Inherited Variation in Vitamin D Genes Is Associated With Predisposition to Autoimmune Disease Type 1 Diabetes

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OBJECTIVE—Vitamin D deficiency [25(OH)D < 50 nmol/L] is commonly reported in both children and adults worldwide, and growing evidence indicates that vitamin D deficiency is associated with many extraskeletal chronic disorders, including the autoimmune diseases type 1 diabetes and multiple sclerosis.

RESULTS—Type 1 diabetic patients have lower circulating levels of 25(OH)D than similarly aged subjects from the British population. Only 4.3 and 18.6% of type 1 diabetic patients reached optimal levels (≥75 nmol/L) of 25(OH)D for bone health in the winter and summer, respectively. We replicated the associations of four vitamin D metabolism genes (GC, DHR7, CYP2R1, and CYP24A1) with 25(OH)D in control subjects. In addition to the previously reported association between type 1 diabetes and CYP27B1 (P = 1.4 × 10^{-3}), we obtained consistent evidence of type 1 diabetes being associated with DHR7 (P = 1.2 × 10^{-3}) and CYP2R1 (P = 3.0 × 10^{-3}).

CONCLUSIONS—Circulating levels of 25(OH)D in children and adolescents with type 1 diabetes vary seasonally and are under the same genetic control as in the general population but are much lower. Three key 25(OH)D metabolism genes show consistent evidence of association with type 1 diabetes risk, indicating a genetic etiological role for vitamin D deficiency in type 1 diabetes.

Vitamin D deficiency is commonly reported in both children and adults (1), and the well-established musculoskeletal consequences include osteomalacia, a softening of bones caused by defective bone mineralization (known as rickets in children), and osteoporosis, a reduced bone mineral density and deterioration in structural bone strength. Other more recently reported consequences are the extraskeletal conditions, which include common cancers (2,3) and coronary artery (4) and autoimmune diseases. The autoimmune or immunemediated diseases include type 1 diabetes, multiple sclerosis, Crohn's disease, and rheumatoid arthritis (5-8). In type 1 diabetes, vitamin D supplementation has been shown to be protective against this chronic disorder (5), caused by T-cell-mediated destruction of insulin-producing β-cells in the pancreas.

The main source of vitamin D is through the action of sunlight (ultraviolet B irradiance) on the skin, which results in the endogenous production of vitamin D3 (cholecalciferol). The only other source is exogenous, through diet as either vitamin D2 (ergocalciferol) or D3. Vitamin D enters the circulation bound to vitamin D-binding proteins (DBPs) and lipoproteins and is released to the liver and hydroxylated to form 25-hydroxyvitamin D [25(OH)D]. A subject's vitamin D status is routinely determined by their levels of 25(OH)D, the inactive circulating form of vitamin D and an established marker of vitamin D availability (7), which has a half-life of 2 weeks (9). 25(OH)D is hydroxylated in the kidneys or in cells of the immune system by the CYP27B1 enzyme (CYP1α) to form 1,25-dihydroxyvitamin D [1,25(OH)2D, calcitriol], the biologically active form responsible for maintaining calcium and phosphorus homeostasis (9).
factor in determining 25(OH)D concentrations. More recently, vitamin D receptor (VDR)-binding sites were reported to be overrepresented near genes associated with type 1 diabetes, Crohn’s disease, and rheumatoid arthritis (13).

Recent evidence indicates that the production and degredation of 1,25(OH)2D is a major signaling component in both the innate (14) and adaptive (15) immune systems. Vitamin D signaling plays an essential role in the activation of monocytes/macrophages in response to infection (14) and possibly in naïve T-cell activation (15,16). These cell populations are central to the development of the autoimmune disease type 1 diabetes (17). However, the relationship between circulating levels of 25(OH)D and immune responsiveness is largely undefined (14).

Type 1 diabetes is a strongly inherited autoimmune disease that affects ~0.4% of European ancestry populations, and incidence has been increasing at 3% per year, with a decreasing trend in age at diagnosis since the 1950s (18). A large number of potential environmental exposures correlate with type 1 diabetes incidence, including viral infection, sanitation and improvements in health care, and dietary intake. The effects of the vitamin D hormone [1,25(OH)2D] in type 1 diabetes was first proposed based upon the observation that incidence rates of type 1 diabetes were negatively correlated with sunlight exposure, resulting in higher incidence at higher latitudes (1), and the distinctive seasonal pattern in type 1 diabetes incidence, with the largest proportion of cases diagnosed during the winter and the lowest during the summer (19). Subsequent evidence includes that type 1 diabetic patients have lower levels of 25(OH)D than age- and sex-matched control subjects (20,21), type 1 diabetic patients have decreased bone mineral density and a greater risk of fractures compared with the general population (22), vitamin D supplementation is reported to be protective against type 1 diabetes (5), the vitamin D hormone has widespread effects in the immune system (14,15,23), and the gene CYP27B1, which encodes the enzyme CYP1α that converts precursor 25(OH)D to 1,25(OH)2D, shows association with type 1 diabetes (24,25) and multiple sclerosis (13,26) risk.

In the current study, we investigate the genetic relationship between vitamin D and type 1 diabetes. This includes a comparison between the vitamin D status of similarly aged type 1 diabetic patients and subjects from the British population and testing genetic variants influencing 25(OH)D metabolism for an association with both circulating levels of 25(OH)D and type 1 diabetes status.

RESEARCH DESIGN AND METHODS

A total of 8,517 British type 1 diabetic case subjects were recruited from pediatric and adult diabetes clinics at 150 National Health Service hospitals across the U.K. as part of the Genetic Resource Investigating Diabetes collection of the Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Immunology Laboratory (www.jdrf.org). The British control subjects consisted of 7,320 subjects drawn from the British control samples with the Wellcome Trust Case-Control Consortium (30) (UKBS-CC) (27,28). The case/control collection was analyzed using a logistic regression model, adjusting for age at bleed, sex, BMI, month of bleed, and geographical region (see below). We note, first, that despite the correlation between age at bleed and age at diagnosis (r = 0.4, age at bleed increases with age at diagnosis) in type 1 diabetic patients, both covariates added to the model (P = 9.5 × 10^{-8} and 0.016, respectively). Second, age at bleed and duration of type 1 diabetes at bleed were highly correlated (r = 0.9; age at bleed increases with duration of type 1 diabetes at bleed), as expected.

Using 25(OH)D concentrations as being severely deficient (<25 mmol/L) (15), deficient (25 mmol/L ≤ 25(OH)D <50 mmol/L), insufficient (50 mmol/L ≤ 25(OH)D < 75 mmol/L), or optimal (≥75 mmol/L) for bone health (33), we defined U.K. seasons as winter (December to February), spring (March to May), summer (June to August), and autumn (September to November).

Statistical analyses. All statistical analyses were performed in either Stata (www.stata.com) or R (www.r-project.org). The type 1 diabetic case subjects with 25(OH)D concentrations were analyzed using linear regression models. The 25(OH)D concentrations were natural log transformed to better approximate a normal distribution, and covariates were selected using forward regression with the following constraints: that we had a limited number of covariates available for both the type 1 diabetic patients; for example, BMI was not available. The log-transformed 25(OH)D concentrations for the type 1 diabetic patients were adjusted for age at bleed, month of bleed, age at diagnosis, and batch and for the UKBS-CC control subjects were adjusted for age at bleed, sex, BMI, month of bleed, and geographical region (see below). We note, first, that the correlation between age at bleed and age at diagnosis (r = 0.4, age at bleed increases with age at diagnosis) in type 1 diabetic patients, both covariates added to the model (P = 9.5 × 10^{-8} and 0.016, respectively). Second, age at bleed and duration of type 1 diabetes at bleed were highly correlated (r = 0.9; age at bleed increases with duration of type 1 diabetes at bleed), as expected.

We imputed unobserved genotypes in the UKBS-CC control Affymetrix version 6.0 data using IMPUTE (34,35) and the reference panel of known CEU haplotypes provided by the International HapMap Project (36). We then tested for an association with 25(OH)D concentrations using SNPTEST (35).

The case/control collection was analyzed using a logistic regression model, adjusted for 12 geographical regions within the U.K. (southwestern, southern, southeastern, London, eastern, Wales, Midlands, north Midlands, northwestern, east, West Riding, northern, and Scotland) to exclude the possibility of confounding by geography. The genotyping and 25(OH)D measurements for case and control subjects. Because the case and control subjects were well matched for region, this stratification resulted in little loss of power (37). The family collection was analyzed using the transmission disequilibrium test.

When testing for an association between type 1 diabetes and an SNP, we performed a 1-degree of freedom (df) likelihood ratio test to determine whether a 1-df multiplicative allelic-effects model or a 2-df genotype-effects model (no specific mode of inheritance assumed) was more appropriate. We
RESULTS
Seasonality of type 1 diabetes diagnosis. We confirmed in 4,127 British type 1 diabetic patients with known month of diagnosis, the previously reported (19) distinct seasonal variation in the incidence of type 1 diabetes (Fig. 1), with the largest proportion (14.0%) of patients diagnosed in January and the lowest (6.4%) in May.

Vitamin D status in type 1 diabetic case subjects compared with the general population. As an indication of vitamin D status within type 1 diabetic patients compared with the general population, we compared 618 type 1 diabetic patients aged 4–18 years with 1,002 NDNS young people aged 4–18 years (32). Figure 2 shows that there was seasonal variation in 25(OH)D concentrations in both NDNS young people and type 1 diabetic patients ($P = 3.9 \times 10^{-33}$ and $1.2 \times 10^{-25}$, respectively), with higher levels in summer and autumn compared with winter and spring.

The majority of NDNS young people surveyed from the general population had suboptimal levels of 25(OH)D (<75 nmol/L) even in the summer months, when only 46.4% had optimal levels of 25(OH)D for bone health ($\geq 75$ nmol/L; Table 1). The suboptimal vitamin D status of the type 1 diabetic patients was even more pronounced with only 18.6% of patients having optimal levels of 25(OH)D in the summer. The lowest proportion of subjects with optimal levels of 25(OH)D was in winter (6.9% of NDNS young people and 3.9% of type 1 diabetic patients). At the health-threatening lower extreme, the highest proportion of subjects with severely deficient levels of 25(OH)D (<25 nmol/L) (12,15) was in winter (6.9% of NDNS young people and 16.5% type 1 diabetic patients), and the lowest proportion in the summer (0.4% of NDNS young people and 1.1% of type 1 diabetic patients) (Table 1).

We fit a logistic regression model to test for an association between vitamin D status and type 1 diabetes risk. We adjusted for season, and the vitamin D status reference group consisted of subjects with optimal levels of 25(OH)D concentrations. The odds ratio (OR) for insufficient subjects was 3.31 (95% CI 2.40–4.56), for deficient subjects was 5.50 (3.89–7.77), and for severely deficient was 8.40 (4.74–14.90) (3-df $P = 1.1 \times 10^{-25}$).

Vitamin D metabolism genes and 25(OH)D concentrations. We replicated the associations of the four 25(OH)D concentration loci (12) (GC [rs2282679], $P = 8.9 \times 10^{-13}$), DHCR7 [rs12785878, $P = 9.9 \times 10^{-4}$], CYP2R1 [rs10741657, $P = 4.4 \times 10^{-3}$], and CYP24A1 [rs6013897, $P = 0.016$], validating both our measurement of vitamin D concentrations and SNP imputation (rs10741657) in 2,610 UKBS-CC control samples (Table 2). In the smaller sample of 720 type 1 diabetic patients, we did not conduct SNP imputation and, consequently, analyzed a proxy SNP for rs2282679 (rs4588, see RESEARCH DESIGN AND METHODS) in GC. We replicated the association of GC (rs4588 $P = 5.2 \times 10^{-13}$) and found some evidence for DHCR7 (rs12785878 $P = 0.036$) and CYP24A1 (rs6013897 $P = 0.054$), thereby validating our measurement of vitamin D concentrations. The SNP effects on 25(OH)D concentrations were consistent between UKBS-CC control and type 1 diabetic patient samples. No evidence was found for CYP2R1 (rs10741657 $P = 0.14$) in the type 1 diabetic patients and for the remaining three vitamin D metabolism genes in UKBS-CC control or type 1 diabetic patient samples (Table 2).

Vitamin D metabolism genes and type 1 diabetes. We tested the four 25(OH)D concentration loci (12) for an association with type 1 diabetes and found evidence of an association with DHCR7 (rs12785878 T>G; OR for minor allele 1.07 [95% CI 1.02–1.13]; $P = 6.8 \times 10^{-10}$ in case/control collections and some evidence (RR 1.10 [0.99–1.21]; $P = 0.067$) in family collections (combined $P = 1.2 \times 10^{-5}$). There was consistent evidence in the case/control and family collections for an association with type 1 diabetes.
at both SNPs in CYP2R1 (combined $P = 3.6 \times 10^{-3}$; Table 3). We also found some evidence for one of the GC SNPs (rs4588 C>A, OR 0.95 [95% CI 0.91–1.00]; $P = 0.050$) in the case/control collection but not in the family collection ($P = 0.71$). No evidence of an association was found in the case/control collection for CYP24A1 (rs6013897 G>A; combined $P = 0.96$).

In the remaining three vitamin D metabolism genes (Table 3), there was only the previously reported association between type 1 diabetes and CYP27B1 (24) (rs10877012 G>A; combined $P = 1.4 \times 10^{-4}$).

**DISCUSSION**

We observed, as have others, the concordance between seasonality of both type 1 diabetes diagnosis (Fig. 1) and 25(OH)D concentrations (Fig. 2), with the highest disease incidence and lowest 25(OH)D concentrations in the winter. We found that type 1 diabetic patients have lower circulating levels of 25(OH)D than similarly aged subjects from the British population (Table 1; Fig. 2), which is consistent with the findings of two previous studies in Italy (21) and Sweden (20). Importantly, the two previous studies compared 25(OH)D concentrations of type 1 diabetic patients measured soon after diagnosis with age- and sex-matched control subjects, and, here, 25(OH)D concentrations were measured at a median time of 5 years (lower and upper quartiles 2 and 8 years, respectively) after diagnosis. This indicates that the circulating levels of 25(OH)D are lower than in the general population soon after diagnosis and remain lower several years after diagnosis, suggesting that the lower levels are not a consequence of the proinflammatory immune system that exists before and shortly after diagnosis (38). In addition, because the two previous studies (20,21) measured 25(OH)D soon after diagnosis, the lower levels are unlikely to be a consequence of treatment with insulin or dietary changes following type 1 diabetes diagnosis.

As the musculoskeletal consequences of vitamin D deficiency are well established, the proportion of young people with severely deficient circulating levels of 25(OH)D is of major concern. Based on the 1997 NDNS of young people aged 4–18 years, >5% (26 of 453; Table 1) of young people in winter and spring are severely deficient.

The comparison of 25(OH)D levels do not take into account covariates such as BMI. Bryden et al. (39) reported, based on 76 type 1 diabetic patients aged 11–18 years (43 male and 33 female), that the BMI of female type 1 diabetic patients was significantly greater than that of the general population, which could be associated with a reduction in 25(OH)D concentrations (40). However, the observed differences between 25(OH)D concentrations in type 1 diabetic patients and the general population are unlikely to be explained by BMI differences alone because we found no difference between 25(OH)D concentrations and type 1 diabetic patient sex ($P = 0.42$), and both male and female type 1 diabetic patients have lower 25(OH)D concentrations than the general population (Table 1).

We replicated the associations of the four 25(OH)D concentration loci in the UKBS-CC control subjects ($P = 0.016$ to 8.9 $\times 10^{-10}$; Table 2), and three of four showed evidence of disease association in the type 1 diabetic patients ($P = 0.054$ to 5.2 $\times 10^{-13}$, Table 2), despite the small sample size (720 type 1 diabetic patients). The consistency of the 25(OH)D concentration loci effects in type 1 diabetic patients and the UKBS-CC control subjects indicate that type 1 diabetes itself is unlikely to confound or mask these genetic associations, a valid concern given that theoretically its treatment and renal complications (41) could effect 25(OH)D concentrations. We note, however, that inconsistent evidence of an association between glycosylated hemoglobin and 25(OH)D levels has been reported (20,40,42).

The four 25(OH)D concentration loci provide an unbiased instrument to test the hypothesis that circulating

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**TABLE 1**

Vitamin D status in 618 type 1 diabetic patients aged 4–18 years compared with 1,002 NDNS young people aged 4–18 years

<table>
<thead>
<tr>
<th>Vitamin D status</th>
<th>Winter (December to February)</th>
<th>Spring (March to May)</th>
<th>Summer (June to August)</th>
<th>Autumn (September to November)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type 1 diabetes</td>
<td>NDNS</td>
<td>Type 1 diabetes</td>
<td>NDNS</td>
</tr>
<tr>
<td>Severe deficient</td>
<td>23 (16.5)</td>
<td>18 (6.9)</td>
<td>79 (56.8)</td>
<td>108 (41.5)</td>
</tr>
<tr>
<td>Deficient</td>
<td>9 (5.9)</td>
<td>8 (4.1)</td>
<td>72 (47.4)</td>
<td>87 (45.1)</td>
</tr>
<tr>
<td>Insufficient</td>
<td>2 (1.1)</td>
<td>1 (0.4)</td>
<td>45 (25.4)</td>
<td>27 (11.4)</td>
</tr>
<tr>
<td>Optimal</td>
<td>5 (3.3)</td>
<td>4 (1.3)</td>
<td>57 (38.0)</td>
<td>47 (15.1)</td>
</tr>
<tr>
<td>Total number of subjects</td>
<td>139</td>
<td>152</td>
<td>177</td>
<td>150</td>
</tr>
</tbody>
</table>

We defined circulating levels of 25(OH)D as being severely deficient (<25 nmol/L) (15), deficient [25 nmol/L ≤ 25(OH)D < 50 nmol/L], insufficient [50 nmol/L ≤ 25(OH)D < 75 nmol/L], or optimal [≥75 nmol/L] for bone health (33).
We assumed a model of multiple allelic effects because this model was not significantly different from the full genotype model for any of the SNPs tested. We report the maximum number of case, control, and family samples genotyped.

**TABLE 2**

Association between SNPs from vitamin D metabolism genes and 25(OH)D concentration (nmol/L)

<table>
<thead>
<tr>
<th>Genes, SNPs, alleles</th>
<th>720 Type 1 diabetic patients</th>
<th>2,610 UKBS-CC control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression model of log vitamin D concentrations adjusted for month of bleed, age at bleed, age at diagnosis, and batch</td>
<td>Regression model of log vitamin D concentrations adjusted for month of bleed, age at bleed, sex, and BMI</td>
</tr>
<tr>
<td></td>
<td>Coefficient for minor allele</td>
<td>Likelihood ratio test</td>
</tr>
<tr>
<td>CYP27A1, rs17470271, T&gt;A</td>
<td>−0.190</td>
<td>0.355</td>
</tr>
<tr>
<td>GC, rs2282679, A&gt;C</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GC, rs4588, C&gt;A</td>
<td>−2.77</td>
<td>0.375</td>
</tr>
<tr>
<td>GC, rs7041, G&gt;T</td>
<td>−1.68</td>
<td>0.347</td>
</tr>
<tr>
<td>DHC7R7, rs12785878, T&gt;G</td>
<td>−0.829</td>
<td>0.395</td>
</tr>
<tr>
<td>CYP2R1, rs10741657, G&gt;A</td>
<td>0.531</td>
<td>0.357</td>
</tr>
<tr>
<td>CYP2R1, rs12794714, G&gt;A</td>
<td>−0.466</td>
<td>0.352</td>
</tr>
<tr>
<td>VDR (FokI), rs2282679, C&gt;T</td>
<td>−0.268</td>
<td>0.366</td>
</tr>
<tr>
<td>VDR (BsmI), rs1544410, G&gt;A</td>
<td>0.401</td>
<td>0.396</td>
</tr>
<tr>
<td>VDR (Cdx2), rs11658820, G&gt;A</td>
<td>−0.0350</td>
<td>0.423</td>
</tr>
<tr>
<td>CYP27B1, rs10877012, C&gt;A</td>
<td>−0.0350</td>
<td>0.423</td>
</tr>
<tr>
<td>CYP24A1, rs2296241, A&gt;G</td>
<td>−0.349</td>
<td>0.353</td>
</tr>
<tr>
<td>CYP24A1, rs6013897, G&gt;A</td>
<td>−0.900</td>
<td>0.467</td>
</tr>
</tbody>
</table>

The SNPs rs7041, rs10741657, and rs12794714 were imputed in UKBS-CC control subjects. We report the maximum number of case and control samples genotyped.

levels of 25(OH)D are linked to type 1 diabetes or, indeed, to any other disease or trait in which a relationship with vitamin D has been proposed. Consequently, we tested the four 25(OH)D concentration loci along with the three remaining vitamin D metabolism genes for an association with type 1 diabetes. In addition to the previously reported association between type 1 diabetes and CYP27B1 (24), we found consistent statistical evidence of type 1 diabetes being associated with DHC7R7 ($P = 1.2 × 10^{-3}$) and CYP2R1 ($P = 3.0 × 10^{-3}$) in both case/control and family collections (Table 3). Importantly, the coefficients of both of these 25(OH)D concentration loci show that the alleles associated with lower levels of 25(OH)D have an increased risk of type 1 diabetes (Tables 2 and 3). There was some evidence for GC (rs4588 $P = 0.050$) in the case/control collection but not in the family population ($P = 0.71$). A study from Germany has also reported an association with rs10741657/CYP2R1 in 203 type 1 diabetic families (RR 0.64 [95% CI 0.48–0.87]; $P = 4 × 10^{-3}$) and in 284 case and 294 control samples (OR 0.78 [0.61–1.00]; $P = 0.05$) (42). We note that the analysis of CYP27B1 included the case/control samples analyzed previously with an additional 196 case and 1,680 control samples and 1,933 of 2,774 families analyzed previously (24). Bailey et al. (24), in the 2,774 case and 1,680 control samples, obtained more evidence of an association between type 1 diabetes and CYP27B1 ($P = 3.9 × 10^{-3}$).

The most associated 25(OH)D concentration locus, GC, only showed some evidence of an effect on type 1 diabetes in the case/control collection, despite the fact that type 1 diabetic patients have lower levels of 25(OH)D than the general population and two other 25(OH)D concentration loci, DHC7R7 and CYP2R1, were associated with type 1 diabetes. One possible explanation is that the GC locus may only affect the levels of 25(OH)D bound to the DBP, without altering the amount of free and unbound 25(OH)D. Most circulating 25(OH)D is bound to DBP (80%), with 20% free and unbound 25(OH)D.

**TABLE 3**

Association between SNPs from vitamin D metabolism genes and type 1 diabetes

<table>
<thead>
<tr>
<th>Gene, SNP, allele</th>
<th>8,517 Case and 10,438 control subjects</th>
<th>1,933 Families</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR for minor allele (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>CYP27A1, rs17470271, T&gt;A</td>
<td>0.98 (0.93–1.02)</td>
<td>0.29</td>
</tr>
<tr>
<td>GC, rs4588, C&gt;A</td>
<td>0.95 (0.91–1.00)</td>
<td>0.050</td>
</tr>
<tr>
<td>GC, rs7041, G&gt;T</td>
<td>0.98 (0.93–1.03)</td>
<td>0.43</td>
</tr>
<tr>
<td>DHC7R7, rs12785878, T&gt;G</td>
<td>1.07 (1.02–1.13)</td>
<td>6.8 × 10⁻³</td>
</tr>
<tr>
<td>CYP2R1, rs10741657, G&gt;A</td>
<td>0.96 (0.92–1.00)</td>
<td>0.079</td>
</tr>
<tr>
<td>CYP2R1, rs12794714, C&gt;T</td>
<td>1.04 (1.00–1.09)</td>
<td>0.064</td>
</tr>
<tr>
<td>VDR (FokI), rs2282679, G&gt;A</td>
<td>0.99 (0.95–1.04)</td>
<td>0.81</td>
</tr>
<tr>
<td>VDR (BsmI), rs1544410, G&gt;A</td>
<td>1.00 (0.95–1.05)</td>
<td>0.92</td>
</tr>
<tr>
<td>VDR (Cdx2), rs11658820, G&gt;A</td>
<td>1.00 (0.94–1.07)</td>
<td>0.96</td>
</tr>
<tr>
<td>CYP27B1, rs10877012, G&gt;A</td>
<td>0.93 (0.89–0.98)</td>
<td>3.1 × 10⁻³</td>
</tr>
<tr>
<td>CYP24A1, rs2296241, G&gt;A</td>
<td>1.00 (0.95–1.05)</td>
<td>0.95</td>
</tr>
<tr>
<td>CYP24A1, rs6013897, G&gt;A</td>
<td>1.00 (0.95–1.05)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

We assumed a model of multiple allelic effects because this model was not significantly different from the full genotype model for any of the SNPs tested. We report the maximum number of case, control, and family samples genotyped.
and 25(OH)D₃ to DBP and VDR, which makes D₃ more bioavailable than D₂ (43). Standard immunoassays detect the bound and unbound forms. Because the relationship between 25(OH)D levels and immune responsiveness remains largely undefined (14,15) and the biological relationship between circulating 25(OH)D and type 1 diabetes risk remains to be determined, we can only assume that 25(OH)D concentrations may be an indirect surrogate for vitamin D signaling within immune cells.

Recent studies suggest that the vitamin D metabolism gene CYP27B1, associated with both type 1 diabetes (24,25) and multiple sclerosis (26), has a role in vitamin D signaling within immune cells (15). Inducible CYP27B1 and VDR expression has been identified within monocytes, macrophages, and T-cells as being critical in responses to mycobacterial infection and possibly in naïve T-cell activation and proliferation (14–16,23). Consequently, the inducibility of CYP27B1 or VDR expression and/or 1,25(OH)₂D concentrations within the immune cells such as monocytes, macrophages, and T-cells could be a relevant quantitative phenotype in additional analyses of the relationship between vitamin D metabolism and the development of autoimmune disease. In such future studies, children with type 1 diabetes–affected siblings and mothers with a family history of type 1 diabetes and their newborns should provide additional insight into the association of vitamin D metabolism and susceptibility to type 1 diabetes and perhaps to other autoimmune diseases, such as multiple sclerosis (7).

Since the advent of GWA studies, great progress has been made in identifying susceptibility loci for autoimmune diseases such as type 1 diabetes (44) and in understanding how susceptibility alleles affect immune systems. The susceptibility alleles of three type 1 diabetes loci collectively provide a relevant example for the current study and for its interpretation: PTPN22 (45) has been associated with lower T-cell signaling and reduced T-cell activation (46), PTPN2 (47) has been associated with lower T-cell interleukin (IL)-2 cytokine signaling (48), and IL2RA (49) has been associated with reduced IL-2 production in memory T-cells (50). These results indicate that inherited impairment or lowering of T-cell signaling and activation is a predisposing phenotype for type 1 diabetes. Recently, von Essen et al. (15) have suggested that severely low circulating levels of 25(OH)D are associated with reduced T-cell activation and proliferation, although there are other considerations to be taken into account in the interpretation of these studies (16). Taken together, these studies indicate a common mechanism in type 1 diabetes predisposition, T-cell hyporesponsiveness, which may be restored to normal levels by vitamin D supplementation to achieve optimal levels of 25(OH)D, a hypothesis that can be tested in future studies.

In conclusion, we have linked the genetic determinants of circulating levels of 25(OH)D (DHCR7 and CYP2R1) and vitamin D signaling in T-cells (CYP27B1) with type 1 diabetes risk. This provides the evidence that vitamin D deficiency of type 1 diabetic patients probably plays a primary, causal role in the pathogenesis of type 1 diabetes and is not secondary to hyperglycemia, diet, or to treatment with insulin (20). However, we cannot yet rule out that treatment with insulin may be responsible for the lowering of circulating levels of 25(OH)D or of CYP27B1 expression within monocytes, macrophages, and T-cells. Consequently, this study supports the potential of vitamin D supplementation as part of a prevention strategy for autoimmune disease and for vitamin D deficiency–related comorbidities in type 1 diabetic patients in later life. Randomized controlled trials of vitamin D supplementation will be required to establish both causality (5) and health benefits for existing type 1 diabetic patients. A first step will be to establish if optimal 25(OH)D concentrations can be achieved in the circulation of patients with type 1 diabetes by oral supplementation and if improved 25(OH)D status alters any of the emerging immunophenotypes being associated with this autoimmune disease.

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