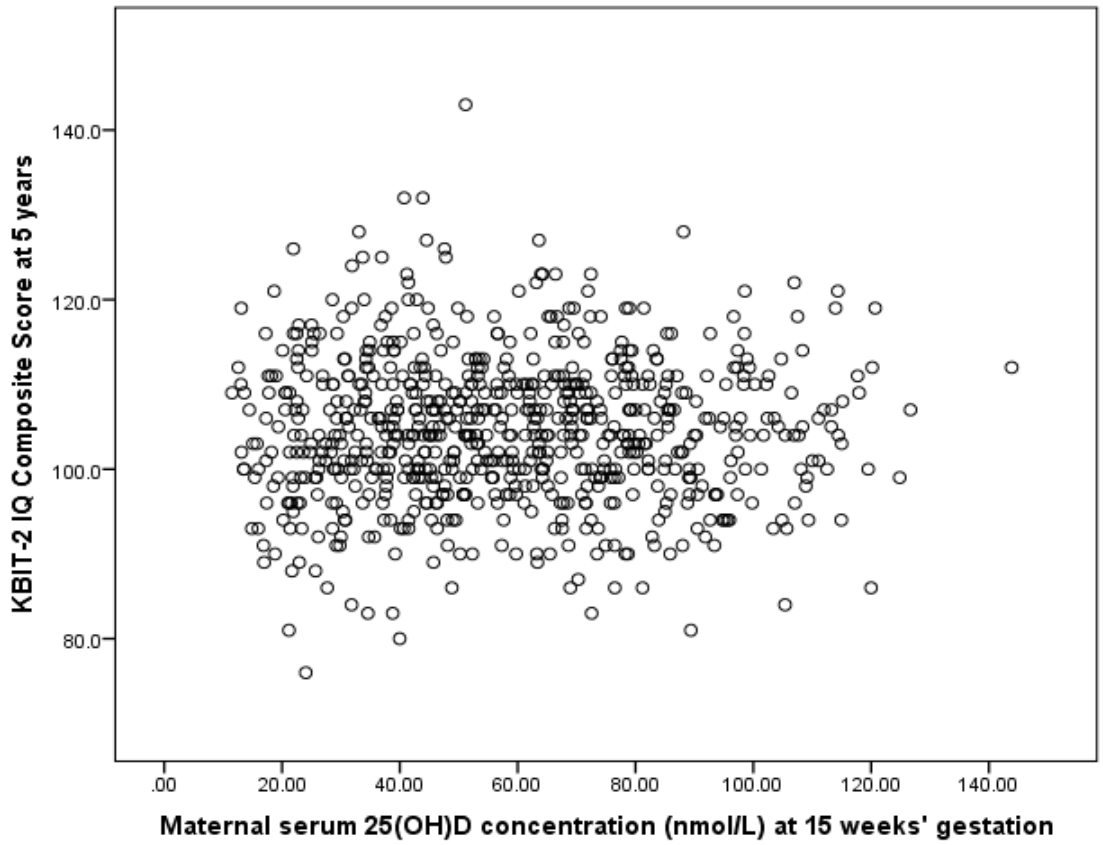
Figure 1 Flow chart of study participants

ND: neurodevelopment

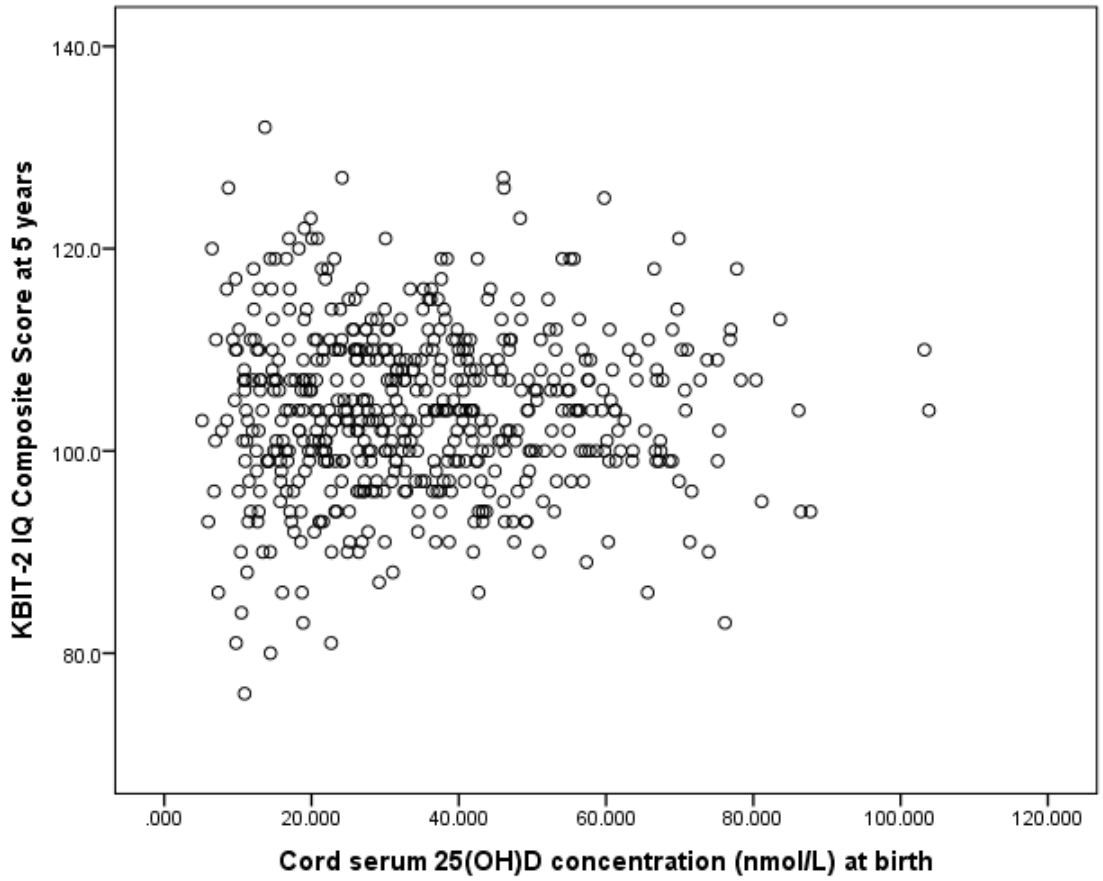


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A


KBIT-2 IQ Composite Score at 5 years
140.0
120.0
100.0
80.0
.00
20.00
40.00
60.00
80.00
100.00
120.00
140.00
Maternal serum 25(OH)D concentration (nmol/L) at 15 weeks' gestation

B


KBIT-2 IQ Composite Score at 5 years
140.0
120.0
100.0
80.0
.000
20.000
40.000
60.000
80.000
100.000
120.000
Cord serum 25(OH)D concentration (nmol/L) at birth

Supplemental Figure 1 Distribution of (A) maternal serum 25-hydroxyvitamin D (25(OH)D) concentrations at 15 weeks' gestation and (B) cord serum 25(OH)D concentrations at birth with Kaufman Brief Intelligence Test, 2nd Edition (KBIT-2) IQ composite scores at five years.

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