Association of the Vitamin D Metabolism Gene CYP2B1 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 (1α,25(OH)2D) could protect from immune destruction of the pancreatic β-cells. 1α,25(OH)2D is derived from its precursor 25-hydroxyvitamin D by the enzyme 1α-hydroxylase encoded by the CYP2B1 gene and is inactivated by 24-hydroxylase encoded by the CYP24A1 gene. Our aim was to study the association between the CYP2B1 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 CYP2B1 variants, including common polymorphisms −1260C>A (rs10877012) and +2838T>C (rs4646536) and 16 tag polymorphisms in the CYP2A41 gene.

RESULTS—We found evidence of association with type 1 diabetes for CYP2B1 −1260 and +2838 polymorphisms, which are in perfect linkage disequilibrium. The common C allele of CYP2B1 −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 × 10−4) and in the fully independent collection of families (relative risk for the C/C genotype 1.33, P = 3.9 × 10−6). The combined P value for an association with type 1 diabetes was 3.8 × 10−6. For the CYP2A41 gene, we found no evidence of association with type 1 diabetes (multitest, P = 0.23).

CONCLUSIONS—The present data provide evidence that common inherited variation in the vitamin D metabolism affects susceptibility to type 1 diabetes.

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 (1α,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 1α,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 CYP2B1 gene on chromosome 12q13.1–q13.3 (11–14), which encodes 1α-hydroxylase, the enzyme that converts 25(OH)D into 1α,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 CYP2B1 gene. Circulating 1α,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 20q13.2-4q13.3. 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 CYP2B1 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; 355 from Finland; 390 from Norway, and 423 from Romania), providing 3,081 parent-child trio genotypes for CYP2B1 −1260 and 2,198 trio genotypes for CYP2B1 +2838. The collection of all DNA samples has been approved by relevant ethical committees. We obtained written informed consent from all participants.
In the CYP27B1 gene, we genotyped three single nucleotide polymorphisms (SNPs), CYP27B1 rs10877012 (located in the 5’ region) and CYP27B1 rs4646536, located in intron 6), which were previously reported (11–14), and rs1176345, 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: 53,450,894.53,482,103), 54 SNPs had major allele frequency (MAF) > 0.05, and 16 were chosen as tag SNPs that capture association of other common variants with rs2 > 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]) 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 nlpop program within 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 t2 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 rs10877012 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 rs10877012 (P = 3.8 \times 10^{-5} 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 rs10877012 (F heterogeneity = 3.44, P = 0.016) but no evidence for heterogeneity in the disease RR between populations above that expected at random (x_{6}^{2} = 3.11, P = 0.79). We found no evidence of regional heterogeneity in the control allele frequencies (F_{1,7261} = 0.86, P = 0.58).

In contrast to other previously published studies (11–14), we found that intronic SNP CYP27B1 rs4646536 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 \times 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 rs10877012 and +2838 with D’ = 1.0 and r2 = 0.99 (we obtained lower P values for CYP27B1 rs10877012 because more samples were genotyped for this SNP than for +2838). To verify genotyping of CYP27B1 rs10877012 and +2838, we directly sequenced 376 case subjects and 200 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 rs10877012 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 rs10877012, 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 1

<table>
<thead>
<tr>
<th>Allele</th>
<th>Case subjects</th>
<th>Control subjects</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4,999 (31.8)</td>
<td>5,836 (33.3)</td>
<td>1.00 (reference)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>10,709 (68.2)</td>
<td>11,680 (66.7)</td>
<td>1.07 (1.02–1.13)</td>
<td>2.9 \times 10^{-3}*</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>767 (9.8)</td>
<td>999 (11.4)</td>
<td>1.00 (reference)</td>
<td></td>
</tr>
<tr>
<td>A/C</td>
<td>3,465 (44.1)</td>
<td>3,838 (43.8)</td>
<td>1.20 (1.08–1.33)</td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>3,622 (46.1)</td>
<td>3,921 (44.8)</td>
<td>1.22 (1.10–1.36)</td>
<td></td>
</tr>
<tr>
<td>Transmitted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untransmitted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are n (%). For the case-control collection, we adopted a genotype model because it was significantly different from the multiplicative allelic effects model (x\textsuperscript{2} = 5.0, P = 0.025). For the family collection, although there was no difference between the models (x\textsuperscript{2} = 5.6, P = 0.056), we adopted the genotype model for consistency with the case-control collection. 1-df likelihood ratio test for multiplicative allelic effects. 3df 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 0.71–0.97]).

**DISECUSSION**

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...
vitamin D–dependent rickets type I (OMIM [Online Mendelian Inheritance in Man] no. 264700), characterized by low concentrations of 1,25(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)D to 1α,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 1α,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 1α,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, 1α,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 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, 1α,25(OH)2D3 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 1α,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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