No association between vitamin D levels around time of birth and later risk of developing oligo- and polyarticular juvenile idiopathic arthritis: a Danish case–cohort study

SU Thorsen1,2, CB Pipper3, M Alberdi-Saugstrup4,5, S Nielsen6, A Cohen6, M Lundqvist6, LC Thygesen7, A Ascherio8, J Svensson1

1Copenhagen Diabetes Research Centre (CPH-DIRECT), Department of Paediatrics, Herlev University Hospital, Herlev, Denmark, 2Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, 3Department of Public Health, Section of Biostatistics, University of Copenhagen, Copenhagen, Denmark, 4Paediatric Rheumatology Clinic, Rigshospitalet, Copenhagen, Denmark, 5Department of Paediatrics, Næstved Hospital, Næstved, Denmark, 6Department of Congenital Disorders, Statens Serum Institute, Copenhagen, Denmark, 7National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark, and 8Department of Nutrition, Harvard TH Chan School of Public Health, Boston, MA, USA

Objectives: Basic and epidemiological studies on rheumatic autoimmune diseases have suggested an association between vitamin D levels around time of birth and disease risk. The literature on vitamin D and juvenile idiopathic arthritis (JIA) is scarce. We hypothesized that low levels of 25-hydroxyvitamin D [25(OH)D] around time of birth would be associated with increased risk of oligo- or polyarticular JIA.

Method: We conducted a case–cohort study of validated cases diagnosed with oligo- and polyarticular JIA (1993–2012) and controls matched on date of birth. Cases and controls were born in the period 1983–2010. Cases were diagnosed using international criteria. The concentration of 25(OH)D was assessed from neonatal dried blood spot (DBS) samples using high-sensitivity liquid chromatography tandem mass spectrometry (LC-MS/MS). Odds ratios (ORs) were calculated using conditional logistic regression and a two-way analysis of variance (ANOVA) was used to test for season and birth year 25(OH)D variations. A total of 300 matched pairs were included in the statistical analyses.

Results: No significant association was found between levels of 25(OH)D and JIA risk in the adjusted model [OR (per 25 nmol/L increase) 1.2, 95% confidence interval (CI) 0.9–1.6, p = 0.2]. 25(OH)D levels were found to fluctuate significantly with season (p < 0.0001) and year (p < 0.0001). The median level of 25(OH)D was 34.4 nmol/L in cases and 31.5 nmol/L in controls.

Conclusions: Our study does not support the hypothesis that a window of vulnerability exists around time of birth with regard to 25(OH)D levels and later JIA risk. Further studies should explore whether 25(OH)D levels during early pregnancy or infancy may influence JIA risk.

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease affecting children and adolescents. JIA is an umbrella term that covers a heterogeneous group of diseases (1). JIA is defined by the onset of arthritis before the age of 16, persistence of symptoms beyond 6 weeks, and the exclusion of other reasons for arthritis (2). The incidence rate in Denmark is 9–16 cases per 100 000 person-years (3). Currently, the International League of Associations for Rheumatology (ILAR) has classified seven main categories of JIA based on clinical features, blood autoantibodies, genetics, and the age of onset (2). Of the seven categories, oligoarticular and rheumatoid factor (RF)-negative polyarticular JIA are the most common. Oligoarticular JIA is further subcategorized into persistent and extended oligoarticular JIA (1, 2). The aetiology of JIA is thought to be multifactorial but still remains a puzzle (4). Approximately one-third of JIA risk is attributable to common genetic variation in human leucocyte antigen (HLA) and non-HLA susceptibility loci (5, 6). Only a few studies have examined environmental triggers, such as early life infections, maternal smoking during pregnancy, breastfeeding, socioeconomic status, and psychosocial factors (7–10). In the search for preventive actions for this chronic and disabling group of diseases, it is necessary to continue to identify possible environmental triggers.

Vitamin D is regarded as a prohormone/secosteroid due to synthesis, protein-bound transportation, and activity in different locations. The vitamin D receptor is located in the nucleus of human cells (e.g. cells of the
immune system), where it modulates gene expression (11, 12). Immune regulatory properties of the active form of vitamin D, namely 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], have been found in animal and human intervention studies, indicating a potential role for vitamin D in the prevention of autoimmune diseases (13–15). Of note, studies have shown that exposure to low levels of vitamin D in utero can cause persistent immune impairment (16–18). Furthermore, vitamin D deficiency and insufficiency during pregnancy and postpartum is a common problem in various populations and at different latitudes including Denmark (19, 20). There is a strong correlation between maternal and neonatal vitamin D levels (21).

To the best of our knowledge, no studies have been conducted regarding the association between vitamin D levels during pregnancy or around the time of birth and later risk of JIA in the offspring. The primary objective of this study was to examine a possible association between levels of 25-hydroxyvitamin D [25(OH)D] at birth and the risk of developing oligo- or polyarticular JIA.

**Method**

**Study design and sample population**

Our study was a case–cohort study with 1:1 matching. Cases were eligible for inclusion in the study if they had been diagnosed with either oligoarticular or polyarticular JIA, classified by either European League Against Rheumatism (EULAR) or ILAR criteria. Only six cases have been diagnosed with EULAR criteria due to onset before 1997 (3). We only included three main categories of JIA based on the prevalence in Denmark and suggested overlapping aetiological pathways; due to sample size issues an a priori decision was made to collapse all cases for the main analysis (22). From now on, oligo- and polyarticular RF-negative and -positive JIA are referred to as JIA. Cases were solely collected from a validated database established by the Paediatric Rheumatology Clinic, Rigshospitalet, Copenhagen, Denmark. With regard to polyarticular JIA RF status, to be categorized as RF negative one negative test is sufficient, but to be categorized as RF positive two tests for RF have to be positive; these have to be taken at least 3 months apart during the first 6 months of disease (2). Most cases originated from the Zealand Region and Capital Region of Denmark and all cases were born in the period 1983–2010. Controls were selected by obtaining a neighbouring neonatal dried blood spot (DBS) sample when cases were identified in the Danish Newborn Screening Biobank (DNSB), thereby matching on date of birth (23). Based on a sample size calculation, one control was collected for each case.

Initially, 401 cases were identified. Sixty-nine cases were either not found in the biobank (n = 65) or belonged to other ongoing projects (n = 4). Three hundred matched pairs were included in the inferential statistical analysis (see flowchart of inclusions and exclusions, Figure 1).

**Assessment of vitamin D status on DBS**

Vitamin D status was assessed by measuring 25(OH)D$_2$ and 25-dihydroxyvitamin D$_3$ [25(OH)D$_3$] in 3.2-mm punches taken from DBS samples. Sample preparation and analysis were performed using high-sensitivity liquid chromatography tandem mass spectrometry (LC-MS/MS) according to a modified version of the analysis presented by Eyles et al (24). The modifications consisted of using commercially available calibrators and controls (Perkin Elmer, Waltham, MA, USA) and an online extraction step. The LC-MS/MS system consisted of an Aria TLX2 system (Thermo Fisher Scientific, Waltham, MA, USA) with two Agilent 1100 binary pumps and two Agilent Quatary pumps (Agilent, Santa Clara, CA, USA) connected to a Thermo TSQ Ultra triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) ion source. Online extraction was performed using a Cyclone P 0.5 × 50 mm Turbo flow column (Thermo Fisher Scientific) and analytical separation was achieved using a Hypersil GOLD 50 × 2.1 mm, 3 µm reversed phase column (Thermo Fisher Scientific).

The lower limit of quantification (LLOQ) was 4 nmol/L for 25(OH)D$_3$ and 3 nmol/L for 25(OH)D$_2$. 25(OH)D levels from DBS are whole blood concentrations. Most 25(OH)D molecules in the bloodstream are protein bound and to approximate and report sera concentrations we corrected the original levels using the formula: S-25(OH)D = 25(OH)D × [1/(1 – 0.61)] (haematocrit fraction for capillary blood)] (25). Total 25(OH)D = 25(OH)D$_3$ + 25(OH)D$_2$.

**Variables**

Information on variables (demography, pregnancy, and birth characteristics) was collected from the National Patient Register, the Medical Birth Register, and the Civil Registration System (26, 27). The following variables were eligible to be included in the model, based on a causal diagram and the literature (8–10, 28): 25(OH)D, gender, parental age at delivery, birth weight and birth length, gestational age, ethnicity, smoking status during pregnancy, and mode of delivery. Inspired by Carlens et al (8), birth weight and gestational age were both arranged into three groups (Table 2). Birth weight and length are both measures of foetal growth and due to possible multicollinearity (r = 0.79, p < 0.001), only birth weight was included in the adjusted model. Unfortunately, we had to omit smoking status, father’s age at delivery, and mode of delivery because of high levels of missing values (80, 112, and 388, respectively).

**Statistical analysis**

All analyses were performed using SAS version 9.2. Descriptive data are expressed as median and interquartile range (IQR) and the measure of effect as odds ratio.
Initially, 401 cases were identified. 266 had oligoarticular and 135 had polyarticular JIA (121 RF–/14 RF+).

332 cases (221 oligoarticular and 111 polyarticular JIA (97 RF–/14 RF+) and 329 controls were available for 25(OH)D analysis.

329 cases (218 oligoarticular and 111 polyarticular JIA (97 RF–/14 RF+) and 325 controls had successful 25(OH)D measurements.

300 matched pairs (202 oligoarticular and 98 polyarticular (84 RF–/14 RF+) were included in the statistical analyses.

Figure 1. Flowchart illustrating the initial inclusions and exclusions. Of the 69 cases excluded, four belonged to other projects and 65 were not found in the biobank.

(OR) with 95% confidence interval (CI). The difference between cases and controls was analysed by conditional logistic regression. The assumptions for logistic regression modelling were fulfilled.

Univariate and multivariate conditional logistic regression analyses were performed to examine the influence of 25(OH)D on risk of JIA and adjust for confounders. To capture a possible non-linear relationship between 25(OH)D levels and JIA risk, we used second-order polynomial and piecewise linear spline regression models, the latter with knots at 12.5, 25, 50, and 75 nmol/L. A likelihood ratio test was performed to check whether the model including splines fitted the data better than the initial linear model. Furthermore, subgroup analyses were performed on both oligo- and polyarticular JIA. In addition, we examined whether 25(OH)D levels fluctuated significantly with year of birth (divided into four equally sized strata: 1983–1995, 1996–1999, 2000–2003, and 2004–2010) and season (coded as: spring (March–May), summer (June–August), autumn (September–November), and winter (December–February)) using a two-way analysis of variance (ANOVA) including an interaction term. Because of a bimodal distribution of age of onset in both oligo- and polyarticular JIA, we conducted a post-hoc analysis using logistic regression with age of onset as the outcome variable (< 6 years = ‘1’ and ≥ 6 years = ‘0’) including 289 cases. Possible interactions between 25(OH)D and gender and ethnicity were examined. In addition, a sensitivity analysis was conducted, where we restricted the sample population to ethnic Danes. A gender-specific analysis was also carried out. The exploratory nature of this study meant that no correction for multiple testing was performed. p < 0.05 was considered statistically significant.

The study was approved by the Danish Ethical Committee (H-4-2013-049) and by the DNSB Steering Committee. According to Danish law, anonymous register-based studies do not require informed consent.

Results
The median level of total 25(OH)D was 34.4 nmol/L in cases and 31.5 nmol/L in controls. The variation in 25(OH)D was also found to be similar between the groups, with an IQR of 29 nmol/L in cases and 25 nmol/L in controls. Other characteristics of the study population are reported in Table 1.
An association between levels of 25(OH)D and JIA risk around time of birth was found in the unadjusted model [OR (per 25 nmol/L increase) 1.3, 95% CI 1.0–1.7] but disappeared in the adjusted model [OR (per 25 nmol/L increase) 1.2, 95% CI 0.9–1.6]. 25(OH)D2 and 25(OH)D3 were initially collapsed yielding a total level. In subanalyses, we excluded 25(OH)D2 because 295 controls and 289 cases had whole blood levels below the validated quantitative range of the assay (LLOQ = 3 nmol/L), but this did not change the results (data not shown). Similar results were obtained between the JIA categories of oligoarticular JIA [OR (per

Table 1. Descriptive statistics for the study population.

| Variables | Oligoarticular | Polyarticular | Control |
|-----------|----------------|---------------|---------|
| 25(OH)D levels (median/IQR, nmol/L) (n)* | 36.4/22.1–51.1 (202) | 28.0/19.8–45.2 (98) | 31.5/19.4–44.5 (300) |
| 25(OH)D3 levels (median/IQR, nmol/L) (n) | 33.9/19.6–47.5 (202) | 26.3/17.3–43.1 (98) | 29.1/17.6–42.5 (300) |
| 25(OH)D2 levels (median/IQR, nmol/L) (n) | 2.0/1.6–2.8 (202) | 1.7/0.1–2.1 (98) | 1.8/1.4–2.5 (300) |

Demographic characteristics

- Female (n/% of total) 135/66.8 74/75.5 150/50
- Age at onset (median/IQR, years) (n) 5/3–9 (193) 8.5/3.5–12 (96) NS
- Mother’s age at child’s birth (median/IQR, years) (n) 29.5/27–33 (202) 29/25–32 (98) 29/26–32 (300)
- Father’s age at child’s birth (median/IQR, years) (n) 32/30–36 (161) 31/28–35 (85) 32/28–35 (242)

Pregnancy and birth characteristics

- Gestational age (median/IQR, weeks) (n) 40/39–40.9 (202) 40/39–41 (98) 40/39–40.9 (300)
- Birth weight (median/IQR, g) (n) 3500/3140–3800 (202) 3420/3190–3750 (98) 3500/3150–3875 (300)
- Birth length (median/IQR, cm) (n) 52/50–53 (202) 52/51–53 (98) 52/50–53 (300)

25(OH)D, 25-Hydroxyvitamin D; 25(OH)D3, 25-dihydroxyvitamin D3; 25(OH)D2, 25-dihydroxyvitamin D2; JIA, juvenile idiopathic arthritis; IQR, interquartile range.

*Numbers of observations in each cell.

Table 2. Univariate and multivariate conditional logistic regression showing the association between 25(OH)D (+ other covariates) and JIA.

| Variable | Univariate | Multivariate |
|----------|------------|--------------|
| 25(OH)D per 25 nmol/L increase | 1.3 (1.0–1.7) 0.02 | 1.2 (0.9–1.6) 0.2 |
| Gender | | |
| Female | 2.4 (1.7–3.4) < 0.0001 | 2.4 (1.7–3.5) < 0.0001 |
| Male | 1.0 | 1.0 |
| Ethnicity | | |
| Ethnic Dane | 3.4 (1.7–6.6) 0.0004 | 3.2 (1.6–6.6) 0.002 |
| Other | 1.0 | 1.0 |
| Gestational age | | |
| < 37 weeks | 0.9 (0.4–1.7) 0.6 | 0.9 (0.4–1.8) 0.7 |
| 37–41.99 weeks | 1.0 | 1.0 |
| ≥ 42 weeks | 1.1 (0.6–2.0) 0.7 | 1.1 (0.6–2.0) 0.8 |
| Birth weight | | |
| < 3000 g | 0.9 (0.6–1.4) 0.6 | 0.9 (0.5–1.4) 0.6 |
| 3000–3999 g | 1.0 | 1.0 |
| ≥ 4000 g | 0.8 (0.5–1.3) 0.3 | 0.9 (0.6–1.5) 0.7 |
| Mother’s age at delivery | | |
| < 25 years | 1.0 | 1.0 |
| 25–35 years | 1.5 (0.9–2.4) 0.1 | 1.2 (0.7–2.0) 0.5 |
| ≥ 35 years | 1.3 (0.7–2.4) 0.5 | 1.1 (0.6–2.3) 0.7 |

25(OH)D, 25-Hydroxyvitamin D; JIA, juvenile idiopathic arthritis; OR, odds ratio; CI, confidence interval.

The odds of having JIA are compared to being a control. The univariate analysis shows marginal differences in the odds and the multivariate analysis shows conditional differences. Covariates included in the multivariate analysis: 25(OH)D, gender, ethnicity, weight (categorical), gestational age (categorical), and mother’s age (categorical). The categories of the covariates that received an OR of 1.0 are the reference categories.
25 nmol/L increase) 1.1, 95% CI 0.8–1.6] and polyarticular JIA [OR (per 25 nmol/L increase) 1.5, 95% CI 0.9–2.4], as shown in Figure 2. Moreover, there was no non-linear relationship between 25(OH)D levels and JIA risk either when including a quadratic term in the adjusted model (p = 0.7) or when using linear splines (p = 0.6).

Overall 25(OH)D levels were found to fluctuate significantly with year (p < 0.0001) and season (p < 0.0001) (Supplementary Figures S1 and S2) but no interaction was found between year and season (p = 0.18). Pairwise comparisons between seasons showed that significantly higher levels were found in the period from June to August.

Some secondary findings need to be highlighted; we found the odds of developing JIA were significantly higher among females than males in both the unadjusted (OR 2.4, 95% CI 1.7–3.4) and adjusted models (OR 2.4, 95% CI 1.7–3.5). No interaction was found between 25(OH)D levels and gender (p = 0.2). Moreover, gender-specific analyses yielded similar results to those of the main analyses (data not shown). Being an ethnic Dane significantly increased the JIA risk in both the unadjusted (OR 3.4, 95% CI 1.7–6.6) and adjusted models (OR 3.2, 95% CI 1.6–6.6) compared to having another ethnic background (45% from Asia excluding Japan, 21% from Europe, 19% from Africa, and 15% from other developing countries). No interaction was found between 25(OH)D levels and ethnicity (p = 0.3).

Sensitivity analyses restricted to ethnic Danes showed similar results to those of the main analyses (data not shown). The remaining covariates were not found to be associated with JIA risk (Table 2).

In the adjusted logistic regression model with age of onset as the outcome variable, no association with 25(OH)D levels was found, when including all covariates from Table 2 and year of birth (results not shown).

Discussion

No conditional association between 25(OH)D levels around time of birth and JIA risk was found. However, previously identified risk factors for JIA (i.e. being female vs. male and/or ethnic Dane vs. other ethnic background) were confirmed (10, 29).

Research in the field of vitamin D and JIA is still in its infancy. Until now, studies have focused on 25(OH)D levels after onset of JIA and the function of 25(OH)D with regard to disease activity. These studies have suggested that patients with JIA have lower levels of 25(OH)D but no effect on disease activity has yet been found (30). These findings might explain the behavioural changes linked to the disease, such as reduced outdoor

Figure 2. Illustrating 25(OH)D levels by case status. Note that whole blood levels of 25(OH)D have been multiplied by a factor 2.56 [1/(1–0.61)] (approximate haematocrit fraction in capillary blood) to yield serum levels of 25(OH)D.
activity and hence less sunlight exposure. Furthermore, levels of 25(OH)D have been inversely related to inflammation (31).

Murine studies have suggested that vitamin D deficiency in utero causes irreversible alterations in the immune system possibly through epigenetic changes, which makes the host more susceptible to breaches in self-tolerance and resultant autoimmune disease (17, 32, 33). Murine models of arthritis have shown that supplementation with high levels of dietary 1,25(OH)2D prevents disease or halts an already ongoing autoimmune response (34).

Human studies both in vitro and in vivo have shown that 1,25(OH)2D and its analogues promote and inhibit regulatory T-cell (Treg) and T-helper (Th)17-cell differentiation and function, respectively (13, 35, 36). A disrupted Th17/Treg balance has been postulated to be crucial for the pathogenesis of JIA (37–40). The immune regulatory effects found in these studies are probably a cause of the high doses of vitamin D applied to the cell cultures or given orally as supplements. The biological variation in 25(OH)D levels may not be sufficient to cause such effects. In our study, only 6% of cases and 3% of controls had 25(OH)D levels above 75 nmol/L.

Rheumatoid arthritis (RA) and other rheumatic diseases have been associated with vitamin D (41). The results of the Iowa Women’s Health Study suggest an inverse association between dietary intake of vitamin D and RA risk (42), although such an association could not be confirmed in a study by Hiraki et al (43). Of note, the Nurses’ Health Study found that higher levels of UV-B radiation around the time of birth and later in life may lower the risk of developing RA; such an effect could be mediated through a vitamin D-dependent pathway and/or a photoimmunological pathway (44–46). Additionally, a large study by Disanto et al suggested that the month of birth influences RA risk. They also found that the seasonal variation in 25(OH)D levels in the third trimester was negatively associated with overall risk of developing autoimmunity (16). The biological basis of the month-of-birth effect found might be due to other environmental triggers than 25(OH)D levels, which fluctuate with season. Furthermore, the negative correlation found between levels of 25(OH)D in the third trimester and the risk of autoimmunity was based on average predicted 25(OH)D levels during gestation, using blood samples from middle-aged women (n = 3787), a method that is inferior to direct quantification of 25(OH)D at the end of pregnancy.

Our study benefits from a clear classification of cases and its novelty. Physicians in a highly specialized rheumatology paediatric clinic validated the cases used in this study. The storage time of DBS ranged from 5 to 32 years in this study, but 25(OH)D levels in DBS have been shown to be stable for at least 40 years (24). Nevertheless, we found that the median level of 25(OH)D in the most recent DBS (2004–2010) was 8–17 nmol/L higher than in the older groups (lowest levels in 1996–1999); temporal degradation or an increase in supplemental intake may be a possible explanation for this finding. Reassuringly, we also found that 25(OH)D levels fluctuate with season, with the highest levels in the summer months, which lends weight to the validity of the assay; prior studies have used a similar method with success (47). Some limitations should be mentioned. First, although 300 matched pairs were used in our study, we cannot exclude the possibility of a false negative error (type 2 error) due to sample size issues (i.e. we reject an association between 25(OH)D levels and JIA risk), even though an association does indeed exist. Second, a single 25(OH)D measurement at one point in time is less likely to reflect longer-term 25(OH)D levels than multiple measurements throughout pregnancy and infancy. Third, we grouped three categories of JIA together (including oligoarticular JIA, which can be further subcategorized into persistent and extended oligoarticular JIA), which may blur associations between 25(OH)D and specific JIA categories. No change in results was found in the subgroup analyses, even though these results are also prone to a false negative error (type 2 error). Fourth, the association with ethnicity is probably due to differences in genetic make-up. We cannot exclude the possibility of residual confounding, but restricting the analyses to only ethnic Danes did not have an impact on our results. Finally, 34% of cases and 37% of controls had 25(OH)D levels below 25 nmol/L, in line with a large Dutch study measuring 25(OH)D levels from cord blood (n = 2324) (20). A child with a high load of susceptibility genes may be more vulnerable to deficient levels of 25(OH)D compared to a child with a neutral or protective genotype. We were unfortunately not able to adjust for genetic susceptibility.

In conclusion, we cannot rule out that an association exists between 25(OH)D levels in early pregnancy or in infancy and later JIA risk. Our study was unable to detect an association between 25(OH)D levels around time of birth with regard to later JIA risk.

Acknowledgements
We would like to express our appreciation to Bent Nørgaard-Pedersen and David M. Hougaard from the Department of Congenital Disorders, Statens Serum Institute, Copenhagen, Denmark, for their support in the initial phases of this project. This work was supported by a scholarship from Copenhagen University and by grants from Herlev University Hospital, the Danish Rheumatism Association, and the Capital Region of Denmark.

References
1. Ravelli A, Martini A. Juvenile idiopathic arthritis. Lancet 2007;369:767–78.
2. Petty RE, Southwood TR, Manners P, Baum J, Du G, Goldenberg J, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 2004;31:390–2.
3. Berntson L, Andersson GB, Fasth A, Herlin T, Kristinsson J, Lahdenne P, et al. Incidence of juvenile idiopathic arthritis in the Nordic countries. A population based study with special reference to the validity of the ILAR and EULAR criteria. J Rheumatol 2003;30:2275–82.

4. Prakken B, Albani S, Martini A. Juvenile idiopathic arthritis. Lancet 2011;377:2138–49.

5. Cobb JE, Hinks A, Thomson W. The genetics of juvenile idiopathic arthritis: current understanding and future prospects. Rheumatology (Oxford) 2014;53:592–9.

6. Hinks A, Cobb J, Marion MC, Prahalad S, Sudman M, Bowes J, et al. Dense genotyping of immune-related disease regions identifies 14 new susceptibility loci for juvenile idiopathic arthritis. Nat Genet 2013;45:664–9.

7. Berkun Y, Paedh S. Environmental factors and the geoepidemiology of juvenile idiopathic arthritis. Autoimmun Rev 2010;9:A319–24.

8. Carlens C, Jacobsson L, Brandt L, Cnattingius S, Stephansson O, Askling J. Perinatal characteristics, early life infections and later risk of rheumatoid arthritis and juvenile idiopathic arthritis. Ann Rheum Dis 2009;68:1159–64.

9. Ellis JA, Ponsonby AL, Pezic A, Chavez RA, Allen RC, Akikusa JD, et al. CLARITY – ChiLhood Arthritis Risk Factor Identification sTudY. Pediatr Rheumatol Online J 2012;10:37.

10. Ellis JA, Munro JE, Ponsonby AL. Possible environmental determinants of juvenile idiopathic arthritis. Rheumatology (Oxford) 2010;49:411–25.

11. Haussler MR, Whifflet GK, Kaneko I, Haussler CA, Hsieh D, Hsieh JC, et al. Molecular mechanisms of vitamin D action. Calcif Tissue Int 2013;92:77–98.

12. Baeke F, Etten EV, Overbergh L, Mathieu C. Vitamin D3 and the immune system: maintaining the balance in health and disease. Nutr Res Rev 2007;20:106–18.

13. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. Nutrients 2013;5:2502–21.

14. Peelen E, Knippenberg S, Maris AH, Tervoort M, Smolders J, Tervaert JWC, et al. Effects of vitamin D on the peripheral adaptive immune system: a review. Autoimmun Rev 2011;10:733–43.

15. Antico A, Tampoa M, Tozzoli R, Bizzaro N. Can supplementation with vitamin D reduce the risk or modify the course of autoimmune diseases? A systematic review of the literature. Autoimmun Rev 2012;12:127–36.

16. Disanto G, Chaplin G, Morahan JM, Giovannoni G, Hyponen E, Ebers GC, et al. Month of birth, vitamin D and risk of immune mediated disease: a case control study. BMC Med 2012;10:69.

17. Yu S, Canover MT. Epigenetic reduction in invariable NKT cells following in utero vitamin D deficiency in mice. J Immunol 2011;186:1384–90.

18. Disanto G, Watson CT, Meier UC, Ebers GC, Giovannoni G, Ramagopalan SV. Month of birth and thymic output. JAMA Neurol 2013;70:527–8.

19. McAree T, Frisina D, Manickavasagar T, Sivalokanathan S, Brenner N, Bassett P, et al. Vitamin D deficiency in pregnancy - still a public health issue. Matern Child Nutr 2013;9:23–30.

20. Vinkhuysen AAE, Eyles DW, Burne TH, Blanken LME, Kruthof CJ, Verhulst F, et al. Prevalence and predictors of vitamin D deficiency based on maternal mid-gestation and neonatal cord blood: the generation r study. J Steroid Biochem Mol Biol. Published online 15 September 2015. doi: 10.1016/j.jsbmb.2015.09.018.

21. Novakovic B, Galati JC, Chen A, Morley R, Craig J, Safery R. Maternal vitamin D predominates over genetic factors in determining neonatal circulating vitamin D concentrations. Am J Clin Nutr 2012;96:188–95.

22. Macauba C, Nguyen K, Milojcic D, Park JL, Mellins ED. Oligoarticular and poliarthritic JIA: epidemiology and pathogenesis. Nat Rev Rheumatol 2009;5:616–26.

23. Norgaard-Pedersen B, Hougaard DM. Storage policies and use of the Danish newborn screening biobank. J Inherit Metab Dis 2007;30:530–6.
44. Arkema EV, Hart JE, Bertrand KA, Laden F, Grodstein F, Rosner BA, et al. Exposure to ultraviolet-B and risk of developing rheumatoid arthritis among women in the nurses’ health study. Ann Rheum Dis 2013;72:506–11.

45. Schwarz T. 25 years of UV-induced immunosuppression mediated by T cells—from disregarded T suppressor cells to highly respected regulatory T cells. Photochem Photobiol 2008;84:10–18.

46. Milliken SVI, Wassall H, Lewis BJ, Logie J, Barker RN, Macdonald H, et al. Effects of ultraviolet light on human serum 25-hydroxyvitamin D and systemic immune function. J Allergy Clin Immunol 2012;129:1544–51.

47. McGrath JJ, Eyles DW, Pedersen CB, Anderson C, Ko P, Burne TH, et al. Neonatal vitamin D status and risk of schizophrenia. Arch Gen Psychiatry 2010;67:889–94.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Supplementary Figure S1. 25(OH)D levels by season of birth.
Supplementary figure S2. 25(OH)D levels by year of birth

Please note that the editors are not responsible for the content or functionality of any supplementary material supplied by the authors. Any queries should be directed to the corresponding author.