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

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

Citation

Published Version
doi:10.2337/db10-1656

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:10361976

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
Inherited Variation in Vitamin D Genes Is Associated With Predisposition to Autoimmune Disease Type 1 Diabetes

Jason D. Cooper, Deborah J. Smyth, Neil M. Walker, Helen Stevens, Oliver S. Burren, Chris Wallace, Christopher Greissl, Elizabeth Ramos-Lopez, Elina Hyppönen, David B. Dunger, Timothy D. Spector, Willem H. Ouwehand, Thomas J. Wang, Klaus Badenhoop, and John A. Todd

OBJECTIVE—Vitamin D deficiency (25-hydroxyvitamin D [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.

RESEARCH DESIGN AND METHODS—We measured 25(OH)D concentrations in 720 case and 2,610 control plasma samples and genotyped single nucleotide polymorphisms from seven vitamin D metabolism genes in 8,517 case, 10,438 control, and 1,933 family samples. We tested genetic variants influencing 25(OH)D metabolism for an association with both circulating 25(OH)D concentrations and disease status.

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, DHCR7, 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 × 10−4), we obtained consistent evidence of association with two type 1 diabetes being associated with DHCR7 (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. Diabetes 60:1624–1631, 2011

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 disease (4) and autoimmune diseases. The autoimmune or immune-mediated 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).

A substantial proportion of 25(OH)D variation has been attributed to genetic factors, with heritability estimates of 28.8% (10) and 43% (11) reported. A recent genome-wide association (GWA) meta-analysis of circulating levels of 25(OH)D in 33,996 samples of European descent from 14 cohorts reported convincing evidence for four loci, namely GC/4p12 (rs2282670 P = 1.9 × 10−109), DHCR7/11q12 (rs12785878 P = 2.1 × 10−2), CYP27B1/11p15 (rs10741657 P = 3.3 × 10−20), and CYP24A1/20q13 (rs6013897 P = 6.0 × 10−10) (12). These single nucleotide polymorphisms (SNPs) are within or near genes involved in vitamin D transport (GC), cholesterol synthesis (DHCR7), and hydroxylation (CYP27B1 and CYP24A1). No loci linked to skin pigmentation were detected, despite being a major

From the 1Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory, Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Addenbrooke’s Hospital, Cambridge, U.K.; the 2Department of Internal Medicine I, Division of Endocrinology, Diabetes, and Metabolism, University Hospital Frankfurt, Frankfurt am Main, Germany; the 3University College London Institute of Child Health, Medical Research Council Centre of Epidemiology for Child Health and Centre for Paediatric Epidemiology and Biostatistics, London, U.K.; the 4Department of Paediatrics, University of Cambridge, Addenbrooke’s Hospital, Cambridge, U.K.; the 5Department of Twin Research and Genetic Epidemiology, King’s College London, London, U.K.; the 6Department of Haematology, University of Cambridge and National Health Service Blood and Transplant, Cambridge, U.K.; Human Genetics, Wellcome Trust Sanger Institute, Genome Campus, Hinxton, U.K.; the 7Division of Cardiology, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts; the 8Harvard Medical School, Boston, Massachusetts; and the 9Framingham Heart Study, Framingham, Massachusetts.

Corresponding author: John A. Todd, john.todd@cimr.cam.ac.uk.

Received 30 November 2010 and accepted 27 February 2011.

DOI: 10.2337/db10-1656

© 2011 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.
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 degradation 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 effect 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 Inflammation Laboratory (www.childhood-diabetes.org.uk/grd.shtml). The British control subjects consisted of 7,320 subjects drawn from the British British control subjects consisted of 7,320 subjects drawn from the British control samples with the Wellcome Trust Case-Control Consortium (30) and the control samples with the Wellcome Trust Case-Control Consortium (30) and the reference panel of known CEU samples (35). The case/control collection was analyzed using a logistic regression model, with 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 data were analyzed by the University of Edinburgh (UKBS-CC) (27,28).

A collection of 1,933 families (2,125 parent-child trios) were also genotyped, a large number of covariates available for 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 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.

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 data were analyzed by the University of Edinburgh (UKBS-CC) (27,28).

A collection of 1,933 families (2,125 parent-child trios) were also genotyped, consisting of 472 multiplex families from the Diabetes U.K. Warren Collection, 80 simplex families from Yorkshire (U.K.), 293 multiplex and simplex families from Northern Ireland (U.K.), 423 simplex families from Romania, 315 multiplex families from the Human Biological Data Interchange (U.S.), and 360 simplex families from Norway. All subjects were of white European ancestry.

Genotyping and 25(OH)D Measurements. Before the GWA meta-analysis of 25(OH)D concentrations (12), we (J.D.C., D.J.S., H.S., E.H., T.D.S., and J.A.T.) genotyped 10 SNPs using TaqMan assays from six genes that encode proteins that were established as major components of vitamin D metabolism (2), to test for an association between vitamin D metabolism genes and circulating levels of 25(OH)D and between vitamin D metabolism genes and type 1 diabetes. The six vitamin D metabolism genes were CYP27A1 (rs14740271), CYP24A1 (rs10877012), CYP27B1 (rs1544410), CYP2R1 (rs11568820), CYP2D6 (rs2285570), vitamin D receptor; rs2282679 in 60 CEU parents; www.1000genomes.org and rs7041 (Asp119Glu; r² = 0.35 with rs2282679) from this gene.

We measured 25(OH)D concentrations for a subset of 2,610 UKBS-CC control samples with the Wellcome Trust Case-Control Consortium (30) Affymetrix version 6.0 chip (www.affymetrix.com) genotype data. We also genotyped 10 SNPs using TaqMan assays from the Diabetes U.K. Warren Collection, 80 simplex families from Yorkshire (U.K.), 293 multiplex and simplex families from Northern Ireland (U.K.), 423 simplex families from Romania, 315 multiplex families from the Human Biological Data Interchange (U.S.), and 360 simplex families from Norway. All subjects were of white European ancestry.

We measured 25(OH)D concentrations for a subset of 2,610 UKBS-CC control samples with the Wellcome Trust Case-Control Consortium (30) Affymetrix version 6.0 chip (www.affymetrix.com) genotype data. We also genotyped 10 SNPs using TaqMan assays from the Diabetes U.K. Warren Collection, 80 simplex families from Yorkshire (U.K.), 293 multiplex and simplex families from Northern Ireland (U.K.), 423 simplex families from Romania, 315 multiplex families from the Human Biological Data Interchange (U.S.), and 360 simplex families from Norway. All subjects were of white European ancestry.

We measured 25(OH)D concentrations for a subset of 2,610 UKBS-CC control samples with the Wellcome Trust Case-Control Consortium (30) Affymetrix version 6.0 chip (www.affymetrix.com) genotype data. We also genotyped 10 SNPs using TaqMan assays from the Diabetes U.K. Warren Collection, 80 simplex families from Yorkshire (U.K.), 293 multiplex and simplex families from Northern Ireland (U.K.), 423 simplex families from Romania, 315 multiplex families from the Human Biological Data Interchange (U.S.), and 360 simplex families from Norway. All subjects were of white European ancestry.

We measured 25(OH)D concentrations for a subset of 2,610 UKBS-CC control samples with the Wellcome Trust Case-Control Consortium (30) Affymetrix version 6.0 chip (www.affymetrix.com) genotype data. We also genotyped 10 SNPs using TaqMan assays from the Diabetes U.K. Warren Collection, 80 simplex families from Yorkshire (U.K.), 293 multiplex and simplex families from Northern Ireland (U.K.), 423 simplex families from Romania, 315 multiplex families from the Human Biological Data Interchange (U.S.), and 360 simplex families from Norway. All subjects were of white European ancestry.

We measured 25(OH)D concentrations for a subset of 2,610 UKBS-CC control samples with the Wellcome Trust Case-Control Consortium (30) Affymetrix version 6.0 chip (www.affymetrix.com) genotype data. We also genotyped 10 SNPs using TaqMan assays from the Diabetes U.K. Warren Collection, 80 simplex families from Yorkshire (U.K.), 293 multiplex and simplex families from Northern Ireland (U.K.), 423 simplex families from Romania, 315 multiplex families from the Human Biological Data Interchange (U.S.), and 360 simplex families from Norway. All subjects were of white European ancestry.

We measured 25(OH)D concentrations for a subset of 2,610 UKBS-CC control samples with the Wellcome Trust Case-Control Consortium (30) Affymetrix version 6.0 chip (www.affymetrix.com) genotype data. We also genotyped 10 SNPs using TaqMan assays from the Diabetes U.K. Warren Collection, 80 simplex families from Yorkshire (U.K.), 293 multiplex and simplex families from Northern Ireland (U.K.), 423 simplex families from Romania, 315 multiplex families from the Human Biological Data Interchange (U.S.), and 360 simplex families from Norway. All subjects were of white European ancestry.

We measured 25(OH)D concentrations for a subset of 2,610 UKBS-CC control samples with the Wellcome Trust Case-Control Consortium (30) Affymetrix version 6.0 chip (www.affymetrix.com) genotype data. We also genotyped 10 SNPs using TaqMan assays from the Diabetes U.K. Warren Collection, 80 simplex families from Yorkshire (U.K.), 293 multiplex and simplex families from Northern Ireland (U.K.), 423 simplex families from Romania, 315 multiplex families from the Human Biological Data Interchange (U.S.), and 360 simplex families from Norway. All subjects were of white European ancestry.
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 × 10^−33 and 1.2 × 10^−25, respectively), with higher levels in winter 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 (≥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% 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 × 10^−35).

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 × 10^−13], DHCGR7 [rs12785878, P = 9.9 × 10^−4], CYP2R1 [rs10741657, P = 4.4 × 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 × 10^−13) and found some evidence for DHCGR7 (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 DHCGR7 (rs12785878 T>G; OR for minor allele 1.07 [95% CI 1.02–1.13]; P = 6.8 × 10^−3) in case/control collections and some evidence (relative risk [RR] 1.10 [95% CI 0.99–1.21]; P = 0.067) in family collections (combined P = 1.2 × 10^−3). There was consistent evidence in the case/control and family collections for an association with 25(OH)D concentrations.
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 T>C; 1.00 [0.95–1.05]; $P = 0.96$).

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>T; 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 $8.9 \times 10^{-13}$; 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

**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>Severe deficiency Group</td>
<td>Type 1 diabetes</td>
<td>23 (16.5)</td>
<td>9 (5.9)</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td></td>
<td>NDNS</td>
<td>18 (6.9)</td>
<td>8 (4.1)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Deficiency Group</td>
<td>Type 1 diabetes</td>
<td>79 (56.8)</td>
<td>72 (47.4)</td>
<td>45 (25.4)</td>
</tr>
<tr>
<td></td>
<td>NDNS</td>
<td>108 (41.5)</td>
<td>87 (45.1)</td>
<td>27 (11.4)</td>
</tr>
<tr>
<td>Insufficient Group</td>
<td>Type 1 diabetes</td>
<td>31 (22.3)</td>
<td>65 (42.8)</td>
<td>97 (54.8)</td>
</tr>
<tr>
<td></td>
<td>NDNS</td>
<td>91 (35.9)</td>
<td>78 (40.4)</td>
<td>99 (41.8)</td>
</tr>
<tr>
<td>Optimal Group</td>
<td>Type 1 diabetes</td>
<td>6 (4.3)</td>
<td>6 (3.9)</td>
<td>33 (18.6)</td>
</tr>
<tr>
<td></td>
<td>NDNS</td>
<td>43 (16.5)</td>
<td>20 (10.4)</td>
<td>110 (46.4)</td>
</tr>
<tr>
<td>Total number of subjects Group</td>
<td>Type 1 diabetes</td>
<td>139</td>
<td>152</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>NDNS</td>
<td>260</td>
<td>193</td>
<td>237</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).
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 DHCR7 (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 collection (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 control samples (OR 0.78 [95% CI 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 families, obtained more evidence of an association between type 1 diabetes and CYP27B1 (2,774 family P = 3.9 × 10⁻³, 1,933 family P = 0.011, Table 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, DHCR7 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 (80–90%) and to albumin (10–20%), with <1% unbound (43). An additional

### Table 2

<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>Coefficient for minor allele</td>
<td>Likelihood ratio test P (1 df)</td>
</tr>
<tr>
<td>CYP27A1, rs17470271, A&gt;T</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>DHCR7, 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), rs2228570, 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>CYP2R1, rs10787012, G&gt;T</td>
<td>-0.0350</td>
<td>0.423</td>
</tr>
<tr>
<td>CYP24A1, rs2296241, G&gt;A</td>
<td>-0.349</td>
<td>0.353</td>
</tr>
<tr>
<td>CYP24A1, rs6013897, T&gt;A</td>
<td>-0.900</td>
<td>0.467</td>
</tr>
<tr>
<td>Coef</td>
<td>0.0176</td>
<td>0.0176</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.

### Table 3

<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) P</td>
<td>RR for minor allele (95% CI) P</td>
</tr>
<tr>
<td>CYP27A1, rs17470271, A&gt;T</td>
<td>0.98 (0.93–1.02)</td>
<td>0.97 (0.89–1.05)</td>
</tr>
<tr>
<td>GC, rs4588, C&gt;A</td>
<td>0.95 (0.91–1.00)</td>
<td>0.98 (0.98–1.07)</td>
</tr>
<tr>
<td>GC, rs7041, G&gt;T</td>
<td>0.98 (0.93–1.03)</td>
<td>0.98 (0.98–1.07)</td>
</tr>
<tr>
<td>DHCR7, rs12785878, T&gt;G</td>
<td>1.07 (1.02–1.13)</td>
<td>1.10 (0.99–1.21)</td>
</tr>
<tr>
<td>CYP2R1, rs10741657, G&gt;A</td>
<td>0.96 (0.92–1.00)</td>
<td>0.97 (0.87–0.95)</td>
</tr>
<tr>
<td>CYP2R1, rs12794714, G&gt;A</td>
<td>1.04 (1.00–1.09)</td>
<td>1.13 (1.04–1.24)</td>
</tr>
<tr>
<td>VDR (FokI), rs2228570, C&gt;T</td>
<td>0.99 (0.95–1.04)</td>
<td>0.92 (0.85–1.00)</td>
</tr>
<tr>
<td>VDR (BsmI), rs1544410, G&gt;A</td>
<td>1.00 (0.95–1.05)</td>
<td>0.93 (0.85–1.01)</td>
</tr>
<tr>
<td>CYP2R1, rs10787012, G&gt;T</td>
<td>1.00 (0.94–1.07)</td>
<td>1.12 (0.91–1.32)</td>
</tr>
<tr>
<td>CYP27B1, rs10787012, G&gt;T</td>
<td>0.93 (0.89–0.98)</td>
<td>0.89 (0.82–0.97)</td>
</tr>
<tr>
<td>CYP24A1, rs2296241, G&gt;A</td>
<td>1.00 (0.95–1.05)</td>
<td>0.95 (0.85–1.01)</td>
</tr>
<tr>
<td>CYP24A1, rs6013897, T&gt;A</td>
<td>1.00 (0.95–1.05)</td>
<td>—</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.
consideration is the difference in affinities of 25(OH)D$_2$ and 25(OH)D$_3$ to DBP and VDR, which makes D$_3$ more bioavailable than D$_2$ (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)$_2$D 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$_3$ 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 fully 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 (50).

ACKNOWLEDGMENTS

The authors thank the U.K. Medical Research Council (MRC) (grant no. G0000934) and the Wellcome Trust (grant no. 068545/Z/02) for funding the collection of DNA for the British 1958 Birth Cohort. The authors acknowledge use of DNA from the UKBS-CC, funded by the Wellcome Trust (grant 076113/C/04/Z), the Wellcome Trust/Juvenile Diabetes Research Foundation (grant 061858), and the National Institutes of Health Research of England. The collection was established as part of the Wellcome Trust Case-Control Consortium. The authors acknowledge use of DNA from the Human Biological Data Interchange and Diabetes U.K. for the U.S. and U.K. multiplex families, respectively; the Norwegian Study Group for Childhood Diabetes (D. Undlien, University of Oslo, and K. Ronningen, Norwegian Institute of Public Health, Norway) for the Norwegian families; D. Savage of the Belfast Health and Social Care Trust; C. Patterson and D. Carson of Queen’s University Belfast; P. Maxwell of Belfast City Hospital for the Northern Irish families; and C. Guja and C. Ionescu-Tirgoviste of the Institute of Diabetes “N Paulescu”, Romania, for the Romanian families. Funding for the project was provided by the Wellcome Trust (award no. 076113). The Cambridge Institute for Medical Research is in receipt of a Wellcome Trust Strategic Award (079895), The Great Ormond Street Hospital/University College London, Institute of Child Health receives a proportion of funding from the Department of Health’s National Institute for Health Research (Biomedical Research Centre Funding). The Medical Research Council provides funds for the MRC Centre of Epidemiology for Child Health and for the vitamin D genetics project (G0001653). The National Diet and Nutrition Survey was funded by the Food Standards Agency and Department of Health and was accessed through the U.K. Data Archive (study no. 4243). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. J.D.C. was funded by the Juvenile Diabetes Research Foundation International and the Wellcome Trust, the National Institute for Health Research Cambridge Biomedical Centre, and the European Union (FP7-NAIMIT; grant agreement no. 241447). D.J.S., N.M.W., H.S., and J.A.T. were funded by the Juvenile Diabetes Research Foundation International, the Wellcome Trust, and the National Institute for Health Research Cambridge Biomedical Centre. J.A.T. is a Senior Investigator of the National Institute for Health Research. K.B. is funded by the European Union (FP7-NAIMIT and FP7-EURADRENAL; grant agreement no. 241447 and 201167) and T.J.W. by the American Heart Association. E.H. is funded by the Department of Health (U.K.) Public Health Career Scientist Award.

T.J.W. has participated in the scientific advisory board of Diasorin. No other potential conflicts of interest relevant to this article were reported.
J.D.C. conducted analyses and wrote the manuscript. D.J.S. contributed to sample handling and genotyping. N.M.W. managed data. H.S. contributed to sample handling and genotyping. O.S.B. conducted bioinformatics. C.W. conducted analyses. J.A.T. wrote the manuscript. All authors reviewed, edited, and discussed the manuscript.

DNA control samples were prepared and provided by S. Ring, R. Jones, and M.W. McAr dife of the University of Bristol; D. Strachan of the University of London; and P. Burton of the University of Leicester. This study makes use of data from the “National Diet and Nutrition Survey: Young People Aged 4 to 18 Years, 1997,” whose principal investigators were from the Social Survey Division of Office for National Statistics; the MRC Resource Centre for Human Nutrition Research; the Ministry of Agriculture, Fisheries, and Food; and the Department of Health. This study also makes use of data generated by the Wellcome Trust Case-Control Consortium. A full list of the investigators who contributed to the generation of the data are available from http://www.wtccc.org.uk. The authors thank P. Clarke, G. Coleman, S. Duley, D. Harrison, S. Hawkins, M. Maisuria, T. Mistry, and N. Taylor from the Juvenile Diabetes Research Foundation (JDRF)/Wellcome Trust Diabetes and Inflammation Laboratory for preparation of DNA samples and H. Schule nburg from the JDRF/Wellcome Trust Diabetes and Inflammation Laboratory for genotyping using Taqman. The authors thank the participation of all the patients, control subjects, and family members. The U.K. Data Archive bears no responsibility for the additional analysis or interpretation of the data. The authors are also grateful to Ann Prentice, MRC Human Nutrition Research, for discussing the measurement of 25(OH)D in the 1997 NDNS.

REFERENCES

26. Wellcome Trust Case Control Consortium. Genome-wide association studies of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661–678
27. Wellcome Trust Case Control Consortium; Australiano-Amercian Spondylitis Consorium (TASC); Bioinformatics of RA Genes Genetics and Genomics Study Syndicate (BRRAGGS) Steering Committee; Breast Cancer Susceptibility Collaboration (UK). Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. Nat Genet 2007;39:1329–1337


47. Todd JA, Walker NM, Cooper JD, et al.; Genetics of Type 1 Diabetes in Finland; Wellcome Trust Case Control Consortium. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet 2007;39:857–864

