Association of the Vitamin D Metabolism Gene *CYP27B1* With Type 1 Diabetes

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OBJECTIVE—Epidemiological studies have linked vitamin D deficiency with the susceptibility to type 1 diabetes. Higher levels of the active metabolite 1α,25-dihydroxyvitamin D ($1\alpha,25(OH)_2D$) could protect from immune destruction of the pancreatic β-cells. $1\alpha,25(OH)_2D$ is derived from its precursor 25-hydroxyvitamin D by the enzyme 1α-hydroxylase encoded by the CYP27B1 gene and is inactivated by 24-hydroxylase encoded by the CYP24A1 gene. Our aim was to study the association between the CYP27B1 and CYP24A1 gene polymorphisms and type 1 diabetes.

RESEARCH DESIGN AND METHODS—We studied 7,854 patients with type 1 diabetes, 8,758 control subjects from the U.K., and 2,774 affected families. We studied four *CYP27B1* variants, including common polymorphisms −1260C>A (rs10877012) and +2838T>C (rs4646536) and 16 tag polymorphisms in the CYP24A1 gene.

RESULTS—We found evidence of association with type 1 diabetes for *CYP27B1* −1260 and +2838 polymorphisms, which are in perfect linkage disequilibrium. The common C allele of *CYP27B1* −1260 was associated with an increased disease risk in the case-control analysis (odds ratio for the C/C genotype 1.22, $P = 9.6 \times 10^{-4}$) and in the fully independent collection of families (relative risk for the C/C genotype 1.33, $P = 3.9 \times 10^{-3}$). The combined *P* value for an association with type 1 diabetes was 3.8×10^{-6}. For the CYP24A1 gene, we found no evidence of association with type 1 diabetes (multilocus test, $P = 0.23$).

CONCLUSIONS—The present data provide evidence that common inherited variation in the vitamin D metabolism affects susceptibility to type 1 diabetes. ***Diabetes*** **56:2616–2621, 2007**

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$1\alpha,25(OH)_2D$, 1α,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; EFSD, European Foundation for the Study of Diabetes; IL, interleukin; MAF, minor allele frequency; NCBI, National Center for Biotechnology Information; SNP, single nucleotide polymorphism; VDR, vitamin D receptor.

Type 1 diabetes is strongly inherited and yet exhibits striking epidemiological features such as seasonality in diagnosis, with more cases diagnosed in the autumn and winter months, and a north-south geographical gradient, suggesting inverse correlation between the amount of sunshine and type 1 diabetes incidence (1,2). Lower serum concentrations of 1α,25-dihydroxyvitamin D ($1\alpha,25(OH)_2D$), the hormonally active form of vitamin D, and of its precursor 25-hydroxyvitamin D (25(OH)D) have been reported at the diagnosis of type 1 diabetes compared with normal control subjects (3–5). Epidemiological studies indicate that vitamin D supplementation in early childhood is associated with decreased type 1 diabetes incidence (6–8). However, a direct role of impaired vitamin D metabolism in the etiology of type 1 diabetes has not been proven. If vitamin D is a significant factor in type 1 diabetes, then it might be expected that common functional sequence polymorphisms in the genes that influence vitamin D action could predispose to the disease. We have previously studied the gene of the vitamin D receptor (VDR), which binds $1\alpha,25(OH)_2D$ and mediates the effects of vitamin D. We found no association between *VDR* sequence variants and type 1 diabetes, in contrast to some other studies with smaller sample sizes (9), and a recently conducted meta-analysis also found no evidence of association (10).

Several studies have reported associations of type 1 diabetes and other autoimmune diseases with polymorphisms in the CYP27B1 gene on chromosome 12q13.1-q13.3 (11–14), which encodes 1α-hydroxylase, the enzyme that converts 25(OH)D into $1\alpha,25(OH)_2D$. However, these results have not been verified. In the present study, we have investigated the association between type 1 diabetes and sequence variants in the CYP27B1 gene. Circulating $1\alpha,25(OH)_2D$ is biologically inactivated through a series of reactions beginning with 24-hydroxylation. Vitamin D 24-hydroxylase is encoded by the CYP24A1 gene located on chromosome 20q13.2-q13.3. Here, we have for the first time also studied the association between type 1 diabetes and *CYP24A1* polymorphisms.

RESEARCH DESIGN AND METHODS

We studied a case-control collection comprising 7,854 patients with type 1 diabetes and 8,758 healthy control subjects from the U.K. The recruitment of these subjects and sample processing have been described elsewhere (15). We also studied *CYP27B1* polymorphisms in a family collection including 2,774 type 1 diabetes families with one or two affected offspring (815 from the U.K. and Northern Ireland, 841 from Finland, 335 from the U.S., 360 from Norway, and 423 from Romania), providing 3,081 parent-child trio genotypes for *CYP27B1* −1260 and 2,198 trio genotypes for *CYP27B1* +2838. The collection of all DNA samples has been approved by relevant ethical committees. We obtained written informed consent from all participants.

TABLE 1
CYP27B1 −1260 allele and genotype frequencies and association test results in 7,854 case, 8,758 control, and 3,081 parent-child trio genotypes

	Case subjects	Control subjects	OR (95% CI)	*P* value
Allele				
A	4,999 (31.8)	5,836 (33.3)	1.00 (reference)	
C	10,709 (68.2)	11,680 (66.7)	1.07 (1.02–1.13)	2.9×10^{-3}*
Genotype				
A/A	767 (9.8)	999 (11.4)	1.00 (reference)	
C/A	3,465 (44.1)	3,838 (43.8)	1.20 (1.08–1.33)	
C/C	3,622 (46.1)	3,921 (44.8)	1.22 (1.10–1.36)	9.6×10^{-4}†
	Transmitted	Untransmitted	RR (95% CI)	
Allele C	1,405 (52.6)	1,264 (47.4)	1.11 (1.03–1.20)	6.4×10^{-3}‡
Genotype				
A/A	274 (8.9)	989 (10.7)	1.00 (reference)	
C/A	1,369 (44.4)	4,055 (43.9)	1.27 (1.08–1.48)	
C/C	1,438 (46.7)	4,199 (45.4)	1.33 (1.12–1.58)	3.9×10^{-3}†

Data are *n* (%). For the case-control collection, we adopted a genotype model because it was significantly different from the multiplicative allelic effects model ($\chi_1^2 = 5.0$, $P = 0.025$). For the family collection, although there was no difference between the models ($\chi_1^2 = 3.6$, $P = 0.056$), we adopted the genotype model for consistency with the case-control collection. *1-df likelihood ratio test for multiplicative allelic effects. †2-df likelihood ratio test for genotype effects. ‡Transmission disequilibrium test. Genotypes for the family-based pseudo-control subjects were estimated as described previously (17).

Genotyping. In the CYP27B1 gene, we genotyped three single nucleotide polymorphisms (SNPs), *CYP27B1* −1260C>A (rs10877012, located in the 5′ region) and *CYP27B1* +2838T>C (rs4646536, located in intron 6), which were previously reported (11–14), and rs8176345, a synonymous SNP in exon 5 that we found by sequencing. We used HapMap data (16) to select tag SNPs that capture common variants in the CYP24A1 gene. Of the 111 HapMap SNPs located in the region (National Center for Biotechnology Information [NCBI] build 34, coordinates chromosome 20: 53,450,894.0.53,482,103), 54 SNPs had minor allele frequency (MAF) >0.05, and 16 were chosen as tag SNPs that capture association of other common variants with $r^2 > 0.8$. *CYP24A1* SNPs were genotyped in up to 5,239 case and 5,539 control subjects (exact numbers for each SNP are shown in Table 3). Genotyping was done using TaqMan (Assay-by-design; Applied Biosystems, Warrington, U.K.; see the online appendix [available at http://dx.doi.org/10.2337/db07–0652]). All genotypes were scored by two researchers independently to minimize error. Genotypes of control subjects and parents did not deviate from Hardy-Weinberg equilibrium above that expected at random ($P > 0.05$).

DNA sequencing. Direct sequencing of nested PCR products from 32 healthy control subjects from the U.K. was performed using an Applied Biosystems 3700 capillary sequencer (Foster City, CA). Polymorphisms were identified using the Staden Package (http://www.mrc-lmb.cam.ac.uk/pubseq/) and mapped to the NCBI human genome build 35.

Statistical analyses. All statistical analyses were performed within Stata statistical package (http://www.stata.com), using additional Stata routines (http://www-gene.cimr.cam.ac.uk/clayton/software/). We analyzed case and control subjects using logistic regression models (17), adjusting for 12 broad geographical regions, to allow for geographic variation in allele frequencies across the U.K. (18). The families were analyzed using the transmission disequilibrium test (19) and conditional logistic regression (17). A score test was used to combine tests from family and case-control studies as described previously (15). We used htstep, htsearch, and haptag programs within Stata 8.2 to select tag SNPs in the CYP24A1 gene. For these SNPs, we performed a multilocus test using mlpop program in Stata 8.2, which tests for association between disease and the tag SNPs due to linkage disequilibrium with one or more causal variants in the region. It contrasts allele frequencies of a nonredundant set of tag SNPs between case and control subjects by use of Hotelling's t^2 test (20,21). We did not apply correction for multiple testing.

RESULTS

Association analysis of the *CYP27B1* polymorphisms. We found evidence that the promoter polymorphism *CYP27B1* −1260 is associated with type 1 diabetes in both the case-control ($P = 9.6 \times 10^{-4}$; C/C genotype, odds ratio [OR] 1.22 [95% CI 1.10–1.36]; Table 1) and the family ($P = 3.9 \times 10^{-3}$; C/C genotype, relative risk [RR] 1.33 [95% CI 1.12–1.58]; Table 1) collections. Consequently, when we combined evidence from both collections, which are fully independent from each other, the combined test provided statistical support for an association between type 1 diabetes and *CYP27B1* −1260 ($P = 3.8 \times 10^{-6}$ for the 2 degree of freedom [df] genotype model, see Table 1 legend). There was evidence of population heterogeneity in the parent allele frequencies of *CYP27B1* −1260 ($F_{3,\ 3486} = 3.44$, $P = 0.016$) but no evidence for heterogeneity in the disease RR between populations above that expected at random ($\chi_6^2 = 3.11$, $P = 0.79$). We found no evidence of regional heterogeneity in the control allele frequencies ($F_{11,\ 7261} = 0.86$, $P = 0.58$).

In contrast to other previously published studies (11–14), we found that intronic SNP *CYP27B1* +2838 was also associated with type 1 diabetes in both collections. The major allele T was associated with increased type 1 diabetes risk in both the case-control ($P = 0.010$; T/T genotype, OR 1.20 [95% CI 1.07–1.36]; Table 2) and the family ($P = 6.1 \times 10^{-4}$; T/T genotype, RR 1.36 [1.11–1.67]; Table 2) collections. The combined *P* value was 8.5×10^{-5} (2-df genotype model, see Table 2 legend).

We noted that in all population samples that we studied, including control subjects from U.K. and parents of the patients from U.K. and Northern Ireland, Norway, or Romania, there is almost perfect linkage disequilibrium between SNPs *CYP27B1* −1260 and +2838 with $D' = 1.0$ and $r^2 = 0.99$ (we obtained lower *P* values for *CYP27B1* −1260 because more samples were genotyped for this SNP than for +2838). To verify genotyping of *CYP27B1* −1260 and +2838, we directly sequenced 376 case subjects and 533 control subjects and found complete concordance in the results. This raised the possibility that in the German and Polish population samples studied previously (11–14), there may have been genotyping error in the analysis of *CYP27B1* −1260 polymorphism. Therefore, in Cambridge, we re-genotyped 120 DNA samples from 36 type 1 diabetes families from the original German laboratory for the two SNPs, obtaining only 88.2% concordance between the two genotype datasets for *CYP27B1* −1260, and this problem was compounded by evidence of data handling errors. Contrary to previous analyses (11,12,14), in these German samples, we found the most perfect linkage disequilibrium

TABLE 2
CYP27B1 +2838 allele and genotype frequencies and association test results in 5,552 case, 7,435 control, and 2,198 parent-child trio genotypes

	Case subjects	Control subjects	OR (95% CI)	*P* value
Allele				
C	3,576 (32.2)	5,031 (33.8)	1.00 (reference)	
T	7,528 (67.8)	9,839 (66.2)	1.08 (1.02–1.14)	7.1×10^{-3}*
Genotype				
C/C	573 (10.3)	877(11.8)	1.00 (reference)	
T/C	2,430 (43.8)	3,277 (44.1)	1.16 (1.03–1.31)	
T/T	2,549 (45.9)	3,281 (44.1)	1.20 (1.07–1.36)	0.010†
	Transmitted	Untransmitted	RR (95% CI)	
Allele T	995 (53.6)	863 (46.4)	1.15 (1.05–1.26)	2.2×10^{-3}‡
Genotype				
C/C	182 (8.3)	709 (10.8)	1.00 (reference)	
T/C	996 (45.3)	2,926 (44.4)	1.27 (1.06–1.53)	
T/T	1,020 (46.4)	2,959 (44.9)	1.36 (1.11–1.67)	6.1×0^{-4}†

Data are *n* (%). For the family collection, we adopted a genotype model because it was significantly different from the multiplicative allelic effects model ($\chi_1^2 = 5.4$, $P = 0.020$). For the case-control collection, although there was no difference between the models ($\chi_1^2 = 1.94$, $P = 0.17$), we adopted the genotype model for consistency with the family collection. †2-df likelihood ratio test for genotype effects. ‡Transmission disequilibrium test. Genotypes for the family-based pseudo-control subjects were estimated as described previously (17).

between the two SNPs (*CYP27B1* −1260 and +2838 SNPs: $D' = 1.00$ and $r^2 = 0.99$) as we report here for all other populations studied, indicative of past genotyping and data analysis errors.

Resequencing of the *CYP27B1* gene. We then resequenced 8 kb of the CYP27B1 gene, including all exons, introns, and 2 kb 5′ and 3′ of the gene, using DNA samples of 32 healthy subjects from U.K. to test for the presence of an obvious candidate for a causal variant, such as an amino acid–changing polymorphism or a splice mutation. We discovered two novel rare SNPs with MAFs <0.01, one in the promoter at position −1138 and one in the 3′-untranslated region (ss67078180 and ss67078183, respectively; http://www.ncbi.nlm.nih.gov/SNP/). We did not genotype these SNPs because even large samples that we studied here were underpowered to demonstrate association of such rare variants. We also found a synonymous SNP rs8176345 in exon 5 with MAF = 0.03 that was not in linkage disequilibrium with the common CYP27B1 SNPs at positions −1260 and +2838 ($r^2 = 0.06$ and 0.06, respectively). We genotyped rs8176345 in a subset of the case-control collection comprising 3,040 type 1 diabetic patients and 3,349 control subjects but obtained no evidence of an association ($P = 0.23$; OR 0.87 [95% CI 0.71–1.09]). We also identified a common promoter SNP rs3782130 at position −1074 with MAF = 0.33. Because we were unable to develop a working high throughput genotyping assay for this SNP, we sequenced it in 376 case subjects and 533 control subjects and found that it was also in almost perfect linkage disequilibrium with SNPs at positions −1260 and +2838 ($r^2 = 0.99$ and 0.97, respectively).

Interaction analyses. We performed case-only gene-gene interaction tests (15) between known type 1 diabetes susceptibility loci and *CYP27B1* −1260. We did not undertake the same analyses for *CYP27B1* −2838 because these SNPs are in almost perfect linkage disequilibrium. There was no consistent evidence of an interaction (that is the deviation from a multiplicative model) for the joint effects of *CYP27B1* −1260 and *INS* rs689 (−23*Hph*I), *PTPN22* rs2476601 (Arg620Trp), or *CTLA4* rs3087243 (Supplementary Table 1). However, there was some evidence for an interaction with *HLA-DRB1* (Supplementary Table 1), but when we analyzed *CYP27B1* −1260 stratified by specific *HLA-DRB1* genotypes, we found that risk ratios were not consistent between the case-control and family collections (Supplementary Table 2). Therefore, we conclude that in conferring risk of type 1 diabetes *CYP27B1* does not interact with the previously known disease genes. We conducted a similar interaction analysis for *CYP27B1* −1260 and seven *VDR* SNPs (*Fok*I, *Apa*I, *Bsm*I, *Taq*I, rs2544043, rs12721366, and rs4303288) (9,10). However, we found no evidence of an interaction (Supplementary Table 1). We also tested *CYP27B1* −1260 for age-at-diagnosis and sex effects in a case-only analysis but did not find evidence for these (Supplementary Table 1) or for parent-of-origin effect in the affected families ($P = 0.76$).

Analysis of the CYP24A1 gene. In the case-control collection, we tested 16 tag SNPs that capture association of the common variants that were present in the CYP24A1 gene in HapMap (Table 3). A multilocus test revealed no evidence of association between *CYP24A1* polymorphisms and type 1 diabetes ($P = 0.23$). Therefore, we did not undertake follow-up genotyping of any of the *CYP24A1* polymorphisms in additional case and control subjects or families.

DISCUSSION

The present study provides the first evidence of association between *CYP27B1* polymorphisms and type 1 diabetes in a fully validated analysis. Our results in the present report indicate what appears to have been technical and analytical errors in the previous studies (11–14). Nevertheless, these initial reports did contribute to our motivation to carry out the current analysis of *CYP27B1* in type 1 diabetes.

Taking into account prior epidemiological and experimental links between vitamin D and type 1 diabetes (3–8,22–27) and the association between *CYP27B1* and type 1 diabetes that we established here, we suggest that common inherited variation in the CYP27B1 gene affects vitamin D metabolism and is an etiological factor that predisposes type 1 diabetes. Rare *CYP27B1* mutations that completely inactivate 1α-hydroxylase are known to cause

TABLE 3
Analysis of 16 tag polymorphisms of the CYP24A1 gene in type 1 diabetic case and control subjects

CYP24A1 polymorphism	Alleles 1/2	Minor allele	Case subjects				Control subjects				MAF	OR (95% CI)
			11	12	22	Total	11	12	22	Total		
rs2762928	T/A	T	92 (1.8)	1,187 (23.5)	3,775 (74.7)	5,054	106 (1.9)	1,283 (23.2)	4,150 (74.9)	5,539	0.13	1.01 (0.93–1.09)
rs2585428	G/A	A	1,453 (29.1)	2,441 (48.9)	1,101 (22.0)	4,995	1,552 (28.0)	2,739 (49.5)	1,247 (22.5)	5,538	0.47	0.98 (0.92–1.03)
rs912505	G/A	G	205 (4.1)	1,735 (34.7)	3,055 (61.2)	4,995	227 (4.2)	1,829 (33.4)	3,413 (62.4)	5,469	0.21	1.04 (0.97–1.11)
rs8124792	G/A	A	4,616 (90.1)	492 (9.6)	14 (0.27)	5,122	4,680 (89.6)	523 (10.0)	21 (0.40)	5,224	0.05	0.95 (0.84–1.08)
rs4809956	T/C	T	175 (3.5)	1,613 (31.8)	3,289 (64.8)	5,077	180 (3.4)	1,707 (32.2)	3,417 (64.4)	5,304	0.19	1.00 (0.93–1.07)
rs2426498	C/G	G	3,816 (75.0)	1,182 (23.2)	87 (1.7)	5,085	4,124 (75.1)	1,258 (22.9)	107 (2.0)	5,489	0.13	0.99 (0.91–1.07)
rs13038432	G/A	G	29 (0.64)	668 (14.7)	3,854 (84.7)	4,551	30 (0.59)	690 (13.5)	4,404 (86.0)	5,124	0.07	1.10 (0.98–1.22)
rs2245153	T/C	C	3,066 (64.3)	1,493 (31.3)	207 (4.3)	4,766	3,274 (65.4)	1,533 (30.6)	198 (4.0)	5,005	0.19	1.05 (0.98–1.13)
rs6022999	G/A	G	258 (5.2)	1,719 (34.8)	2,970 (60.0)	4,947	259 (4.8)	1,956 (36.2)	3,188 (59.0)	5,403	0.23	0.98 (0.92–1.05)
rs6127118	G/A	A	2,670 (56.7)	1,769 (37.5)	273 (5.8)	4,712	3,022 (59.7)	1,761 (34.8)	277 (5.5)	5,060	0.23	1.10 (1.03–1.18)
rs3787557	T/C	C	3,522 (74.4)	1,135 (24.0)	80 (1.7)	4,737	3,772 (74.6)	1,175 (23.2)	108 (2.1)	5,055	0.14	1.00 (0.92–1.09)
rs2762939	C/G	C	258 (5.6)	1,788 (38.5)	2,596 (55.9)	4,642	309 (6.1)	1,909 (37.4)	2,888 (56.6)	5,106	0.25	1.01 (0.94–1.08)
rs6068810	T/G	T	8 (0.17)	297 (6.3)	4,447 (93.6)	4,752	6 (0.12)	349 (7.2)	4,489 (92.7)	4,844	0.04	0.87 (0.75–1.02)
rs2181874	G/A	A	2,848 (57.5)	1,819 (36.7)	289 (5.8)	4,956	3,102 (57.0)	1,994 (36.6)	346 (6.4)	5,442	0.25	0.97 (0.91–1.03)
rs2244719	T/C	C	1,361 (26.9)	2,629 (51.9)	1,074 (21.2)	5,064	1,506 (27.5)	2,734 (49.8)	1,246 (22.7)	5,486	0.48	0.97 (0.92–1.03)
rs2248359	T/C	T	919 (17.54)	2,430 (46.4)	1,890 (36.1)	5,239	817 (15.3)	2,592 (48.7)	1,918 (36.0)	5,327	0.40	1.05 (0.99–1.11)

Data are *n* (%). Multilocus test (20,21), $F_{16,11183} = 1.24$, $P = 0.23$.

vitamin D–dependent rickets type I (OMIM [Online Mendelian Inheritance in Man] no. 264700), characterized by low concentrations of $1\alpha,25(OH)_2D$ (28,29). We hypothesize that the presence of the *CYP27B1* −1260 C allele or another variant in linkage disequilibrium with it (such as two that we have studied here, *CYP27B1* +2838 in intron 6 and rs3782130 in the 5′ region) reduces the level of the active 1α-hydroxylase and conversion of 25(OH)Dto $1\alpha,25(OH)_2D$, leading to increased predisposition to type 1 diabetes. Recently, preliminary data have suggested that type 1 diabetic patients carrying at *CYP27B1* −1260 risk genotype CC had lower CYP27B1 mRNA levels in the peripheral blood mononuclear cells compared with healthy control subjects with the CC genotype (30). Functional roles of the *CYP27B1* polymorphisms should be investigated in further experiments, evaluating their effects on 1α-hydroxylase activity and $1\alpha,25(OH)_2D$ concentration, in particular, in the immune cells, such as dendritic cells and monocytes, that underpin immune responses (31,32).

Given our evidence that variation in the CYP27B1 gene etiologically contributes to type 1 diabetes risk, other genes that control vitamin D metabolism are also biologically plausible candidates, and studies of their association with type 1 diabetes are required. Here, we investigated the CYP24A1 gene that encodes vitamin D 24-hydroxylase, an enzyme that inactivates $1\alpha,25(OH)_2D$, and found no evidence of association. Studies of *CYP27A1* or *CYP2R1* that encode vitamin D 25-hydroxylases and of the vitamin D–binding protein gene (33,34) are also needed.

In the immune system, $1\alpha,25(OH)_2D$ has been shown to suppress production of the interleukin (IL)-12, IL-2, tumor necrosis factor-α, and γ-interferon cytokines; to activate expression of transforming growth factor-β1 and IL-4 cytokines, thereby inhibiting Th1-type responses; and to induce regulatory T-cells (35). It can also regulate differentiation and maturation of dendritic cells critical in induction of T-cell–mediated immune responses (36). These immunomodulatory effects may explain the reported protective effects of vitamin D in type 1 diabetes (37). In the animal models, $1\alpha,25(OH)_2D_3$ and its analogs have been effective in prevention of autoimmune diabetes (23–27) and of other autoimmune diseases (38–42). Epidemiological studies in humans also indicate that intake of vitamin D and its high circulating levels are associated with a lower risk of rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus (43–45). Genetic studies reported association of the *CYP27B1* polymorphisms with Addison's disease, Hashimoto's thyroiditis, and Graves' disease (12,13), but these results await confirmation. The possibility that *CYP27B1* and $1\alpha,25(OH)_2D$ may be involved in multiple autoimmune diseases suggests that effects of vitamin D on type 1 diabetes involve immune regulation, but this does not rule out additional effects, such as protection of pancreatic β-cells and their functions.

Our study indicates that genetic variation in the vitamin D metabolism is an etiological factor in type 1 diabetes. This evidence justifies further experiments investigating molecular and cellular actions of vitamin D and mechanisms of its protective effect in type 1 diabetes. Epidemiological studies indicate that vitamin D supplementation in early childhood may reduce type 1 diabetes risk (6–8). Given that vitamin D insufficiency is more common among children and young adults than was previously thought

(46), its correction may be a viable approach to prevent type 1 diabetes or delay its development.

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REFERENCES

1. Knip M, Veijola R, Virtanen SM, Hyoty H, Vaarala O, Akerblom HK: Environmental triggers and determinants of type 1 diabetes. *Diabetes* 54:S125–S136, 2005
2. Dahlquist G, Mustonen L: Childhood onset diabetes: time trends and climatological factors. *Int J Epidemiol* 23:1234–1241, 1994
3. Baumgartl HJ, Standl E, Schmidt-Gayk H, Kolb HJ, Janka HU, Ziegler AG: Changes of vitamin D3 serum concentrations at the onset of immune-mediated type 1 (insulin-dependent) diabetes mellitus. *Diabetes Res* 16: 145–148, 1991
4. Pozzilli P, Manfrini S, Crino A, Picardi A, Leomanni C, Cherubini V, Valente L, Khazrai M, Visalli N: Low levels of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 in patients with newly diagnosed type 1 diabetes. *Horm Metab Res* 37:680–683, 2005
5. Littorin B, Blom P, Scholin A, Arnqvist HJ, Blohme G, Bolinder J, Ekbom Schnell A, Eriksson JW, Gudbjornsdottir S, Nystrom L, Ostman J, Sundkvist G: Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). *Diabetologia* 49:2847–2852, 2006
6. EURODIAB: Vitamin D supplement in early childhood and risk for type I (insulin-dependent) diabetes mellitus: the EURODIAB substudy 2 study group. *Diabetologia* 42:51–54, 1999
7. Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM: Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 358:1500–1503, 2001
8. Stene LC, Joner G: Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. *Am J Clin Nutr* 78:1128–1134, 2003
9. Nejentsev S, Cooper JD, Godfrey L, Howson JM, Rance H, Nutland S, Walker NM, Guja C, Ionescu Tirgoviste C, Savage DA, Undlien DE, Ronningen KS, Tuomilehto Wolf E, Tuomilehto J, Gillespie KM, Ring SM, Strachan DP, Widmer B, Dunger D, Todd JA: Analysis of the vitamin D receptor gene sequence variants in type 1 diabetes. *Diabetes* 53:2709–2712, 2004
10. Guo SW, Magnuson VL, Schiller JJ, Wang X, Wu Y, Ghosh S: Meta-analysis of vitamin D receptor polymorphisms and type 1 diabetes: a HuGE review of genetic association studies. *Am J Epidemiol* 164:711–724, 2006
11. Lopez ER, Regulla K, Pani MA, Krause M, Usadel KH, Badenhoop K: CYP27B1 polymorphisms variants are associated with type 1 diabetes mellitus in Germans. *J Steroid Biochem Mol Biol* 90:155–157, 2004
12. Lopez ER, Zwermann O, Segni M, Meyer G, Reincke M, Seissler J, Herwig J, Usadel KH, Badenhoop K: A promoter polymorphism of the CYP27B1 gene is associated with Addison's disease, Hashimoto's thyroiditis, Graves' disease and type 1 diabetes mellitus in Germans. *Eur J Endocrinol* 151:193–197, 2004
13. Kurylowicz A, Badenhoop K: CYP27B1 gene polymorphism is associated with Graves' disease in a Polish population study. *Thyroid* 15:1107–1108, 2005
14. Pani MA, Regulla K, Segni M, Krause M, Hofmann S, Hufner M, Herwig J, Pasquino AM, Usadel KH, Badenhoop K: Vitamin D 1alpha-hydroxylase (CYP1alpha) polymorphism in Graves' disease, Hashimoto's thyroiditis and type 1 diabetes mellitus. *Eur J Endocrinol* 146:777–781, 2002
15. Smyth D, Cooper JD, Collins JE, Heward JM, Franklyn JA, Howson JM, Vella A, Nutland S, Rance HE, Maier L, Barratt BJ, Guja C, Ionescu Tirgoviste C, Savage DA, Dunger DB, Widmer B, Strachan DP, Ring SM, Walker N, Clayton DG, Twells RC, Gough SC, Todd JA: Replication of an association between the lymphoid tyrosine phosphatase locus (*LYP/PTPN22*) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes* 53:3020–3023, 2004
16. International HapMap Consortium: A haplotype map of the human genome. *Nature* 437:1299–1320, 2005
17. Cordell HJ, Clayton DG: A unified stepwise regression procedure for evaluating the relative effects of polymorphisms within a gene using case/control or family data: application to HLA in type 1 diabetes. *Am J Hum Genet* 70:124–141, 2002
18. Clayton DG, Walker NM, Smyth DJ, Pask R, Cooper JD, Maier LM, Smink LJ, Lam AC, Ovington NR, Stevens HE, Nutland S, Howson JM, Faham M, Moorhead M, Jones HB, Falkowski M, Hardenbol P, Willis TD, Todd JA: Population structure, differential bias and genomic control in a large-scale, case-control association study. *Nat Genet* 37:1243–1246, 2005
19. Spielman RS, McGinnis RE, Ewens WJ: Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52:506–516, 1993
20. Chapman JM, Cooper JD, Todd JA, Clayton DG: Detecting disease associations due to linkage disequilibrium using haplotype tags: a class of tests and the determinants of statistical power. *Hum Hered* 56:18–31, 2003
21. Vella A, Cooper JD, Lowe CE, Walker N, Nutland S, Widmer B, Jones R, Ring SM, McArdle W, Pembrey ME, Strachan DP, Dunger DB, Twells RC, Clayton DG, Todd JA: Localization of a type 1 diabetes locus in the IL2RA/CD25 region by use of tag single-nucleotide polymorphisms. *Am J Hum Genet* 76: 5, 2005
22. Brekke HK, Ludvigsson J: Vitamin D supplementation and diabetes-related autoimmunity in the ABIS study. *Pediatr Diabetes* 8:11–14, 2007
23. Mathieu C, Laureys J, Sobis H, Vandeputte M, Waer M, Bouillon R: 1,25-Dihydroxyvitamin D3 prevents insulitis in NOD mice. *Diabetes* 41: 1491–1495, 1992
24. Mathieu C, Waer M, Laureys J, Rutgeerts O, Bouillon R: Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3. *Diabetologia* 37:552–558, 1994
25. Gregori S, Giarratana N, Smiroldo S, Uskokovic M, Adorini L: A 1alpha,25-dihydroxyvitamin D(3) analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. *Diabetes* 51:1367–1374, 2002
26. Zella JB, McCary LC, DeLuca HF: Oral administration of 1,25-dihydroxyvitamin D3 completely protects NOD mice from insulin-dependent diabetes mellitus. *Arch Biochem Biophys* 417:77–80, 2003
27. Mathieu C, Adorini L: The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. *Trends Mol Med* 8:174–179, 2002
28. Kitanaka S, Takeyama K, Murayama A, Sato T, Okumura K, Nogami M, Hasegawa Y, Niimi H, Yanagisawa J, Tanaka T, Kato S: Inactivating mutations in the 25-hydroxyvitamin D3 1alpha-hydroxylase gene in patients with pseudovitamin D-deficiency rickets. *N Engl J Med* 338:653–661, 1998
29. Wang JT, Lin CJ, Burridge SM, Fu GK, Labuda M, Portale AA, Miller WL: Genetics of vitamin D 1alpha-hydroxylase deficiency in 17 families. *Am J Hum Genet* 63:1694–1702, 1998
30. Ramos-Lopez E, Bruck P, Jansen T, Pfeilschifter JM, Radeke HH, Badenhoop K: CYP2R1-, CYP27B1- and CYP24-mRNA expression in German type 1 diabetes patients. *J Steroid Biochem Mol Biol* 103:807–810, 2007
31. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zugel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL: Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 311:1770–1773, 2006
32. Overbergh L, Stoffels K, Waer M, Verstuyf A, Bouillon R, Mathieu C: Immune regulation of 25-hydroxyvitamin D-1alpha-hydroxylase in human monocytic THP1 cells: mechanisms of interferon-gamma-mediated induction. *J Clin Endocrinol Metab* 91:3566–3574, 2006
33. Pani MA, Donner H, Herwig J, Usadel KH, Badenhoop K: Vitamin D binding

protein alleles and susceptibility for type 1 diabetes in Germans. *Autoimmunity* 31:67–72, 1999
34. Ongagna JC, Pinget M, Belcourt A: Vitamin D-binding protein gene polymorphism association with IA-2 autoantibodies in type 1 diabetes. *Clin Biochem* 38:415–419, 2005
35. Jones G, Strugnell SA, DeLuca HF: Current understanding of the molecular actions of vitamin D. *Physiol Rev* 78:1193–1231, 1998
36. Penna G, Adorini L: 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol* 164:2405–2411, 2000
37. Mathieu C, Gysemans C, Giulietti A, Bouillon R: Vitamin D and diabetes. *Diabetologia* 48:1247–1257, 2005
38. Lemire JM, Archer DC: 1,25-Dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. *J Clin Invest* 87:1103–1107, 1991
39. Cantorna MT, Hayes CE, DeLuca HF: 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc Natl Acad Sci U S A* 93:7861–7864, 1996
40. Larsson P, Mattsson L, Klareskog L, Johnsson C: A vitamin D analogue (MC 1288) has immunomodulatory properties and suppresses collagen-induced arthritis (CIA) without causing hypercalcaemia. *Clin Exp Immunol* 114: 277–283, 1998
41. Cantorna MT, Hayes CE, DeLuca HF: 1,25-Dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. *J Nutr* 128:68–72, 1998
42. Cantorna MT, Munsick C, Bemiss C, Mahon BD: 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. *J Nutr* 130:2648–2652, 2000
43. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A: Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA* 296:2832–2838, 2006
44. Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG: Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. *Arthritis Rheum* 50:72–77, 2004
45. Kamen DL, Cooper GS, Bouali H, Shaftman SR, Hollis BW, Gilkeson GS: Vitamin D deficiency in systemic lupus erythematosus. *Autoimmun Rev* 5:114–117, 2006
46. Holick MF: Resurrection of vitamin D deficiency and rickets. *J Clin Invest* 116:2062–2072, 2006