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Vitamin D Binding Protein Gene Polymorphisms and its Association with Type 2 Diabetes Mellitus in an Iranian Population

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Abstract:

Polymorphisms of Vitamin D-binding protein (DBP) may represent a risk factor for susceptibility to Type 2 Diabetes Mellitus (T2DM). Two polymorphisms are common at codons 420 (ACG to AAG) and 416 (GAT to GAG) in the DBP gene. The present study aimed to assess variants of DBP at codons 420 and 416 utilizing polymerase chain reaction-restriction fragment length polymorphism(PCR-RFLP), which were digested by Sty I (codon 420) and Hae III (codon 416) restriction enzyme. For this purpose, 240 patients were recruited along with 159 controls. The genotype frequency of Glu/Glu at codon 416 in control and patient groups was 25.16% and 32.92% respectively. Moreover, the genotype frequency of Lys/Lys was 6.67% in patients and 1.89% in controls at codon 420. A significant difference was found between control and patient groups in genotype frequencies at codon 420 ($p<0.05$). It was found that there might be an association between the vitamin D-binding protein gene and T2DM.

INTRODUCTION

Diabetes is among the most common metabolic disorders, for which the number of suffering patients is estimated as 200 million people all over the world. There are two main classifications for Diabetes: type 1 and type 2 (1 ,2). Type 2 diabetes mellitus is non-insulin-based and the main cause of 90% of the extensive disease (3). It can be affected by interactions between the environment and genes (4). The usually identified genes are T2DM CAPN10, KCNJ11, PPARG, ABCC8, HNF4A, HNF1A, GCK, PC-1/ENPPI, PTPN1, IRS, and LMNA (5). Vitamin D has a key role in the control of bone metabolism and mineral homeostasis. In addition to its main roles in physiological development, the endocrine system of vitamin D contributes to several pathological progressions like autoimmune disease, coronary artery disease, and T2DM (6,7) . Recently, a huge deal of attention has been paid to the correlation between T2DM and vitamin D disorders (8,9). According to uncertain available data in this regard, the vitamin D pathway affects the evolution of diabetes. 1,25-dihydroxy vitamin D3 can bind to β-cells thus affecting insulin release (10). Experimentally, it was revealed that vitamin D deficiency will reduce the secretion of insulin (11). There is a relation between insulin disorder and the reduced levels of DBP (12). According to a survey, insulin secretion can be improved by vitamin D supplements (13). There is also an association between vitamin D levels and genes of DBP, VDR, CYP24A1, CYP2R1, CYP27A1, and CYP27B1(14-17). DBP is critical for vitamin

D metabolism and affects the levels of vitamin D and insulin release in β-cells (18,19). Vitamin D binding protein also known as group-specific component protein (GC), is a multifunctional serum glycoprotein. Considerably, DBP is a series of the albumin, α-albumin/afamin gene, and α-fetoprotein family. DBP has a molecular weight of almost 52-59 kDa. It has been synthesized as a glycoprotein in the liver (20,21). The human DBP gene localizing on 4q12-q13 includes 12 introns and 13 exons. DBP gene is highly polymorphic with 3 common variants (GC1S, GC1F, and GC2) and more than 124 scarce variants (22). These three usual variants are resultant of sequence variations at codons 420 and 416 in exon 11 of the DBP gene. At codon 416, aspartic acid is replaced by glutamic acid via a nucleotide substitution from GAT to GAG. Moreover, threonine is replaced by lysine through a nucleotide substitution of ACG to AAG at codon 420 (23). In the southern coastal part of Iran (Bandaries populations), there is a considerably higher prevalence of diabetes than its mean prevalence in the country (12% and 7.7%, respectively). Furthermore, the Bandaries' inhabitants seem to be negroid and different ethnically from the northern and central white population of Iran. In this study we sought to explore the frequency of these SNPs in T2DM patients.

Methods

Collection of samples

This study was performed on 240 T2DM patients and 159 non-diabetics as a control group (age range of 30–70 years for both groups). All the participants were natives of southern Iran (Bushehr Province) referring to the Persian Gulf Tropical Medicine Research Center and Fatemeh Zahra University Hospital in Iran. The medical-ethical committee of Bushehr University of Medical Sciences verified the study and each participant confirmed the informed consent. Data collection was performed through anthropometric measurement and a questionnaire. WHO criteria and definitions were utilized for non-diabetic and diabetic cases (Fasting Plasma Glucose ^{3}7.0 mmol/l or 126mg/dl, as well as 2-h post-load plasma glucose ^{3}11.1 mmol/l or 200mg/dl during a 75-g Oral Glucose Tolerance Test).

DNA extraction and polymorphism determination

The blood samples were collected from participants who were in the night fasting condition. Genomic DNA of blood samples was extracted utilizing QIA amp DNA Mini Kit (Germany), based on the "blood and body fluid spin protocol" and the manufacturer's recommendation. PCR-RFLP was used to examine polymorphisms at codons 420 (ACG to AAG substitution) and 416 (GAT to GAG substitution) in exon 11 of the DBP gene. Gene Runner software was used to design forward and reverse primers for the DBP gene followed by acquiring gene sequences from Gene Bank (http://www.ncbi.nlm.nih.gov). Table 1 presents the oligonucleotide primers utilized in this work. PCR amplification was conducted in a 15μL reaction mixture containing 0.4 mM of dNTP, 1.5 μL 10 X PCR buffer, 20 pmol of each forward and reverse primers, 0.5 units of Taq polymerase, 40 ng of DNA template, and 1.6 mM MgCl2 via PCR thermocycler model (Techne TC-512). The PCR conditions were as follows: the first denaturation cycle of DNA for 5 min at 95°C after 35 cycles, each containing 35 s of denaturation at 95°C,and 20 s annealing at 52°C, and 30s extension at 72°C, and the ultimate extension cycle of 72°C for 10 min.PCR products were 483bp (base pairs), and each PCR product (10μL) was separately digested with 1μL of the HaeIII and StyI restriction enzyme for 1 h at 37°C. Table 1 represents the results of PCR-RFLP analyzed on ethidium bromide-stained 3% agarose gel. Linkage disequilibrium was determined between codons 420 and 416 in the DBP gene by Haploview software. The chi-square goodness-of-fit test was used to assess Hardy-Weinberg Law. A p-value less than 0.05 is particularly regarded to be statistically significant.

Table 1. PCR-RFLP and primer sequences for DBP gene

Gene	Primer sequences 5′-3′	PCR product size (bp)	Detection methods
DBP	F -ACTAGTAGTAAGACCTTA R-GATTGGAGTGCATACGTT	Normal allele: 483 Mutant allele: 186, 298	**PCR-RFLP** **HaeIII**
		Normal allele: 483 Mutant allele: 178, 305	**PCR-RFLP** **StyI**

RESULTS

Table 2 presents clinical characteristics revealing no difference between control and patient groups. Nevertheless, patients had higher levels of insulin than control groups and there was a significant difference between aforementioned groups (P=0.02). Hardy-Weinberg equilibrium was the basis for the genotype and allele frequencies in control and case at codons 416 and 420. Moreover, there was linkage disequilibrium for the mentioned codons in both groups (Table 3). No significant differences were found between control and patient groups in the frequency of haplotypes in the DBP gene at codons 416 and 420 (Table 4). Moreover, the odds ratio for haplotypes is determined (Table 5). It should be noted that no significant association was found in allele frequencies of the study population (Table 6). A considerable association was revealed between patient and control groups by the genotype frequencies of Thr –Thr/ Thr – Lys/ Lys- Lys in the DBP gene at codon 420 (P<0.05). However, no significant relationship was found at codon 416 in genotype frequencies (Table 7). For Glu/Glu (codon 416) and Lys/Lys (codon 420), the genotype frequency of patients was higher significantly than controls.

DISCUSSION

DBP is essential for the role of vitamin D in the cell. There are no considerable studies in the field of the relationship between T2DM and vitamin D. However, evidence suggested that vitamin D can enhance the sensitivity to insulin and the survival of pancreatic cells (24) . Some studies have reported a relation between lower rates of vitamin D in serum and increasing T2DM(25). In this work, the relation of T2DM with the DBP gene was studied in Bushehr province. Gcls, Gc1f, and Gc2 can be referred to as prevalent variants in the DBP gene(22). These three variants are in the codons 420 and 416 in exon 11 in the DBP gene(23).

Table 2. The clinical characteristics of the groups of patients and controls

Features	Patients (n=240)	Controls (n=159)	p-value
Female/male ratio	124/116	76/83	0.4
Age (years)	41.02 ± 9.52	41.59 ± 9.84	0.6
BMI (kg/m2)	26.96 ± 4.61	26.13 ± 4.52	0.08
Fasting glucose (mg/dl)	81.2 ± 9.24	81.84 ± 10.37	0.5
2 h-OGTT (mg/dl)	92.47 ± 17.18	92.29 ± 17.49	0.9
Insulin (µIU/ml)	8.24 (5.84–15.07)	6.97 (5.83–11.12)	0.02

Table 3. Analysis of linkage disequilibrium between 420 and 416 codon

	|D'|	r2	LOD	LOD_p_value	Chi_square	p_value
control	0.92674	0.35657	64.56365	0	113.3897	0
case	0.58411	0.12608	32.37624	0	60.51963	0
total	0.73278	0.20845	84.47336	0	166.3392	0

Table 4. Haplotype frequencies in DBP gene at codons 416 and 420

Haplotype	T2DM patients	Controls
Asp–Thr	78 (27%)	32 (21%)
Asp–Lys	52 (17.7%)	40 (26.2%)
Glu–Thr	146 (50.1%)	79 (52%)
Glu–Lys	15 (5.2%)	2 (0.8%)
	P-Value=0.3	

Table 5. The odds ratio in haplotypes of the DBP gene

		of OR	of OR
Glu/Thr	0.979	0.737	1.3
Asp/Lys	1.301	0.926	1.828
Asp/Thr	0.661	0.471	0.926
Glu/Lys	6.156	1.418	26.717

Table 6. The allele frequencies of the DBP gene at codons 416 and 420 in the study population

Allele		
Codon 416		
	ASP	**Glu**
T2DM patients	214(44.58%)	266(55.42%)
Controls	150(47.17%)	168(52.83%)
P-Value=0.5		
Codon 420		
	Thr	**Lys**
T2DM patients	370(77.08%)	110(22.92%)
Controls	232(72.96%)	86(27.04%)
P-Value=0.2		

Table 7. The genotype frequencies of the DBP gene at codons 416 and 420 in the study population

Genotype			
Codon 416			
	Asp/Asp	**Asp/Glu**	**Glu/Glu**
T2DM patients	53(22.08%)	108(45%)	79(32.92%)
Controls	31(19.5%)	88(55.34%)	40(25.16%)
P- Value=0.1			
Codon 420			
	Thr/Thr	**Thr/Lys**	**Lys/Lys**
T2DM patients	146(60.83%)	78(32.5%)	16(6.67%)
Controls	76(47.8%)	80(50.31%)	3(1.89%)
P<0.05			

Some studies have been performed on the relationship between this gene with T2DM.

Hirai et al. in a study on the Japanese population found that the DBP gene is effective in getting susceptible to T2DM (26). Moreover, the relation between the DBP variant and type II diabetes was reported by Wang et al (27). in The Chinese population in Shanghai and Shao et al (28). in China, Najing province. It is revealed the effects of DBP genotype on the fasting insulin level in Dogrib Indians. Baier et al (23). represented the relation between the DBP variants to oral glucose tolerance in nondiabetic Pima Indians. However, the relation of T2DM to the DBP gene was not proved by Malecki et al(29). in the Polish population, Ye et al(30). in French Caucasians and Klupa et al(18). in White Americans of European Origin. DBP variants in the Asian population were along with T2DM significantly (31). In the present work, we found no meaningful difference between the patient and healthy population on the frequency of haplotypes and alleles in codons 420 and 416. Nevertheless, we found no significant differences regarding genotype frequency in codon 416. Considering genotype frequency in codon 420, a significant difference was identified in our population studied. Wang et al(31). reported a significant relation between T2DM codons 416 and 420 in DBP variants in the Asian population. They also revealed that people with the Lys allele were highly affected by diabetes in the Asian population. More interestingly, in most studies, the frequency of the Glu-Lys haplotype was close to zero. The frequency of this haplotype in control and patient groups were reported near 0% by Malecki et al(29). and Ye et al. (30). Moreover, this haplotype frequency was reported as 7% and 0% in White Americans of European Origin population in patient and control groups, respectively [18]. Rahman et al(32). declared the lowest level of vitamin D for type 2 diabetic patients and also the highest frequency of Glu/Glu (codon 416) and Lys/Lys (codon 420) (32). Nevertheless, the rate of Glu-Lys haplotype frequency was 5.2% and 0,8% in the patient and healthy groups respectively in our study. Based on the genotype frequency, in codon 420 there was a significant difference between patient and healthy population in our study. It should be noted that diabetes is a disease caused by the interaction of genes, lifestyle, and environment (33). Generally, more studies are required with different populations to obtain a reliable conclusion on the relationship between gene DBP variants and T2DM.

Conflict of interest

There are no conflicts of interest.

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