# Maternal and Newborn Vitamin D–Binding Protein, Vitamin D Levels, Vitamin D Receptor Genotype, and Childhood Type 1 Diabetes

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*Diabetes Care 2019;42:553–559 | https://doi.org/10.2337/dc18-2176*


## OBJECTIVE

**Circumstantial evidence links 25-hydroxy vitamin 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 liquid chromatography–tandem mass spectrometry, and DBP using polyclonal radioimmunoassay, in cord blood and maternal plasma samples collected at delivery and midpregnancy. We genotyped mother and child for variants in or near genes involved in vitamin D metabolism (*GC*, *DHCR7*, *CYP2R1*, *CYP24A1*, *CYP27B1*, and *VDR*). Logistic regression was used to estimate odds ratios (ORs) adjusted for potential confounders.**

## 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 μmol/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.**


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

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*Received 18 October 2018 and accepted 2 January 2019*

*This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc18-2176/-/DC1.*

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-hydroxy vitamin D [25(OH)D], the clinical biomarker of vitamin D status (2). A second hydroxylation to the biologically active form calcitriol [1,25$(OH)_2$D] occurs in the kidneys and probably in other target cells. The biological effects of 1,25$(OH)_2$D are mediated by VDR (encoded by *VDR*) (3). A recent study reported an association between 25(OH)D in early childhood and later islet autoimmunity (a surrogate end point for T1D) that depended on *VDR* genotype (4). VDR-binding sites are overrepresented near genetic regions associated with T1D (5), and several single nucleotide polymorphisms (SNPs) 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 DBP (encoded by *GC*), influence circulating 25-hydroxy vitamin 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 with control subjects 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.

## RESEARCH DESIGN AND 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% of eligible mothers participated) nationwide from 1999 to 2008 (last birth in 2009). The current study uses data from repeated questionnaires (using version VIII of the MoBa data files) and biomarker analyses of maternal and cord blood samples (17). All participating mothers gave written informed consent. The establishment of the MoBa study and data collection in MoBa were previously based on a license 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 (REK Sør-Øst, Oslo, Norway) approved the current study. Children who developed T1D by 5 February 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 comprised the group of T1D case subjects and 576 mother/child pairs from a random sample with available blood samples of the cohort comprised the group of control subjects (Fig. 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 those of the whole MoBa cohort, with the exception of a lower proportion of cesarean section and premature birth (19).

### Blood Sampling

Maternal blood samples were collected in EDTA tubes at hospital laboratories at enrollment around pregnancy week 18 (median 18.5 weeks [interquartile range (IQR) 19.4–17.9]) and again soon after delivery (median 1 day [IQR 3–1] [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 and 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-Aldrich, St. Louis, MO) as previously described (19). Analyses of plasma 25-hydroxyvitamin $D_3$ and $D_2$ were done at the internationally certified Statens Serum Institut, using a liquid chromatography–tandem mass spectrometry and the MSMS Vitamin D Kit (PerkinElmer, Inc., Waltham, MA) for mass spectrometry as previously described (8). The seasonally adjusted (deseasonalized using cosinor modeling 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, we had participants genotyped for selected SNPs using a custom GoldenGate Assay (Illumina, San Diego, CA) as previously described in detail in (22). Briefly, SNPs in the vitamin D pathway (see Supplementary Table 1) were genotyped: five SNPs in or near *CYP2R1*, *CYP24A1*, *CYP27B1*, *GC*, and *DHCR7*, associated with 25(OH)D (7), and two SNPs in or near *VDR* (rs1544410 and 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). 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 ref. 23).

### Other Covariates

Information on birth weight, maternal age at delivery, and delivery mode was obtained from the nationwide Medical

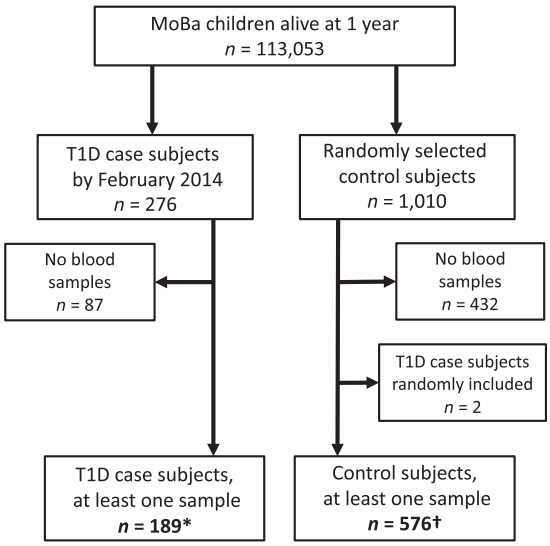


**Figure 1**—Formation of the analysis sample. *148 had three blood samples, 38 had two blood samples, and 3 had one blood sample available for 25(OH)D and DBP testing. There were 174 midpregnancy, 174 postpartum, and 175 cord blood samples. †456 had three blood samples, 111 had two blood samples, and 9 had one blood sample available for 25(OH)D and DBP testing. There were 532 midpregnancy, 525 postpartum, and 542 cord blood samples.

Birth Registry of Norway (24). Information regarding maternal prepregnancy BMI and smoking during pregnancy was obtained from midpregnancy 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. Investigations of the association between the DBP-to-25(OH)D ratio and T1D risk, and potential gene-environment interactions, were secondary aims. The main exposure was estimated average DBP (μmol/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 CI, we used bootstrapping with 10,000 replicates and calculated the percentiles for 95% CIs for the odds ratios (ORs). The average predicted DBP was centered at pregnancy week 36. This centering has little or no consequences for further modeling and statistical significance of our results but was chosen for better comparison 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 midpregnancy and postpartum), DBP quartiles to assess linearity, and the DBP-to-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-to-DBP ratio. We tested interactions of 25(OH)D and selected vitamin D pathway SNPs (see Supplementary Table 1), non-HLA GRS for T1D or offspring HLA genotype. We also tested the interaction between DBP and the *GC* SNP rs2282679, and offspring T1D. These interactions were chosen a priori on the basis of biological plausibility.

We used a 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 versus none, as well as less than or equal to the median versus greater than the median of the T1D non-HLA GRS in control subjects, 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, prepregnancy BMI, smoking in pregnancy, and age at delivery (see Supplementary Fig. 1 for a directed acyclic graph of these). As a sensitivity analysis, we also included maternal T1D, birth weight, 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 midpregnancy, postpartum, and in cord blood samples is shown in Fig. 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 (Supplementary Fig. 2).

### 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 1 (Table 2). However, in analysis of each sample type separately, higher DBP level in the postpartum sample was associated with lower offspring T1D risk (adjusted odds ratio 0.80 [95% CI 0.67–0.95], $P = 0.01$), while DBP midpregnancy or in cord blood was not (Table 2).

The 25(OH)D-to-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 postpartum sample (Table 2). This suggestive association disappeared after further adjustment for DBP, suggesting that the association with the 25OHD-to-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 not otherwise

**Table 1—Characteristics of case subjects with childhood T1D and randomly selected control subjects in MoBa**

| | Control subjects ($n$ = 576) | Case subjects ($n$ = 189) |
|---|---|---|
| Age 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 T1D | 0 (0.0) | 7 (3.7) |
| Preterm birth | 19 (3.3) | 10 (5.3) |
| Missing data | 1 (0.2) | 1 (0.5) |
| Birth weight (g) | | |
| <2,500 | 8 (1.4) | 7 (3.7) |
| 2,500–4,500 | 539 (93.6) | 174 (92.1) |
| >4,500 | 29 (5.0) | 8 (4.2) |
| Parity | | |
| No previous births | 248 (43.1) | 93 (49.2) |
| One birth | 215 (37.3) | 57 (30.2) |
| Two or more births | 113 (19.6) | 39 (20.6) |
| Maternal age (years) | 30 (17–42) | 30 (19–42) |
| Maternal non-Norwegian ethnicity | 30 (5.2) | 10 (5.3) |
| Maternal smoking during pregnancy | | |
| Nonsmoker 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 prepregnancy 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 | | |
| 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 data | 55 (9.5) | 17 (9.0) |
| Child's non-HLA T1D GRS‖ | 61.2 (45.7–76.3) | 63.2 (45.4–78.6) |
| Missing data | 19 (3.5) | 12 (6.8) |
| Child's 25(OH)D GRS | 3 (0–8) | 3 (0–8) |
| Missing data | 20 (3.7) | 13 (7.4) |
| Cesarean section¶ | 59 (10.2) | 36 (19.0) |

Data are median (range) or $n$ (%). *Diagnosis date of the last case subject included: 3 February 2014. †Median age at diagnosis of T1D case subjects was 5.7 years (range 0.7–12.7). ‡Including those who 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) cesarean section.

appreciably change our estimates but resulted in wider CIs (Supplementary Table 2). Likewise, including more adjustment variables as a sensitivity analysis (maternal T1D, birth weight, 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 has been presented previously (8) (for completeness shown in Supplementary Table 2). 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 [Supplementary Table 3]). 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 GRS for T1D, or HLA genotype (Supplementary Table 3). Further, no interaction was detected between DBP and *GC*(*DBP*) SNP rs2282679 (data not shown).

## CONCLUSIONS

In this case-control study nested within a large prospective pregnancy cohort, we found that higher maternal DBP levels at delivery, but not in midpregnancy or in child's cord blood, were 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. With the larger size of the current study, we were 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

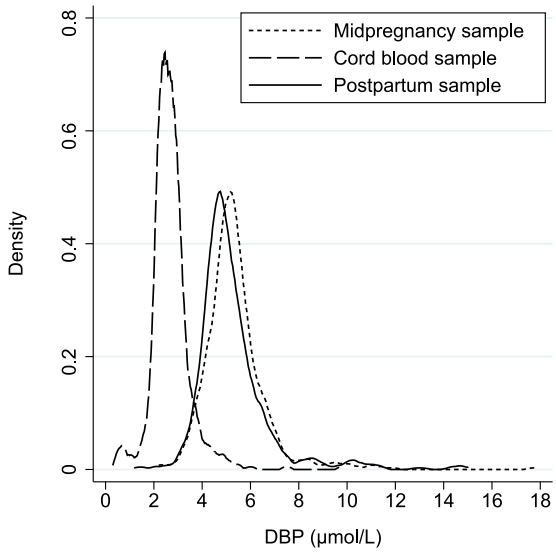


**Figure 2—**Distribution of vitamin DBP concentrations in maternal and cord blood plasma samples from randomly selected control subjects ($n$ = 576) in MoBa. The maternal delivery (postpartum) sample was collected at median 1 day (IQR 0–3) after delivery.

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 toward 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 factors operating late in pregnancy could influence both maternal DBP and offspring T1D risk.

**Table 2—Association between exposures and T1D**

| | OR (95% CI) | aOR (95% CI)* | *P* |
|---|---|---|---|
| DBP, per 1 μmol/L increase | | | |
| Predicted maternal DBP† | 0.74 (0.39–1.22) | 0.49 (0.18–1.02) | NS‡ |
| Midpregnancy | 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-to-DBP ratio | | | |
| Midpregnancy | 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 |

*P* value shown for adjusted analysis. aOR, adjusted OR. *Adjusted for child's HLA genotype and sex and maternal ethnicity, age, prepregnancy BMI, cesarean section, and smoking. †Using maternal (midpregnancy and postpartum) samples in a mixed model to predict maternal DBP values at gestational week 36. Owing to the reduction of the sampling variation in prediction of maternal DBP, the predicted values have a lower SD of 0.35 (while, e.g., DBP in the postpartum samples has an SD of 1.52). This results in a greater observed estimate, as an increase per unit is roughly equivalent to 3 SD in this analysis. We used bootstrapping (10,000 replications) to obtain unbiased 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 (Supplementary Table 3).

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), suggest that DBP should be more intensively studied in relation to T1D. The interaction of 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 would be ideal as a basis for recommendations. However, well-powered randomized controlled trials 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 4 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 ref. 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 time points. 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 mechanisms involved. Further studies in independent cohorts are needed 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 supports 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.

**Acknowledgments**. The authors are grateful for Dr. Torcelino-Iszatt, Norwegian Institute of Public Health, for English language editing. The authors are grateful for all the participating families in Norway who take part in this ongoing cohort study. The authors are thankful for the Norwegian Childhood Diabetes Registry and the Norwegian Childhood Diabetes Study Group. The authors are thankful for Dr. K. Gillespie and G. Mortimer (Diabetes and Metabolism, School of Clinical Sciences, Southmead Hospital, University of Bristol, Bristol, U.K.) for the HLA typing.

**Funding**. MoBa is supported by the Norwegian Ministry of Health and Care Services and the Ministry of Education and Research, National Institutes of Health/National Institute of Environmental Health Sciences (contract no. N01-ES-75558), National Institutes of Health/National Institute of Neurological Disorders and Stroke (grant no. UO1 NS 047537-01 and grant no. UO1 NS 047537-06A1). The Norwegian Childhood Diabetes Registry is funded by the South-Eastern Norway Regional Health Authority. K.S. was supported by an unrestricted grant from the Oak Foundation, Geneva, Switzerland. Costs of all data acquisition, including laboratory assays in MoBa (the substudy PAGE [Prediction of Autoimmune Diabetes and Celiac Disease in Childhood by Genes and Perinatal Environment]), were supported by Norwegian Research Council grant 2210909/F20 (to L.C.S.). The study was in part supported by funds from the European Research Council, Stiftelsen Kristian Gerhard Jebsen, and the Research Council of Norway (to P.R.N.). Data from the Norwegian Patient Registry have been used for this publication. Genotyping in this study was performed at the Genomics Core Facility, Norwegian Radium Hospital, Oslo, Norway.

The interpretation and reporting of these data are the sole responsibility of the authors, and endorsement by the Norwegian Patient Registry is not intended and should not be inferred. The authors alone are responsible for the content and writing of the manuscript.

**Duality of Interest**. No potential conflicts of interest relevant to this article were reported.

**Author Contributions**. G.T. performed data cleaning and preparation, performed statistical analyses, and drafted the manuscript. G.T., G.J., K.S., and L.C.S. conceived and designed the study. G.T., K.S., and L.C.S. planned statistical analyses. G.T. and L.C.S. performed literature research. S.R.D. and A.S.C. performed measurement of 25(OH)D, DBP measurement, and experimental procedures. P.R.N., G.J., and T.S. acquired incident T1D data. K.S. and L.C.S. acquired pregnancy cohort data. K.S. and L.C.S. obtained funding. All authors interpreted data, revised the manuscript critically for important intellectual content, and gave final approval of the version to be published. G.T. and L.C.S. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Prior Presentation**. Parts of this study were presented in abstract form at the Immunology of Diabetes Society Congress 2018, London, U.K., 25–29 October 2018.

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