Maternal and Newborn Vitamin D-Binding Protein, Vitamin D levels, Vitamin D Receptor genotype, and Childhood Type 1 Diabetes

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Abstract

Objective

Circumstantial evidence links 25-hydroxyvitamin D (25[OH]D), vitamin D-Binding Protein (DBP), vitamin D-associated genes and type 1 diabetes (T1D), but no studies have jointly analyzed these. We aimed to investigate whether DBP levels during pregnancy or at birth were associated with offspring T1D, and whether vitamin D pathway genetic variants modified associations between DBP, 25(OH)D and T1D.

Research Design and Methods

From a cohort of >100,000 mother/child-pairs, we analyzed 189 pairs where the child later developed T1D, and 576 random control pairs. We measured 25(OH)D using LC-MS/MS, and DBP using polyclonal radioimmunoassay, in cord blood and maternal plasma samples collected at delivery and mid-pregnancy. We genotyped mother and child for variants in or near genes involved in vitamin D metabolism (*GC, DHCR7, CYP2R1, CYP24A1, CYP27B1, VDR*). Logistic regression was used to estimate odds ratios adjusted for potential confounders (aORs).

Results

Higher maternal DBP levels at delivery, but not in other samples, were associated with lower offspring T1D risk (OR=0.86, 95%CI: 0.74-0.98, per µM/L increase). Higher cord blood 25(OH)D levels were associated with lower T1D risk (OR=0.87, 95%CI: 0.77-0.98 per 10 nmol/L increase) in children carrying the *VDR* rs11568820 *G/G* genotype (P[interaction]=0.01 between 25(OH)D level and rs11568820). We did not detect other gene-environment interactions.

Conclusions

Higher maternal DBP level at delivery may decrease offspring T1D risk. Increased 25(OH)D levels at birth may decrease T1D risk, depending on *VDR* genotype. These findings should be replicated in other studies. Future studies of vitamin D and T1D should include *VDR* genotype and DBP levels.

Introduction

Type 1 diabetes (T1D) often presents in childhood and is associated with increased mortality (1). Vitamin D, vitamin D receptor (VDR), vitamin D-binding protein (DBP) and genetic polymorphisms associated with vitamin D metabolism have separately been suggested to influence the risk of T1D development (2). No studies have jointly analyzed vitamin D, DBP and genetic polymorphisms, which is necessary to relate any, or all, of these factors to T1D risk.

Vitamin D is converted in the liver to 25-hydroxyvitamin D (25(OH)D), the clinical biomarker of vitamin D status (2). A second hydroxylation to the biologically active form calcitriol ($1,25(OH)_2D$) occurs in the kidneys, and probably in other target cells. The biological effects of $1,25(OH)_2D$ are mediated by vitamin D Receptor (VDR, encoded by *VDR)* (3). A recent study reported an association between 25(OH)D in early childhood and later islet autoimmunity (a surrogate endpoint for T1D) which depended on *VDR* genotype (4). VDR binding sites are overrepresented near genetic regions associated with T1D (5), and several single nucleotide polymorphisms (SNP) in or near genes involved in the vitamin D pathway (*CYP2R1, CYP27B1* and *DHCR7*) have been associated with T1D (6). These SNPs, and SNPs in or near genes encoding Vitamin D-binding protein (DBP, encoded by *GC*) influence circulating 25-hydroxyvitamin D (25(OH)D) concentration (7). We recently reported no association between 25(OH)D status and offspring T1D risk (8). In this study, we aimed to investigate whether the association may be modified by vitamin D pathway and VDR SNPs.

DBP is a multifunctional protein that is the major carrier of vitamin D and its metabolites in the circulation, and is the precursor of the macrophage activating factor Gc-MAF (9). The circulating DBP concentration nearly doubles during

pregnancy (10), and DBP appears to increase plasma half-life of 25(OH)D (11). It is not yet established whether the free fraction of 25(OH)D is a better marker for 25(OH)D status than the total 25(OH)D level (12). Lower DBP levels in sera from patients with T1D compared to controls have been reported (13), and recent studies suggest that DBP is a possible autoantigen in T1D (14; 15). Only one previous study has investigated maternal DBP during pregnancy and offspring T1D risk, reporting higher maternal DBP to be associated with lower offspring T1D risk (10).

We aimed to jointly study maternal and newborn DBP, 25(OH)D and SNPs in the vitamin D pathway to test the following hypotheses: 1) Higher maternal or newborn DBP levels predict lower risk of childhood T1D and 2) The association between maternal or newborn 25(OH)D (or DBP) and childhood T1D risk is modified by genetic variants in the vitamin D pathway (including *VDR)*. In addition, we hypothesized that a higher maternal or offspring 25(OH)D relative to DBP (surrogate for free 25[OH]D) predicts lower risk of childhood T1D.

Methods

Study sample

We designed a nested case-control study in the Norwegian Mother and Child Cohort Study (MoBa) (16), which recruited ~114,000 pregnant mothers (41% eligible mothers participated) nationwide from 1999-2008 (last birth in 2009). The current study uses data from repeated questionnaires (using version VIII of the MoBa datafiles) and biomarker analyses of maternal and cord blood samples (17). All participating mothers gave written informed consent. The establishment and data collection in MoBa was previously based on a licence from the Norwegian Data Inspectorate and approval from The Regional Committee for Medical Research Ethics. It is now based on regulations related to the Norwegian Health Registry Act. The Regional Committee for Medical Research Ethics approved the current study. Children who developed T1D by February 5, 2014 were identified with a high degree of ascertainment by register linkage to the Norwegian Childhood Diabetes Registry (18). In all, 189 mother/child-pairs were T1D cases, and 576 mother/child-pairs from a random sample with available blood samples of the cohort were used as controls (Figure 1). Characteristics of the study participants in analysis are given in Table 1. Baseline characteristics for those with available blood samples were largely similar to the whole MoBa cohort, with the exception of a lower proportion of caesarean section and premature birth (19).

Blood sampling

Maternal blood samples were collected in EDTA tubes at hospital laboratories at enrolment around pregnancy week 18 (median 18.5, interquartile range (IQR) 19.4 – 17.9 weeks) and again soon after delivery (median 1 day, IQR 3 – 1 days; hereafter

referred to as postpartum). Plasma was separated before overnight shipment to the MoBa biobank. Immediately after birth, a blood sample was taken from the umbilical cord vein, shipped and plasma separated upon arrival. All samples were stored at -80°C until analysis (20).

Laboratory assays of DBP and 25(OH)D

Plasma concentration of DBP was determined using a competitive radioimmunoassay at the Oslo University Hospital Hormone Laboratory (Oslo, Norway) with a polyclonal antibody (anti-Gc-globulin, Dako, Glostrup, Denmark) and purified Gc-globulin (Sigma Chemicals, St. Louis, MO), as described previously (19). Analyzes of plasma 25-hydroxyvitamin D_3 and $-D_2$ were done at the internationally certified Statens Serum Institut (Copenhagen, Denmark), using a liquid chromatography tandem mass spectrometry (LC-MS/MS) and the MSMS Vitamin D kit (PerkinElmer, Inc., Waltham, MA) for mass spectrometry, as described previously (8). The seasonally adjusted (deseasonalized using cosinor modelling as described in (21)) sum of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 was used as the exposure variable (hereafter referred to as 25(OH)D).

Genotyping assays and genetic risk scores

To account for established T1D susceptibility markers, participants were genotyped for selected SNPs using a custom Illumina Golden Gate assay (Illumina, San Diego, CA), as described in detail in (22). Briefly, SNPs in the vitamin D pathway (see Supplemental Table S1) were genotyped: five SNPs in or near *CYP2R1, CYP24A1, CYP27B1, GC* and *DHCR7,* associated with 25-hydroxyvitamin D (7), and two SNPs in or near *VDR* (rs1544410, rs11568820). A vitamin D deficiency genotype score for

the mother and child was calculated by summing the risk alleles across five non-*VDR* SNPs (7). Human leukocyte antigen (HLA) class II genotype was imputed using the HLA*IMP:02 web service and subsequently confirmed by allele specific PCR (details given in (22)) . HLA genotypes were categorized as shown in Table 1. A non-HLA T1D genetic risk score (GRS), weighted by the increased risk reported per risk allele, was calculated on the basis of 51 non-HLA SNPs associated with T1D (details given in the online supplement to (23)).

Other covariates

Information on birth weight, maternal age at delivery, and delivery mode was obtained from the nationwide Medical Birth Registry of Norway (24). Information regarding maternal pre-pregnancy body mass index (BMI) and smoking during pregnancy was obtained from mid-pregnancy questionnaires. The questionnaires can be accessed at www.fhi.no/moba. Data on maternal T1D were obtained from questionnaires and the Norwegian Patient Registry. The variables were categorized as shown in Table 1.

Statistical methods

All statistical analyses were planned a priori. We applied logistic regression with offspring T1D as outcome. Our main aim was to estimate the association between DBP levels and offspring T1D risk. Investigating the association between the DBP/25(OH)D ratio and T1D risk, and potential gene-environment interactions, were secondary aims. The main exposure was estimated average DBP (µM/L) during pregnancy. First, we used a linear mixed effects random intercept model, including DBP measurements from both maternal samples, gestational age and days since

delivery as predictors, to predict the average maternal DBP concentration. Secondly, we used this predicted average as the exposure in a logistic regression analysis with offspring T1D as the outcome. To account for the variance in both the predicted average DBP and logistic regression analysis and obtain unbiased confidence intervals (CI), we used bootstrapping with 10 000 replicates and calculated the percentiles for 95% CIs for the odds ratios. The average predicted DBP was centered at pregnancy week 36. This centering has little or no consequences for further modelling and statistical significance of our results, but was chosen to better compare with our earlier study (10). For more details, see our earlier publication(22)We also investigated DBP concentration in each sample type separately (cord blood at birth, and maternal samples from mid-pregnancy and postpartum), DBP quartiles to assess linearity, and the DBP/25(OH)D ratio (used as a proxy for free 25(OH)D) in each sample type as exposures. The season-adjusted 25(OH)D concentration was used in the analysis of 25(OH)D and the 25(OH)D/DBP ratio. We tested interactions between 25(OH)D and selected vitamin D pathway SNPs (see Supplemental Table S1), non-HLA GRS for T1D or offspring HLA genotype. We also tested interactions between DBP, the *GC* SNP rs2282679 and offspring T1D. These interactions were chosen *a priori* on the basis of biological plausibility.

We used clustered sandwich estimator to account for correlation between siblings. SNPs were coded as additive (0, 1 or 2 alleles) variables (unless combined), and 25(OH)D and DBP levels were analyzed as continuous variables. Offspring HLA and T1D non-HLA GRS were included as dichotomous variables (carrying at least one HLA DR3-DQ2 or DR4-DQ8 haplotype vs none, and ≤median vs >median of the T1D non-HLA GRS in controls, respectively) in the gene-environment interaction.

Adjustment variables

The following covariates were included in our primary adjustment model: child`s HLA genotype, sex, cesarean delivery, maternal ethnicity, pre-pregnancy (BMI), smoking in pregnancy and age at delivery (see Supplemental Figure S1 for a directed acyclic graph of these). As a sensitivity analysis, we also included maternal T1D, birthweight, birth year, sample batch, and number of 25(OH)D lowering alleles in *GC* polymorphisms rs2282679 and rs222040 as adjusting variables.

Results

The distribution of DBP in mid-pregnancy, postpartum and cord blood samples is shown in Figure 2. There were weak, but statistically significant ($p<0.05$) correlations between DBP concentration in different sample types, and between DBP and 25(OH)D in control children (Supplemental Figure S2).

DBP and T1D risk

Higher estimated average maternal DBP concentrations at gestational week 36 were not significantly associated with lower risk of offspring T1D, with the 95% CI including one (Table 2). However, when analyzing each sample type separately, higher DBP level in the postpartum sample was associated with lower offspring T1D risk (aOR 0.80, 95% CI 0.67 – 0.95, p = 0.01), while DBP mid-pregnancy or in cord blood were not (Table 2).

The 25(OH)D/DBP ratio, as a proxy for “free” 25(OH)D was not associated with offspring T1D risk in any sample type, with the exception of a borderline statistically significant association after adjustment in the post-partum sample (Table 2). This suggestive association disappeared after further adjustment for DBP, suggesting that the association with the 25OHD/DBP ratio was spurious (data not shown). Mutually adjusting 25(OH)D and DBP levels for each other in the same sample, or adjusting maternal postpartum DBP and cord blood 25(OH)D for each other, did otherwise not appreciably change our estimates, but resulted in wider CIs (Supplemental Table S2). Likewise, including more adjusting variables as a sensitivity analysis (maternal T1D, birthweight, birth year, sample batch or number of risk alleles in *GC*

polymorphisms) did not appreciably change the estimates, but resulted in wider CIs (data not shown).

Interactions with genetic markers

An overall lack of associations between 25(OH)D in pregnancy, or at birth, and childhood T1D have been previously presented (8) (for completeness shown in Supplemental Table S2). In the current study, we found that the association between cord blood 25(OH)D and childhood T1D differed significantly by child's *VDR* rs11568820 genotype (p[interaction]=0.01, Supplemental Table S3). Higher 25(OH)D levels at birth had an inverse association on offspring T1D in children homozygous for the *VDR* rs11568820 *G/G* genotype (Table 2). Maternal 25(OH)D remained not associated with offspring T1D risk in mothers or children homozygous for the rs11568820 *G/G* genotype (data not shown). No other significant interaction was detected between 25(OH)D and SNPs in the vitamin D pathway, vitamin D deficiency score, non-HLA genetic risk score for T1D or HLA genotype (Supplemental Table S3). Further, no interaction was detected between DBP and *GC(DBP)* SNP rs2282679 (data not shown).

Discussion

In this case-control study nested within a large prospective pregnancy cohort, we found that higher maternal DBP levels at delivery, but not in mid-pregnancy or in child's cord blood, was associated with lower risk of offspring T1D. We also found that in children homozygous for the *VDR* rs11568820 *G/G* genotype, higher 25(OH)D levels at birth predicted a lower risk of developing T1D. These findings must be interpreted with caution, and should be replicated in independent studies.

In an independent Norwegian nested case-control study, the only previous study of maternal DBP in relation to childhood T1D, , higher DBP levels in the third trimester were associated with decreased offspring T1D risk (10). The current study replicates and extends these findings by reporting that newborn (cord blood) DBP levels were not associated with childhood T1D risk. The current study is larger, able to control for additional possible confounders such as HLA genotype and BMI, and able to investigate possible interactions with genetic variants. While 25(OH)D levels in pregnancy or at birth overall were not associated with the risk of childhood T1D (8), we now report that in children homozygous for the *VDR* rs11568820 *G/G* genotype, higher 25(OH)D levels at birth predicted decreased risk of developing T1D. We did not observe interactions with other SNPs previously reported to modify the effect of vitamin D (25), or reported to be associated with vitamin D levels or T1D(25). While this gene-environment finding must be interpreted with caution, Norris et al. (4) reported a similar interaction between 25(OH)D in early childhood and *VDR* genotype (rs7975232) in the association with islet autoimmunity. rs11568820 is believed to result in lower VDR expression (26). We speculate that low levels of $1,25(OH)_2D$ and VDR could increase risk of autoimmunity, as they together inhibit T-cell proliferation

(27). Increased 25(OH)D (which is correlated with $1,25(OH)_2D$ (28)) levels in pregnancy could offset lower *VDR* expression, as $1,25(OH)_2D$ regulates *VDR* expression (29; 30). While our study suggests an interaction with the child's VDR genotype and not maternal genotype, a Finnish study reported that the maternal VDR SNP rs1544410 was associated with offspring T1D risk (31). No significant association between maternal rs1544410 and offspring T1D risk was observed in our study (data not shown). There are few established environmental factors associated with DBP levels. Interestingly, DBP has been reported to be important in production of the antimicrobial peptide cathelicidin in monocytes by regulating bioavailability of 25(OH)D (32). We hypothesize that low DBP levels towards the end of pregnancy could influence antimicrobial response and inflammation in the mother, which could predispose for offspring autoimmunity. It is also plausible that another unknown factor operating late in pregnancy could influence both maternal DBP and offspring T1D risk.

The gene-environment interaction observed could explain the inconsistent results of 25(OH)D levels and T1D risk in the few previous studies in the field (8; 33; 34). Several polymorphisms and haplotypes in *VDR* have been suspected of an association with T1D (see (25)); rs11568820 and other polymorphisms might be markers of a certain *VDR* genotype and not be relevant to the observed association by themselves. Although *VDR* genotype was not associated with T1D in a large genetic study (6), a potential association could be influenced by the participants 25(OH)D status, as suggested by Ponsonby et al. (35). Consistent with our observation, interactions between rs11568820 and 25(OH)D have been reported in colorectal cancer (36). Similarly, winter sun exposure interacted with a Cdx-2 VDR

polymorphism in multiple sclerosis (37). Multiple sclerosis, like T1D, has an overrepresentation of VDR binding sites near disease-associated genetic regions (5).

The association between maternal DBP levels in late pregnancy or in postpartum and lower offspring T1D risk, reported in this and in an independent Norwegian study (10) warrants further investigation regarding possible mechanisms, and replication in non-Norwegian populations. Our data, taken together with earlier studies linking DBP to T1D (13-15), suggests DBP should be more intensively studied in relation to T1D. The interaction between the rs11568820 *G/G* genotype, which is the most frequent in our study (68.4%), cord blood 25(OH)D levels and T1D should be investigated in another study. If replicated, vitamin D supplementation should probably be recommended to all infants and pregnant women, regardless of genotype, although a large scale randomized controlled trial (RCT) would be ideal as a basis for recommendations. However, well-powered RCTs to prevent T1D are extremely costly and time consuming, and should therefore be carefully planned based on the best available preclinical and observational data. The Norwegian national guidelines recommend vitamin D supplementation from four weeks of age, and recommend a daily intake of 10 µg vitamin D in pregnant women. In the MoBa cohort, 63% of the mothers did not reach the recommended vitamin D intake (38), and 17% of children did not use vitamin D supplementation (see Table 2 in (22)), which shows that there is room for improvement.

The strengths of this study include its prospective design and repeated measurements, which allowed us to assess DBP and 25(OH)D concentrations at

different timepoints. Our large sample size with information on HLA genotype and genetic variation in the vitamin D pathway allowed us to examine interactions with genetic markers. Limitations of the study include, as in any observational study, the possible presence of unknown confounding factors. The nested sample was generally representative for the whole cohort, but our results might not be generalizable to the general population or populations of non-European origin. Further studies are needed to replicate and expand upon these findings.

Our findings indicate that children whose mothers have higher DBP levels at the end of pregnancy are at a decreased risk of developing T1D. DBP has not been studied extensively in the context of T1D and more work is required to elucidate potential mechanism involved. Further studies in independent cohorts are need for replication of this observation, and experimental studies are needed to investigate potential mechanisms. The decreased risk of T1D for children homozygous for *VDR* rs11568820 *G/G* and high 25(OH)D levels at birth support the current recommendations for vitamin D intake for pregnant women and infants. These findings must be interpreted with caution and more evidence is required to validate these results. Regardless, potential future vitamin D studies should consider including *VDR* genotype.

Conflicts of interest

No other potential conflict of interest relevant to this article was reported. The authors alone are responsible for the content and writing of the paper.

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Interpretation of data: All authors.

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Table 1: Characteristics of cases with childhood type 1 diabetes and randomly selected controls in the Norwegian Mother and Child Cohort Study.

	Controls (n = 576)	Cases (n = 189)
Median age (range) at end of follow-up* (years)	11.7 (7.7–17.1)	12.7 (8.0–16.6) †
Female sex	285 (49.5%)	93 (49.2%)
Maternal type 1 diabetes	0 (0.0%)	7 (3.7%)
Preterm birth	19 (3.3%)	10 (5.3%)
Missing data	1 (0.2%)	1 (0.5%)
Birthweight (in grams)		
<2500 g	8 (1.4%)	7 (3.7%)
2500-4500 g	539 (93.6%)	174 (92.1%)
>4500 g	29 (5.0%)	8 (4.2%)
Parity		
No previous births	248 (43.1%)	93 (49.2%)
One	215 (37.3%)	57 (30.2%)
Two or more	113 (19.6%)	39 (20.6%)
Maternal age in years (median, range)	30 (17–42)	30 (19–42)
Maternal non-Norwegian ethnicity	30 (5.2%)	10 (5.3%)
Maternal smoking during pregnancy		
Non-smoker at end of pregnancy‡	469 (81.4%)	161 (85.2%)
Smoked at end of pregnancy	76 (13.2%)	20 (10.6%)
Missing data	31 (5.4%)	8 (4.2%)
Maternal pre-pregnancy BMI (kg/m^2)		
<25	379 (65.8%)	97 (51.3%)
25-30	109 (18.9%)	49 (25.9%)
>30	40 (6.9%)	28 (14.8%)
Missing data	48 (8.3%)	15 (7.9%)
Child's HLA§ genotype and genetic risk score		
Protective (DQ6)	168 (29.2%)	3 (1.6%)
Neutral (any other HLA not mentioned)	111 (19.3%)	5 (2.6%)
Increased risk (≥1 copy of either DQ8 or DQ2)	212 (36.8%)	93 (49.2%)
High risk (DQ8/DQ2 heterozygote)	30 (5.2%)	71 (37.6%)
Missing HLA genotype	55 (9.5%)	17 (9.0%)
Child's non-HLA T1D GRS‖ (median, range)	61.2 (45.7 – 76.3)	63.2 (45.4 – 78.6)
Missing non-HLA T1D GRS score	19 (3.5%)	12 (6.8%)
Child's 25(OH)D GRS (median, range)	3 (0 – 8)	3 (0 – 8)
Missing 25(OH)D GRS score	20 (3.7%)	13 (7.4%)
Caesarean section¶	59 (10.2%)	36 (19.0%)

* The diagnosis date of the last case included - February 3, 2014

† The median age at diagnosis of T1D cases was 5.7 (range 0.7 – 12.7) years

‡ Including those that quit smoking shortly before or during pregnancy

§ Groups defined as protective (carrying at least one copy of HLA DQA1*01:02-DQB1*06:02-DRB1*15:01 [DQ6-DR15]), increased risk (at least one copy of HLA DQA1*03-DQB1*03:02-

DRB1*04 [DQ8-DR4] or DQA1*05:01-DQB1*02:01-DRB1*03:01 [DQ2-DR3], but not both haplotypes), high risk (HLA DQ2-DR3/DQ8-DR4) or neutral (any other genotype).

|| Weighted score, calculated by multiplying the number of risk alleles in 51 non-HLA SNPs with their reported risk per allele.

¶ Includes unknown (n = 1), emergency (n = 55) and elective (n = 39) caesarean section

Table 2: Association between exposures and type 1 diabetes

DBP, per 1 µM/L increase	OR, 95% CI	aOR, 95% CI*	P-value
Predicted maternal DBP†	0.74 (0.39 – 1.22)	0.49 (0.18 – 1.02)	n.s ‡
Mid-pregnancy	1.03 (0.91 – 1.16)	0.96 (0.79 – 1.16)	0.65
Cord blood	0.98 (0.81 – 1.20)	0.87 (0.67 – 1.14)	0.32
Postpartum	0.86 (0.74 – 0.98)	0.80 (0.67 – 0.95)	0.01
25(OH)D / DBP ratio			
Mid-pregnancy	1.00 (0.96 – 1.04)	1.04 (0.98 – 1.09)	0.17
Cord blood	0.99 (0.97 – 1.01)	1.00 (0.97 – 1.04)	0.81
Postpartum	1.01 (0.98 – 1.04)	1.05 (1.00 – 1.10)	0.049
25(OH)D, per 10 nmol/L increase, stratified by *VDR* rs11568820§			
Cord blood, AA/AG	1.18 (1.00 – 1.40)	1.17 (0.95 – 1.44)	0.15
Cord blood, GG	0.87 (0.77 – 0.98)	0.85 (0.72 – 1.00)	0.047

aOR: adjusted Odds Ratio; CI: Confidence Interval. P-value shown for adjusted analysis.

* adjusted for child`s HLA genotype, sex, maternal ethnicity, age, pre-pregnancy BMI, caesarean section and smoking.

† using maternal (mid-pregnancy and postpartum) samples in a mixed model to predict maternal DBP values at gestational week 36. Due to the reduction of the sampling variation when predicting maternal DBP, the predicted values have a lower standard deviation (SD) of 0.35 (while e.g. DBP in the postpartum samples has a SD of 1.52).This results in a greater observed estimate, as an increase per unit is roughly equivalent to 3 standard deviations in this analysis. We used bootstrapping (10 000 replications) to obtain un-biased CIs, and present bias-corrected CIs.

‡ as these results arise from bootstrapping estimations, a p-value is not provided.

§ there was a statistically significant interaction (p[interaction]=0.01) between rs11568820 and 25(OH)D (Supplemental Table S3).

Figure Legends

Figure 1: Formation of the analysis sample

* : 148 had 3 blood samples, 38 had 2 blood samples and 3 had one blood sample available for 25(OH)D and DBP testing. There were 174 mid-pregnancy, 174 postpartum and 175 cord blood samples.

† : 456 had 3 blood samples, 111 had 2 blood samples and 9 had one blood sample available for 25(OH)D and DBP testing. There were 532 mid-pregnancy, 525 postpartum and 542 cord blood samples.

Figure 2: Distribution of vitamin D-binding protein (DBP) concentrations in maternal and cord blood plasma samples.

Figure 2 shows the distribution of vitamin D-binding protein (DBP) concentrations in maternal and cord blood plasma samples from randomly selected controls (n=576) in the Norwegian Mother and Child Cohort Study. The maternal delivery (postpartum) sample was collected at median 1 day (interquartile range 0–3 days) after delivery.

Online-only supplement to:

Maternal and Newborn Vitamin D-Binding Protein, Vitamin D levels, Vitamin D Receptor genotype, and Childhood Type 1 Diabetes

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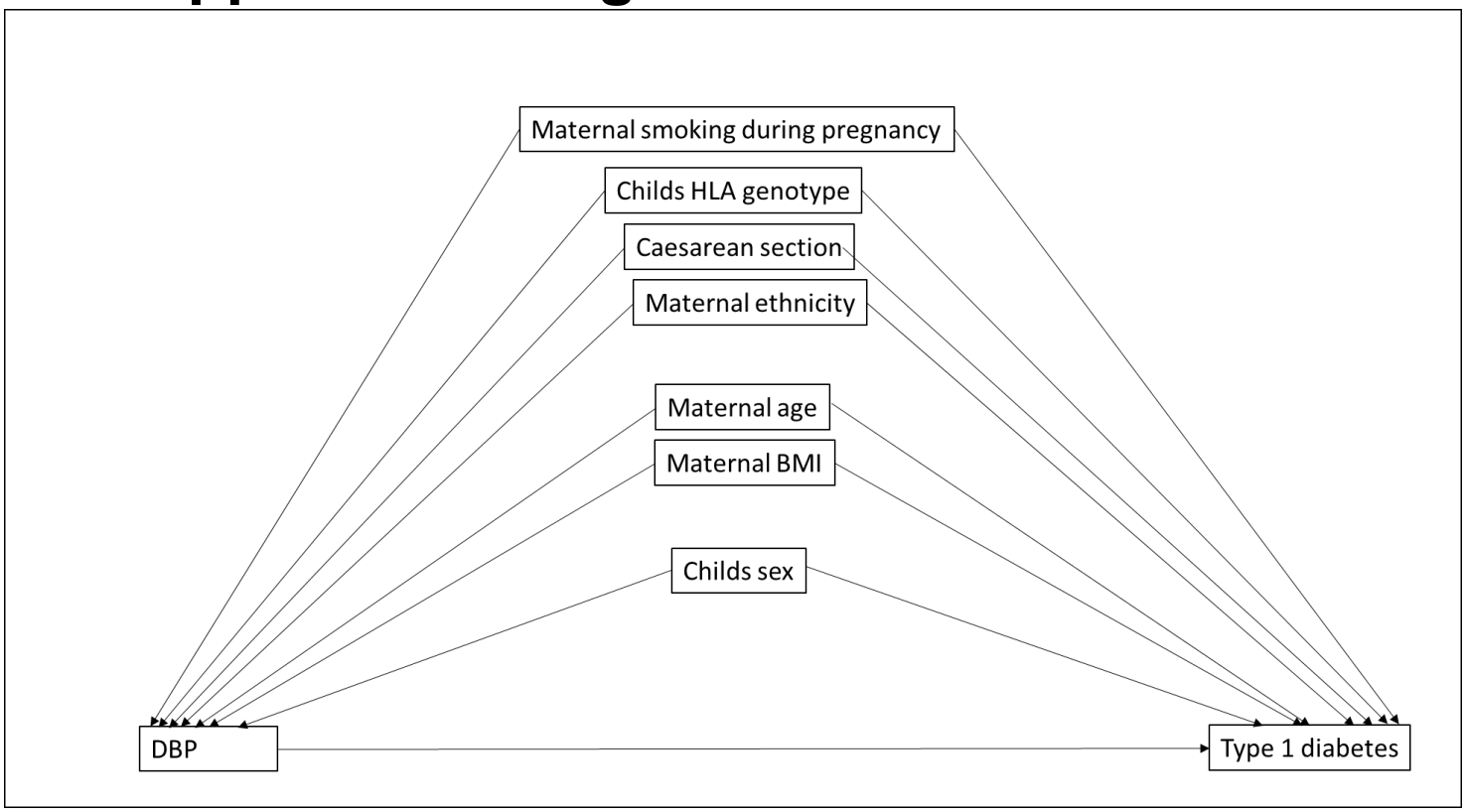
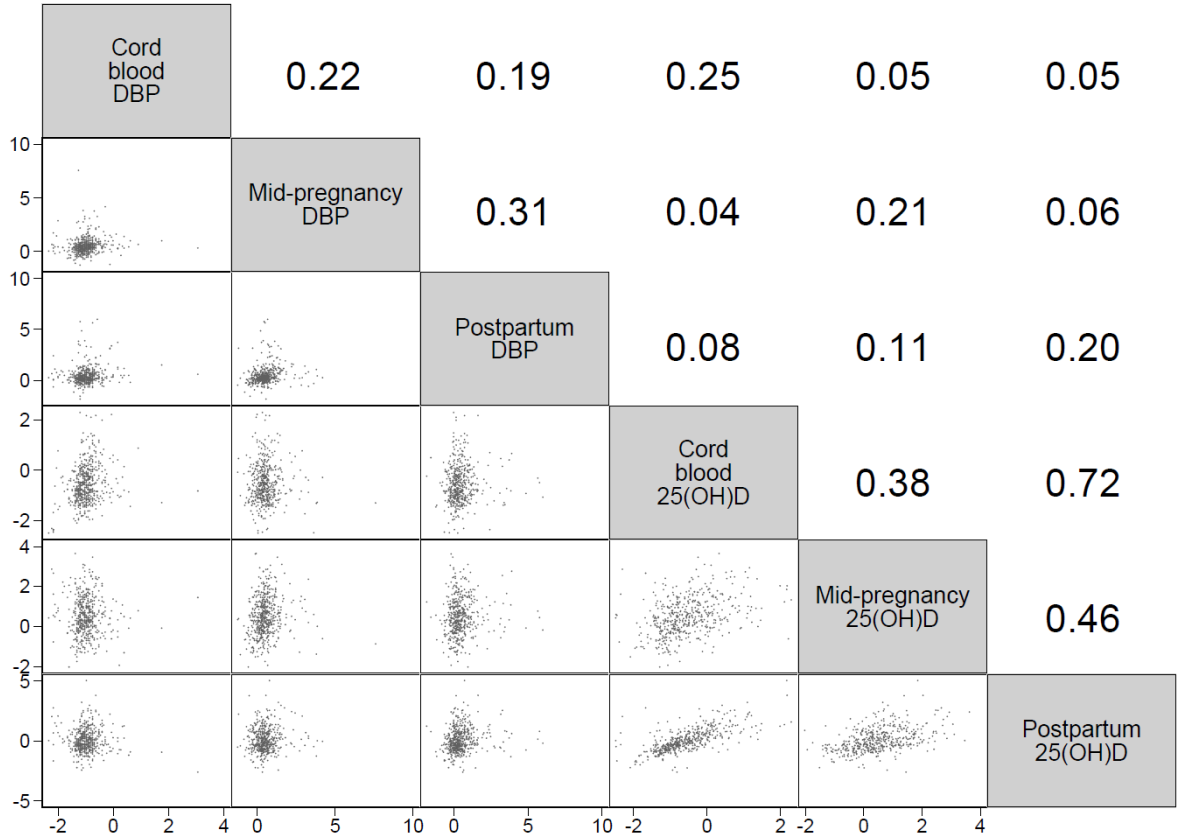
Supplemental Figure S1: Directed



Acyclic Graph

Supplemental Figure 1 shows a hypothetical Directed Acyclic Graph to illustrate how we hypothesized the relationships between DBP, adjustment covariates and Type 1 diabetes.

Supplemental Figure S2: Correlation matrix



This figure shows Spearmans' rho between 25(OH)D (deseasonalized) and DBP z-scores in control children (as correlation estimates based on cases can be biased). Cord blood and postpartum 25(OH)D have high correlation (0.72), with the rest having weaker correlations. All correlations above 0.1 were significant ($p<0.05$).

Supplemental Table S1: 25(OH)D metabolism SNPs

SNP*	Gene	Genotype	Child N(%)	Maternal N(%)
rs10741657	*CYP2R1*	AA	81 (14.9%)	65 (12.2%)
		AG	252 (46.3%)	248 (46.4%)
		GG	192 (35.3%)	198 (37.1%)
		Missing	19 (3.5%)	23 (4.3%)
rs703842	*CYP27B1*	AA	204 (37.5%)	199 (37.3%)
		AG	257 (47.2%)	253 (47.4%)
		GG	63 (11.6%)	58 (10.9%)
		Missing	20 (3.7%)	24 (4.5%)
rs6013897	*CYP24A1*	AA	323 (59.4%)	300 (56.2%)
		AT	180 (33.1%)	178 (33.3%)
		TT	22 (4.0%)	33 (6.2%)
		Missing	19 (3.5%)	23 (4.3%)
rs2282679†	*GC(DBP)*	AA	293 (53.9%)	282 (52.8%)
		AC	185 (34.0%)	195 (36.5%)
		CC	47 (8.6%)	35 (6.6%)
		Missing	19 (3.5%)	22 (4.1%)
rs12785878	*DHCR7*	AA	244 (44.9%)	228 (42.7%)
		AC	223 (41.0%)	238 (44.6%)
		CC	58 (10.7%)	46 (8.6%)
		Missing	19 (3.5%)	22 (4.1%)
rs1544410	*VDR*‡	AA	177 (32.5%)	172 (32.2%)
		AG	246 (45.2%)	239 (44.8%)
		GG	101 (18.6%)	100 (18.7%)
		Missing	20 (3.7%)	23 (4.3%)
rs11568820	*VDR*§	AA	22 (4.0%)	11 (2.1%)
		AG	131 (24.1%)	144 (27.0%)
		GG	372 (68.4%)	355 (66.5%)
		Missing	19 (3.5%)	24 (4.5%)

N and % calculated from control mother and child dyads.

* In addition the following SNPs were tested, but not used for further analyses due to linkage disequilibrium with other SNPs for the respective loci; *CYP2R1: rs2060793 and rs12794714; CYP27B1: rs4646536; GC:* rs222040 and rs1352846; *DHCR7: rs3829251*

† rs2282679 is a near perfect proxy for the coding SNP rs4588. rs2282679 C allele is linked to lower serum 25(OH)D levels.

‡ Also known as *VDR*-BsmI polymorphism

§ Also known as *VDR*-CdX2 polymorphism

Supplemental Table S2: Supplemental Results

25(OH)D, per 1 nmol/L increase	Cases/Controls† (N)	OR, 95% CI	aOR, 95% CI*	P-value
Mid-pregnancy	174/531	1.00 (0.99 – 1.01)	1.01 (1.00 – 1.01)	0.23
Cord blood	175/542	1.00 (0.99 – 1.01)	0.99 (0.98 – 1.01)	0.38
Postpartum	174/524	1.00 (0.99 – 1.01)	1.00 (0.99 – 1.01)	0.75
25(OH)D and DBP mutually adjusted				
25(OH)D, cord blood	174/537	0.99 (0.97 – 1.00)	0.99 (0.97 – 1.00)	0.12
DBP, cord blood		0.98 (0.73 – 1.30)	0.90 (0.63 – 1.30)	0.58
25(OH)D, cord blood	162/494	1.00 (0.99 – 1.01)	0.99 (0.98 – 1.01)	0.42
DBP, postpartum		0.89 (0.77 – 1.02)	0.83 (0.69 – 1.00)	0.047
Mutually adjusted, restricted to VDR rs11568820 GG genotype†				
25(OH)D, cord blood	112/367	0.99 (0.97 – 1.00)	0.99 (0.97 – 1.00)	0.12
DBP, cord blood		0.98 (0.73 – 1.30)	0.90 (0.63 – 1.30)	0.58
25(OH)D, cord blood	105/335	0.99 (0.97 – 1.00)	0.98 (0.96 – 1.00)	0.08
DBP, postpartum		0.90 (0.75 – 1.08)	0.82 (0.65 – 1.03)	0.09

aOR: adjusted Odds Ratio; CI: Confidence Interval

* adjusted for child`s HLA genotype, sex, maternal ethnicity, age, pre-pregnancy BMI, caesarean section and smoking.

† the number of cases and controls included in the unadjusted analysis.

Supplemental Table S3: Gene – Environment interactions

Exposure	SNP	Interaction estimate (95% CI)	p-value
Maternal* 25(OH)D	*CYP2R1* -rs10741657	1.00 (0.99 - 1.01)	0.84
	CYP27B1 - rs703842	1.00 (0.99 - 1.02)	0.68
	CYP24A1 - rs6013897	1.01 (0.99 - 1.02)	0.39
	GC(DBP) - rs2282679	1.00 (0.98 - 1.01)	0.77
	DHCR7 - rs12785878	1.01 (1.00 - 1.02)	0.13
	VDR - rs1544410	1.00 (0.98 - 1.01)	0.44
	VDR - rs11568820	1.00 (0.99 - 1.02)	0.84
	25(OH)D GRS†	1.00 (0.99 -1.01)	0.74
Offspring 25(OH)D	*CYP2R1* -rs10741657	1.01 (0.99 - 1.02)	0.39
	CYP27B1 - rs703842	0.99 (0.98 - 1.01)	0.52
	CYP24A1 - rs6013897	1.01 (0.99 - 1.03)	0.37
	GC(DBP) - rs2282679	1.00 (0.98 - 1.02)	0.87
	DHCR7 - rs12785878	1.00 (0.98 - 1.02)	0.90
	VDR - rs1544410	0.99 (0.98 - 1.01)	0.50
	VDR - rs11568820	**0.98 (0.96 - 0.99)**	**0.01**
	25(OH)D GRS†	1.00 (0.99 -1.01)	0.74
	Non-HLA GRS‡	1.00 (1.00 -1.00)	0.23
	HLA§	1.00 (0.96 - 1.05)	0.80

CI: confidence interval

*: Tested in mid-pregnancy sample.

†: a risk score for low vitamin D levels, calculated by summing the risk alleles in non-VDR SNPs shown above. See [1] for detail.

‡: non-HLA genetic risk score, calculated by multiplying the number of risk alleles with the OR for type 1 diabetes per each established non-HLA risk SNP (n=51), then summing this per child. Please see our previous publication [2] for a list of non-HLA risk SNPs for type 1 diabetes and their ORs.

§: Coded as dichotomous variable: protective or baseline vs risk (carrying at least one HLA DR3-DQ2 or DR4-DQ8 allele)

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