Variations in the Vitamin D-Binding Protein (Gc Locus) and Risk of Type 2 Diabetes Mellitus in French Caucasians

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Electrophoretic variants of the vitamin D-binding protein (DBP) have been reported to be associated with type 2 diabetes mellitus (DM) or with prediabetic phenotypes in several non-Caucasian populations. Two frequent missense polymorphisms at codons 416 (Asp → Glu) and 420 (Thr → Lys) are the genetic basis for the 3 common electrophoretic variants of DBP (Gc1F, Gc1S, and Gc2) and the resulting circulating phenotypes (Gc1F/Gc1F, Gc1F/Gc1S, Gc1S/Gc1S, Gc1F/Gc2, Gc1S/Gc2, and Gc2/Gc2). In this study, we investigated the association of these polymorphisms with type 2 DM in French Caucasian subjects. Variations at codons 416 and 420 were examined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Allele frequencies at both codons did not differ in type 2 DM patients and in control subjects (Asp416: 42.4% *v* 46.2%, respectively, *P* = .33; Lys420: 25.5% *v* 29.0%, respectively, *P* = .31). Distribution of genotypes at both codons, of the haplotypes defined by the 2 codons, and of the DBP phenotypes defined by the haplotypes were also similar in diabetic and control subjects. In conclusion, our study suggests that genetic variants of the DBP gene are not associated with the susceptibility to type 2 DM in French Caucasians.

THE VITAMIN D endocrine system plays an important role in the mechanism of insulin release and in the maintenance of glucose tolerance. Pancreatic β cells express both the specific cytosolic/nuclear vitamin D receptor (VDR),[1] a member of the steroid receptor superfamily, and a putative membrane vitamin D receptor (mVDR).[2] It was shown in experimental animals that vitamin D deficiency is associated with impaired insulin secretion, and that this insulin secretion defect is normalized by 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] administration.[3,4] These observations were confirmed in humans.[5] Correlations between serum concentrations of vitamin D, plasma glucose, and insulin secretion were observed in elderly Dutchmen[6] and in Asian subjects living in London,[7] and supplementation with vitamin D was found to improve insulin secretion in vitamin D-deficient subjects.[5,7]

The circulating metabolites of vitamin D bind with high-affinity to the vitamin D-binding protein (DBP),[8] a single-chain serum glycoprotein also known as group-specific component protein (Gc). Studies in knock-out mice demonstrated the important role of DBP in maintaining stable serum stores of vitamin D metabolites and modulating their bioavailability, activation, and end-organ responsiveness.[9] DBP is encoded by the Gc gene, a member of a multigene cluster that includes albumin and α-fetoprotein genes, located at chromosome 4q11-q13.[10,11]

Sequence variations at codons 416 and 420 in exon 11 of the Gc gene give rise to 3 major electrophoretic variants of DBP, termed Gc1 fast (Gc1F), Gc1 slow (Gc1S), and Gc2.[12,13] These variants differ by amino acid sequence, as well as by attached polysaccharide structures. They also differ by the binding affinity for vitamin D and its metabolites, with the Gc2 variant presenting the lowest affinity.[14,15] Combinations of the 3 DBP variants result in 6 common circulating phenotypes (Gc1F/Gc1F, Gc1F/Gc1S, Gc1S/Gc1S, Gc1F/Gc2, Gc1S/Gc2, and Gc2/Gc2). Several studies in non-Caucasian populations have suggested preferential association of some of these DBP phenotypes with type 2 diabetes mellitus (DM) or with glucose or insulin levels.[16-21] These findings prompted us to investigate the role of Gc gene and DBP variants in the genetic susceptibility to type 2 DM in French Caucasians.

SUBJECTS AND METHODS

Subjects

We studied a group of 237 unrelated Caucasian subjects with overt type 2 DM consecutively recruited at the diabetes department and outpatient clinics of Hôpital Necker, Paris, France. The control group consisted of 143 unrelated Caucasian subjects without known history of diabetes, recruited among members of CEPH family reference panel (1 per family) and among spouses of subjects with type 2 DM. Demographic and clinical characteristics of patients and controls are shown in Table 1. The study was approved by the ethical committee of Hôpital Necker (CCPPRB Paris Necker).

DNA Studies

Genomic DNA was extracted from peripheral blood samples by standard procedures. Polymorphisms at codons 416 (GA**T** to GA**G**; Asp → Glu) and 420 (A**C**G to A**A**G; Thr → Lys) in exon 11 of the Gc gene were examined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with primers 5′-AAATAATGAG-CAAATGAAAGAAGAC-3′ (forward) and 5′-CAATAACAGCAAA-GAAATGAGTAGA-3′ (reverse). PCR conditions were an initial denaturation at 94°C for 5 minutes and 35 cycles at 94°C for denaturation, 51°C for annealing, and 72°C for extension, each step lasting 45 seconds, with a final extension for 7 minutes at 72°C. Amplification yields a 483-bp fragment that for the glutamic acid allele at codon 416 contains a *Hae*III site (297/186 bp), and for the lysine allele at codon 420 contains a *Sty*I site (305/178 bp). PCR products were subjected to

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Table 1. Demographic and Clinical Profile of Patients and Controls

	Diabetic Subjects	Controls
Subjects (n)	237	143
Sex (M/F)	54%/46%	50%/50%
Age (yr)	63 ± 11	61 ± 16
BMI (kg/m^2)	29.3 ± 6.0	—
Age of diagnosis of diabetes (yr)	51 ± 11	—
Duration of diabetes (yr)	13 ± 10	
Treatment (OHA/insulin)	61%/39%	—

NOTE. Data expressed as mean ± SD.
Abbreviation: OHA, oral hypoglycemic agents.

enzymatic digestion separately with *Hae*III and *Sty*I endonucleases, and resolved on 2.0% agarose gel electrophoresis.

Data Analysis

Four haplotypes are defined by the variants at codons 416 and 420 (Asp-Lys, Asp-Thr, Glu-Lys, and Glu-Thr). The frequencies of these haplotypes in diabetic patients and control subjects were estimated from the genotypes with the EH software (ftp://linkage.rockefeller.edu/software/eh).[22] Linkage disequilibrium between the 2 loci was estimated with Lewontin's equations.[23,24] The pair of haplotypes carried by each subject was determined as follows: for individuals homozygous at both loci, or heterozygous at only 1 locus, the haplotypes were directly deduced from the genotypes. For subjects heterozygous at both loci, the phase is unknown. However, the estimated frequency of the Glu-Lys haplotype is extremely low, both in diabetic and control subjects (0.000002 to 0.000005; see Table 3) due to linkage disequilibrium between the 2 loci. Therefore, all subjects heterozygous at both loci were assumed to carry Asp-Lys and Glu-Thr haplotypes. DBP variants were deduced from the haplotypes as previously described: Gc1F, Gc1S, and Gc2 correspond, respectively, to Asp-Thr, Glu-Thr, and Asp-Lys at codons 416 and 420.[13]

Results are expressed as means ± SD. Contingency table χ^2 tests were used to compare allele, genotype and haplotype frequencies, and other qualitative data. Student's *t* test was used to compare quantitative data. Statistics were performed with the JMP software (SAS Institute Inc, Carey, NC).

Table 2. DBP Allele and Genotype Distribution in Diabetic and Control Subjects

	Diabetic Subjects (%) (N = 237)	Controls (%) (N = 143)	*P*
Allele frequency			
Codon 416			
Asp	42.4	46.2	
Glu	57.6	53.8	.33
Codon 420			
Lys	25.5	29.0	
Thr	74.5	71.0	.31
Genotype frequency			
Codon 416			
Asp/Asp	18.1	19.6	
Asp/Glu	48.5	53.1	
Glu/Glu	33.4	27.3	.46
Codon 420			
Lys/Lys	8.4	9.8	
Lys/Thr	34.2	38.5	
Thr/Thr	57.4	51.7	.56

NOTE. Genotypes are in Hardy-Weinberg equilibrium.

Table 3. Distribution of DBP Haplotypes Defined by Variants at Codons 416 and 420

Codons 416-420	Estimated Frequency		Counted Frequency	
	Diabetic Patients	Controls	Diabetic Patients	Controls
Asp-Lys	.255272	.290205	.2553 (121)	.2902 (83)
Asp-Thr	.168779	.171333	.1688 (80)	.1713 (49)
Glu-Lys	.000002	.000005	0 (0)	0 (0)
Glu-Thr	.575947	.538457	.5759 (273)	.5385 (154)

NOTE. Data expressed as frequency (and no. of subjects). χ^2 = 1.259, P = .53.

RESULTS

There were no significant differences between diabetic and control subjects in the distribution of alleles or genotypes at codons 416 and 420 of the Gc gene (Table 2). Genotypes in both codons were in Hardy-Weinberg equilibrium in patients and in controls. The 2 loci were in strong linkage disequilibrium in both groups and in the combined population ($D' = 0.51$; χ^2 290, 3 *df*, $P < .0001$). Estimated and counted frequencies of the 4 possible haplotypes defined by these 2 variants are shown in Table 3. Haplotype frequencies were not significantly different in patients and controls. Estimated and counted frequencies were similar in each group of subjects. Distribution of DBP phenotypes in diabetic and control subjects did not differ significantly (Table 4). These analyses were also performed in more clinically homogeneous subgroups of patients, stratified either in terciles of age of diagnosis of diabetes or by the presence or absence of obesity. Results were similar in these subgroups to results in the whole group of patients and in the controls (data not shown).

The age of diagnosis of diabetes, requirement of insulin therapy, body weight, body mass index (BMI), and the prevalence of obesity, hypertension, or dyslipidemia were compared in diabetic carriers of the different genotypes and DBP phenotypes. No differences in these traits were observed when patients were analyzed as 1 single group or when they were stratified by age of diagnosis of diabetes or by the presence or absence of obesity.

DISCUSSION

We have observed no association of the Gc locus, encoding DBP, with type 2 DM in French Caucasians. The frequencies of alleles, genotypes, and haplotypes of 2 missense variants in exon 11 of the Gc gene, as well as the distribution of the DBP

Table 4. Distribution of Gc Variants Defined by DBP Haplotypes

Gc Variants	Frequencies	
	Diabetic Patients	Controls
1F/1F	.0253	.021
1F/1S	.2152	.2238
1S/1S	.3333	.2727
1F/2	.0717	.0769
1S/2	.2701	.3077
2/2	.0844	.0979

NOTE. The Gc variants 1F, 1S, and 2 are defined by the Asp-Thr, Glu-Thr, and Asp-Lys haplotypes, respectively. χ^2 1.83, 5 *df*, P = .87.

circulating phenotypes defined by these variants, were similar in diabetic and control subjects. These results contrast with those of a few studies in non-Caucasian populations that showed an association of these variants or of their related circulating phenotypes with type 2 DM or with prediabetic traits. Increased prevalence of Gc1 phenotypes was observed in Polynesian subjects with type 2 DM.[16] Different distribution of DBP phenotypes was also observed in type 2 diabetics as compared with nondiabetic Japanese subjects,[21] although these differences were only marginally significant. Studies in Pima Indians showed linkage between microsatellite markers in the region of the Gc locus and fasting glucose and insulin levels.[25] No association between DBP variants and type 2 DM was found in this ethnic group, but an association of Gc1F homozygosity with a higher incremental glucose response during an oral glucose tolerance test (OGTT) was observed in nondiabetic subjects.[20] This higher glucose excursion was not associated with differences in insulin secretion, insulin sensitivity, or endogenous glucose production, and thus, its pathophysiologic mechanism remains unexplained. In a study of a small group of nondiabetic Dogrib Indians from Canada, homozygous subjects for the Gc1F variant presented the lowest levels of fasting insulin.[18] These results were adjusted for differences in sex, age, and BMI, but glucose levels were not reported in that study and were not taken into account in the analyses. Finally, in a study of nondiabetic Hispanic-Americans and Anglos from Colorado, the highest levels of fasting glucose were observed among individuals with the Gc1F phenotype.[19] Here again, the results were only marginally significantly different and fasting insulin or C-peptide, and glucose, insulin, and C-peptide levels during an OGTT did not show DBP-related differences. On the other hand, no association between these missense variants and type 2 DM was found in white Americans of European origin,[26] which is in agreement with our own data in French Caucasians. Moreover, in that study, no differences in fasting plasma glucose or BMI were observed in carriers of different genotypes.

The relationship of these genotypes and DBP variants with glucose or insulin levels could not be assessed in our population, as all subjects have moderate to severe diabetes and were treated by oral hypoglycemic agents and/or insulin. Their glucose or insulin levels, if measured, would mainly reflect the treatment of diabetes and/or the deleterious effects of chronic hyperglycemia on beta-cell function (glucotoxicity). We did not observe any significant association of the different alleles, genotypes, or DBP variants with other phenotypic traits such as the age of diagnosis of diabetes, requirement of insulin therapy, the BMI, or the prevalence of related cardiovascular risk factors such as obesity, arterial hypertension, or dyslipidemia.

These contrasting results might be related to the different ethnic background of the population samples in the different studies. Heredity of most cases of type 2 DM is believed to be polygenic, resulting from the simultaneous action of several unfavorable alleles, and probably also multigenic, meaning that many different combinations of gene defects may exist among diabetic patients, especially in the context of different ethnic backgrounds. It is noteworthy that Gc1F homozygosity, which was shown to be associated with prediabetic traits in Pima and Dogrib Indians, is much more prevalent in these ethnic groups (24.5% and 8.7%, respectively) than in Caucasians (2.9% in our study).[18,20] It is also possible that these contrasting results might be related to bias due to the relatively small number of subjects tested in all of these investigations. Our study might have lacked power to detect a minor contribution of this locus to the susceptibility of type 2 DM. Thus, neither a type 1 (false positive) nor a type 2 (false negative) error can be totally excluded in any of these investigations.

In conclusion, our study suggests that polymorphisms in the Gc gene, encoding DBP, are not associated with the susceptibility to type 2 DM in French Caucasians. However, as the vitamin D endocrine system is involved in the regulation of insulin secretion and in the maintenance of glucose tolerance, larger studies with more detailed phenotypes regarding insulin secretion and insulin sensitivity are required to properly investigate the interactions of DBP variants with these traits in nondiabetic and diabetic Caucasians.

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