Variations in Vitamin D-Binding Protein (Group-Specific Component Protein) Are Associated with Fasting Plasma Insulin Levels in Japanese with Normal Glucose Tolerance*

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ABSTRACT

The locus of the vitamin D-binding protein (DBP; also known as group-specific component protein or Gc) gene, chromosome 4q12, has been reported to be associated with glucose metabolism in several ethnic groups, including Pima Indians. We have recently reported the association of the DBP genotype with type 2 diabetes mellitus in Japan. The aim of this study was to investigate whether genetic variations of DBP have any influence on glucose metabolism without secondary effects of hyperglycemia or diabetes mellitus using 82 Japanese with normal glucose tolerance. The variations of the DBP gene (Gc 1F, 1S, and 2) were determined by PCR-restriction fragment length polymorphism. Fasting plasma insulin concentration and homeostasis model assessment, an index of insulin resistance, were significantly different based on the DBP genotype (P < 0.01 and P < 0.05, respectively). The people with Gc 1S-2 (5.73 ± 2.57 μU/mL) and 1S-1S (5.30 ± 3.46 μU/mL) had significantly higher fasting plasma concentrations than those with 1F-1F (2.84 ± 1.67 μU/mL) (P < 0.01 and P < 0.03, respectively). There was no significant difference in plasma glucose concentration, body mass index, total cholesterol, triglyceride, and blood pressure. In conclusion, genetic variations of DBP are associated with insulin resistance in Japanese with normal glucose tolerance, which might contribute to the development of type 2 diabetes. (J Clin Endocrinol Metab 85: 1951–1953, 2000)

VITAMIN D-BINDING PROTEIN (DBP), also known as group-specific component protein (Gc), is a multifunctional serum glycoprotein (1). DBP is the major serum transport protein for the vitamin D sterols (2). There are three major electrophoretic variants of the DBP glycoprotein, which differ by amino acid substitutions, as well as attached polysaccharide. These variants are called Gc 1F, Gc 1S, and Gc 2, since DBP has been historically known as group-specific component protein (Gc), and they are characterized by polymorphisms in exon 11 (Asp/Glu at codon 416, Thr/Lys at codon 420) (1).

The DBP gene maps to chromosome 4q12. It has recently been found that the locus of the DBP gene was linked to plasma glucose and insulin concentrations in nondiabetic Pima Indians (3). DBP is associated with type 2 diabetes in seven Polynesian Island populations (4). In a Hispanic-American/Anglos population of the San Luis Valley in Colorado, a variation in DBP is associated with elevated plasma glucose (5), and in Dogrib Indians, DBP is associated with both fasting insulin and glucose concentrations (6, 7). Furthermore, recently, variations of DBP have been found to be associated with oral glucose tolerance in nondiabetic Pima Indians (8). We have reported that the DBP genotype is associated with type 2 diabetes mellitus in Japan (9). Thus, the purpose of this study was to investigate whether genetic variations of DBP have any influence on glucose metabolism without secondary effects of hyperglycemia or diabetes mellitus using Japanese subjects with normal glucose tolerance.

Subjects and Methods

Eighty-two Japanese with normal glucose tolerance (47 males and 35 females) were employed in this study. They all showed normal glucose tolerance by 75 g oral glucose tolerance test. The criteria used in this study was the former diagnostic criteria of the Japanese Diabetes Society (1982), in which normal glucose tolerance was defined by a plasma glucose concentration of lower than 110 mg/dL before glucose load and 120 mg/dL at 2 h after 75 g glucose oral administration. Their clinical characteristics are shown in Table 1. The study protocol was approved by the Tohoku University Institutional Review Board. Informed consent was obtained from each subject.

Determination of DBP gene polymorphism

DBP polymorphisms in exon 11 [Asp(GAT)/Glu(GAG) at codon 416, Thr(ACG)/Lys(AAG) at codon 420] were determined by PCR-restriction fragment length polymorphism. DNA was extracted from blood. A region of exon 11 was specifically amplified by PCR using the primers: forward, 5′-ACATGACTAAGACCTTA-3′; and reverse, 5′-GATGGGAGGTCCCATACGT-3′. The PCR product was digested by HaeIII and StyI. Digested DNA fragments were electrophoresed by agarose gel and visualized by ultraviolet light. In the amplified region, the Gc 1F allele has neither HaeIII nor StyI site. The Gc 1S allele has the HaeIII but not the StyI site. The Gc 2 allele has the StyI but not the HaeIII site. DBP genotypes were determined by these polymorphic patterns.
Biochemical analysis

The blood glucose concentration was determined by glucose oxidase method. Plasma insulin concentration was measured by RIA.

Homeostasis model assessment (HOMA)

Insulin resistance was assessed by calculating HOMA(R) using the fasting plasma glucose and insulin concentrations (10). HOMA(R) is calculated as fasting plasma glucose concentration (mg/dL) × fasting plasma insulin concentration (μU/mL)/405.

Statistical analysis

Statistical analysis was performed by one-way ANOVA and Fisher’s PLSD test.

Results

The fasting plasma insulin concentrations were significantly different according to the DBP genotype ($P < 0.01$)

| Genotype | No. | BMI (kg/m²) | Fasting glucose (mg/dL) | Fasting insulin (μU/mL)* | HOMA(R)* |
|----------|-----|-------------|-------------------------|--------------------------|----------|
| 1F-1F    | 19  | 22.3 ± 2.6  | 86.3 ± 8.6              | 2.84 ± 1.67              | 0.63 ± 0.39 |
| 1F-1S    | 20  | 22.8 ± 3.1  | 89.4 ± 8.1              | 3.98 ± 3.16              | 0.89 ± 0.70 |
| 1F-2     | 21  | 23.0 ± 2.1  | 89.1 ± 5.1              | 3.14 ± 1.49              | 0.70 ± 0.38 |
| 1S-1S    | 6   | 22.4 ± 2.2  | 90.3 ± 6.7              | 5.30 ± 3.46              | 1.20 ± 0.84 |
| 1S-2     | 12  | 23.3 ± 2.1  | 89.2 ± 8.1              | 5.73 ± 2.57              | 1.26 ± 0.57 |
| 2-2      | 4   | 23.3 ± 3.4  | 85.5 ± 3.7              | 3.83 ± 1.16              | 0.81 ± 0.27 |

Fasting plasma insulin concentrations and HOMA(R) are significantly different according to the DBP genotype.

Discussion

DBP has been reported to be associated with diabetes mellitus or glucose metabolism in several ethnic groups, including Pima Indians (4–8). We have reported that DBP is associated with type 2 diabetes mellitus in Japan (9). Especially Gc 1S-2 was associated with type 2 diabetes mellitus. In this report, we studied the association between DBP genetic variations and glucose metabolism in Japanese people with normal glucose tolerance to exclude the possible effects of hyperglycemia or diabetes mellitus. The results show that there was a significant difference in fasting plasma insulin concentrations based on the DBP genotype. Especially Gc 1S-2 and 1S-1S were associated with higher fasting serum insulin concentrations and HOMA(R), which is an index of insulin resistance. Insulin resistance is often linked to obesity.
However, there was no significant difference in BMI based on the DBP genotype. Thus, the association between DBP and the fasting insulin level is thought to be independent of BMI or obesity. Japanese people with type 2 diabetes mellitus are relatively lean compared with those in other ethnic groups, such as Caucasian and Pima Indians. DBP variations could affect insulin sensitivity without an effect on obesity. This result and our previous data that the DBP genotype is associated with type 2 diabetes mellitus in Japan suggest that the variations of DBP play an important role in insulin resistance and may contribute to the development of type 2 diabetes mellitus in Japan.

The mechanisms of the association are not clear at present. However, there are several possibilities. DBP is a carrier protein for vitamin D hormone and the affinity of DBP for 1,25(OH)2 vitamin D3 and 25-OH-vitamin D3 differs depending on the genotype of DBP (11). 1,25(OH)2 vitamin D3 is essential for normal insulin secretion (12). It enhances insulin sensitivity in the people with renal failure (13), and the level of 25-OH-vitamin D3 is associated with insulin resistance (14). Thus, it is possible that DBP affects glucose metabolism by modulating the action of metabolites of vitamin D. Another possibility is a different role of DBP. DBP is a carrier for vitamin D hormone and the affinity of DBP for vitamin D3, and 25-hydroxy-vitamin D. Another possibility is a different role of DBP. DBP variations could affect glucose metabolism by modulating the action of metabolites of vitamin D. Another possibility is a different role of DBP. DBP variations could affect glucose metabolism by modulating the action of metabolites of vitamin D.

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