Association of Vitamin D Receptor Gene BsmIG>A Polymorphisms in Patients with Type 2 Diabetes Mellitus

Tip 2 Diabetes Mellitus'lu Hastalarda Vitamin D Reseptör Geni BsmIG>A Polimorfizmlerinin İlişkisi

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Abstract

Background: The etiology of type 2 diabetes mellitus (T2DM) is likely to involve defects of both insulin secretion and insulin signaling. Thus, *VDR* gene involved in its metabolic pathway has regarded as good candidates for T2DM. In this study, it was aimed to determine the relation between c.1024+283G>A (*Bsm*I, rs1544410) polymorphisms in the intron 8 of *VDR* gene and T2DM in a Turkish population.

Methods: Seventy-two patients with T2DM (39 females, mean age 58.2±3.0 and 33 males, mean age 55.9±5.0) and 169 healthy subjects (79 females, mean age 56.4±6.8 and 90 males, mean age 55.8±6.7) were included into this study. DNA was isolated from these individuals. Then, the *VDR* NM_000376.2:c.1024+283G>A (g.63980G>A) polymorphism was analyzed by DNA amplification with polymerase chain reaction and endonuclease digestion with *Bsm*I.

Results: The study findings demonstrated that the frequency of the *VDR*: c.1024+283 AA genotype in T2DM patients (25.0%) was not significantly increased compared to healthy controls (16.6%) (OR (95%CI): 1.678 (0.859-3.281), p=0.152)). In the same way, the frequency of the other genotypes and alleles in this polymorphic site were not statistically significant between both study groups.

Conclusions: The scanned c.1024+283G>A polymorphic site in *VDR* gene did not represent a major risk factor for T2DM in the population investigated.

Key words: Vitamin D receptor, genetic polymorphism, diabetes mellitus, polymerase chain reaction

Özet

Amaç: Tip 2 diabetes mellitus'un (T2DM) etiyolojisinde, hem insulin salgısı hem de insulin sinyal bozukluklarının bulunması olasıdır. Bu nedenle buradaki metabolik yolakla ilişkili olan *VDR* geni, T2DM için iyi bir aday olarak göz önüne alınmaktadır. Bu çalışma ile, Türk toplumunda T2DM ile *VDR* geni sekizinci intronu üzerinde bulunan c.1024+283G>A (*Bsm*IA>G, rs1544410) polimorfizmleri arasındaki ilişkinin belirlenmesi amaçlandı. **Materyal ve Metod:** Bu çalışmaya, 72 T2DM hastası (39'u kadın, ortalama yaş 58.2±3.0 ve 33'ü erkek, ortlama yaş 55.9±5.0) ile 169 sağlıklı birey (79'u kadın, ortalama yaş 56.4±6.8 ve 90'ı erkek, ortalama yaş 55.8±6.7) dahil edildi. Bu bireylerden DNA izole edildi. Daha sonra *VDR* NM_000376.2:c.1024+283G>A (g.63980G>A) polimorfizmi, DNA'nın polimeraz zincir reaksiyonu ile çoğaltılması ve *Bsm*I endonükleaz ile kesilmesiyle analiz edildi.

Bulgular: Çalışma sonuçları, T2DM'lu hastalarda *VDR*:c.1024+283 AA genotip oranı (%25.0), sağlıklı kontrollerle (%16.6) karşılaştırıldığında anlamsız şekilde yüksek olduğunu gösterdi (OR (95%CI): 1.678 (0.859-3.281), p=0.152)). Aynı şekilde, bu polimorfik yerin diğer genotip ve allel oranları, hasta ve sağlıklı bireyler arasında istatitiksel olarak anlamlı değildi.

Sonuç: *VDR* geninde taranan c.1024+283G>A polimorfik yer, araştırılan toplumdaki T2DM için büyük bir risk faktörü olduğunu göstermedi.

Anahtar kelimeler: Vitamin D reseptörü, genetik polimorfizm, diabetes mellitus, polimeraz zincir reaksiyonu

Introduction

Diabetes mellitus type 2 or type 2 diabetes (T2DM), called non-insulin-dependent diabetes mellitus (NIDDM), is a metabolic disorder that is characterized by insulin resistance, relative insulin deficiency, and hyperglycemia (1-3). It is reported that the number of people with diabetes worldwide exceeds 200 million, most of them being patients with T2DM. In the societies of the industrialized world, the prevalence of this disease has reached a few percent of entire populations and is still growing (4).

The vitamin D receptor gene (VDR) is located on chromosome 12 (12q13.11) in human, and contains 10 exons. More than 100 single nucleotide polymorphisms (SNPs) have been described with its 63494-bp sequence. Eight exons (3-10) and six alternatively spliced region are distributed in functionally relevant areas, including the promoter region. The VDR: c.1024+283G>A polymorphic site is present in the non-coding sequence (in intron 8) (5). Furthermore, the vitamin D (1,25-dihydroxyvitamin D3) receptor is an intracellular hormone receptor that specifically binds

the active form of vitamin D (1,25-dihydroxyvitamin D3 or calcitriol), interacts with target-cell nuclei to produce a variety of biologic effects, also functions as a receptor for the secondary bile acid lithocholic acid, which is hepatotoxic and a potential enteric carcinogen (6).

Vitamin D deficiency has been shown to impair insulin synthesis and secretion in human and animal models of diabetes, suggesting a role in the development of T2DM (6, 7). These findings are confirmed in animal models, which demonstrate that pancreatic insulin secretion is inhibited by vitamin D deficiency (8). It has been shown that genetic factors carry a major share of an individual's risk for T2DM (9). Recent studies demonstrate that the VDR gene c.1024+283G>A polymorphisms are associated with type 1 diabetes mellitus (T1DM) in Germans, but not in Indian Asians (10, 11).

Previous studies have revealed that the distributions of genotype frequencies of c.1024+283G>A polymorphism do not differ between persons with and without T2DM in the different populations, such as English (12), Indian (13, 14), Chinese (15), French (16), Hungarian (17), American (18), Polish (19, 20), and Turkish (21). On the other hand, several reports have demonstrated an association of the VDR gene polymorphisms and T2DM in Germans (9), and Bangladeshi Asians (12). In addition, a study indicates that polymorphisms of the VDR gene may play a role in the pathogenesis of T2DM by influencing the secretory capacity of β-cells (17).

The goal of this study was to determine whether there was the association of c.1024+283G>A polymorphism of the VDR gene with T2DM in a Turkish population using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Materials and methods

Subjects and DNA Extraction

Seventy-two patients with T2DM (39 females, mean age 58.2±3; and 33 males, mean age 55.9±5), who were determined with fasting plasma glucose (FPG) levels ≥126mg/dl after 12 hours fasting, and confirmed by repeated testing on a different day according to the world health organization criteria (23), at the Internal Medicine Unit in Harran University Hospital. Additionally, all patients were analyzed for body mass index (BMI), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), hemoglobin A1c (HbA1c). One hundred sixty-nine healthy controls (79 females, mean ages: 56.4±6.8; and 90 males; mean ages: 55.86.7), who did not have any disease, were tested for FPG. Control individuals with FPG levels more than 100 mg/dl, and a positive family history of diabetes were discarded from the study. The T2DM patients and healthy controls were matched for age, sex, and all patients were tested all patients for the demographic and clinical profiles. EDTA-blood was taken from these individuals. EDTA-blood was taken from these subjects, and genomic DNA was extracted from nucleated cells by using a standard salting out procedure, as described by Miller et al (24).

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

The c.1024+283G>A (g.63980G>A or BsmIG>A or rs1544410) polymorphic site of the VDR gene (NM_000376.2, GI: 7421) was amplified by using a touchdown PCR technique, and scanned by RFLP analysis. PCR reaction was carried out in a 10-l reaction volume containing 1xPCR buffer, 2 mM MgCl2, 0.2 mM each deoxynucleotide triphosphate (dNTPs, Fermentas, St. Leon-Rot, Germany), 30 ng of DNA, 0.2 µM of each primer (in exon 7: 5'-CAACCAAGACTACAAGTACCGCGTCAGTGA-3', and in intron 8: 5'-AACCAGCGGGAAGAGGTCAAGGG-3') (BioBasic Inc, Ontario, Canada), and 0.3 unit of Taq DNA polymerase (Fermentas) (25). The touchdown PCR conditions: initial denaturation at 94˚C for 3 min, followed by 12 cycles at 94˚C for 30s, 72-61˚C for 30s (decreasing 1˚C per cycle), 72˚C for 30s, and 20 cycles at 94˚C for 30s, 60˚C for 30s, 72˚C 30s, and a final extension at 72˚C for 5 min.

A half of the PCR product (5-µl) was digested in a 20-µl reaction volume for two hours with 1.5 Units of BsmI at 37˚C (Fermentas). The digested PCR product was separated on 2% agarose gel, and was analyzed using Alpha-Imager System (AlphaInnotech, San Leandro, California USA). The profiles of the VDR gene with BsmI; G allele yielded fragments of a 646-bp and 176-bp, and A allele yielded 822-bp (Fig. 1).

Statistical analysis

Student's t-test was used to define differences in means of demographic and clinical characteristics by using the SPSS statistics program (Table 1, and 3). Genotype and allele frequencies of the c.1024+283G>A polymorphic site of the VDR gene were tested for Hardy-Weinberg equilibrium by using chi-square test. Genotype and allele frequencies observed in this study was analyzed with Fisher's exact test by using the SPSS statistics program. Statistical significance was determined as $p<0.05$. The odds ratio (OR) was calculated to measure the strength of the association observed (Table 2).

Ethics

The institutional review board approved the study, and written informed consents were obtained from all patients. The study complied with the Helsinki Declaration.

Results

The VDR gene c.1024+283G>A (in intron 8) polymorphism was investigated in 72 patients with T2DM and 169 healthy controls. The BMI was significantly different between patients with T2DM and

healthy individuals (p<0.05) (Table 1).

The distribution of the genotypes for this polymorphic site was consistent with the Hardy-Weinberg equilibrium in the T2DM (p=0.77) and control groups (p=0.07). Table 2 indicated that there was no significant association with the genotype and allele frequencies in T2DM patients, compared with healthy group. The c.1024+283 AA genotype (homozygous) was not a risk factor for T2DM (Table 2).

However, comparison without and with polymorphic patients by using Student's t-test, there was no significance in age, BMI, FPG, TC, TG, HDL-c, LDL-c, HbA1c, and family history of T2DM (p>0.05) (Table 3).

Discussion

The vitamin D endocrine system is pleiotropic, and plays an important role in skeletal metabolism, including intestinal calcium absorption and regulation of osteoblast differentiation, modulate the immune response, insulin secretion, the renin/angiotension system, and also growth of cancer cells (2-4). Vitamin D deficiency affects insulin synthesis and secretion in humans and in animal models of diabetes, suggesting a role in the development of T2DM (6, 8).

Although the genetics of T2DM are complex and not clearly defined, genetic factors have been recognize to carry a major share of an individual's risk for T2DM (9, 18, 20). These reports point out some of the single nucleotide polymorphisms in the VDR gene affect the insulin secretion, and indicate a risk for T2DM (17, 20). Pani et al have shown that there is significant transmission disequilibrium for several the VDR haplotypes derived from the combined analysis of the BsmI site in T1DM in the German population (10). In addition, McDermott et al have been found no association between BsmI and T1DM in Indian population (11). On the other hand, polymorphisms of the VDR receptor gene may play a role in the pathogenesis of T2DM by influencing the secretory capacity of β-cells, which is independent of vitamin D status (17, 20).

In our study, we investigated the association of c.1024+283G>A polymorphisms of the VDR gene in patients with T2DM and healthy controls in a Turkish population. Though BMI level in T2DM patients (30.1±4.3) was significantly different from healthy group (25.1±2.4) (Table 1), we did not observe any significant difference between the frequencies of c.1024+283G>A genotypes and alleles of VDR gene in both of the study groups (Table 2). Furthermore, although the c.1024+283 AA genotype (25.0%) and A allele (52.1%) frequencies of VDR gene in patients with T2DM was higher than in healthy subjects (16.6% and 45.9%, respectively), these differences were not significant (p=0.152 and 0.232, respectively) (Table 3). VDR multiple polymorphisms had been investigated for the association with T2DM in several populations. Our results were compatible with those reported in the literature for various populations (12-21), except a few studies with some special conditions (12, 16, 18, 22) (Table 2). Dilmec et al indicate that VDR c.1025-49G>T (ApaI) and c.1056T>C (TaqI) genotypes and alleles are not associated with the same T2DM patients in a Turkish population (21). Thus, in Turkish population, VDR gene polymorphisms containing c.1024+283G>A, c.1025-49G>T, and c.1056T>C polymorphic sites with the point a relationship between T2DM could not be determined. Although our results were in accordance with the studies in English, Indian, French, Chinese, American, and Polish populations, they presented some differences from our findings. The variants of the VDR are not a major gene for T2DM in French population. However, the polymorphisms in the VDR gene are associated with susceptibility to obesity in subjects with early-onset T2DM (16). Additionally, Cyganek et al demonstrate that no difference in the distribution of either the VDR genotypes or alleles have seen between patients with and without Diabetic Retinopathy with T2DM in Polish population (20). Although our findings were congenial with these studies in the various populations, they were not amicable with some studies. Ortlepp et al indicate that the VDR BsmI AA genotype is associated with altered fasting glucose levels in young men with low physical activity (9). Hitman et al. demonstrate that there is an association between the VDR gene polymorphisms and insulin secretion in Bangladeshi Asians, categorized as at risk for T2DM, which is independent of vitamin D status (12). Speer et al. point out that polymorphisms of the VDR gene may play a role in the pathogenesis of T2DM by influencing the secretory capacity of β-cells (17).

It is possible to conclude from these results that, although there is no relation between polymorphism and T2DM in general, there is an association between polymorphism and a specific aspect or presentation of this disease, such as obesity, glucose intolerance, and insulin secretion in individuals.

In comparison without (c.1024+283GG genotype) and with polymorphic (c.1024+283AA genotype) T2DM patients, there was no association in age, BMI, FPG, TC, TG, HDL-c, LDL-c, HbA1c, and family history of T2DM (p<0.05) (Table 2). It is also worth mentioning that our study on VDR gene c.1024+283G>A is the first study performed in patients with T2DM in a Turkish population. Related to clinical data, our results are consistent with some studies (13, 16, 18).

In conclusion, our study suggested that the BsmI polymorphism in the VDR gene was not associated with the susceptibility to T2DM. Further studies in larger populations are required to clarify the interactions of the VDR polymorphisms with T2DM due to the different findings from various populations.

Table 1 : The distribution of demographic characteristics in T2DM patients and control group

	T2DM patients	Healthy controls	*p*- value
Subjects (n)	72	169	
Gender (M/F)	33/39	90/79	
Age (years)	57.1±10.8	56.1±6.8	0.386
BMI (kg/m^2)	30.1±4.3	25.1±2.4	0.001

Values are mean ± standard deviation, M: male, F: female.

Table 2: Genotype and allele frequencies of *VDR* gene *Bsm*I polymorphism among patients with T2DM and healthy subjects

SNP Genotype/Allele	T2DM patients (n=72)	Healthy controls (n=169)	X^2	OR (95% CI)	*p-values*
c.1024+283G>A					
Genotypes					
GG	15 (20.8%)	42 (24.9%)		Reference	
GA	39 (54.2%)	99 (58.6%)	0.402	0.836 (0.479-1.456)	0.570
AA	18 (25.0%)	28 (16.6%)	2.324	1.679 (0.859-3.281)	0.152
Alleles					
G	69 (47.9%)	183 (54.1%)		Reference	
A	75 (52.1%)	155 (45.9%)	1.569	1.283 (0.868-1.897)	0.232

Abbreviations: X^2 = Chi-square, OR = Odds ratio, CI = Confidence int erval, SNP = Single Nucleotide Polymorphism.

Table 3 : The demographic and clinical profiles for those with and without polymorphic T2DM patients for *VDR* gene

	***VDR*:c.1024+283G>A**		
Genotype	GG	AA	*p- value*
Numbers	15	18	
Age (years)	58.1±10.2	57.2±12.6	0.812
BMI (kg/m^2)	30.1±4.1	29.1±3.6	0.462
FPG (mg/dl)	258.5±100.0	219.0±89.9	0.241
TC (mg/dl)	227.7±47.9	204.7±42.6	0.154
TG (mg/dl)	175.7±67.0	176.9±58.2	0.954
HDL-C (mg/dl)	54.0±7.7	53.3±8.4	0.800
LDL-C (mg/dl)	142.6±43.9	144.4±42.6	0.904
HbA1c (%)	9.2±2.0	8.9±2.1	0.644
No. of family history	12	11	0.253

Values are mean ± standard deviation, GG genotype: wild type (not polymorphic), AA genotype: polymorphic.

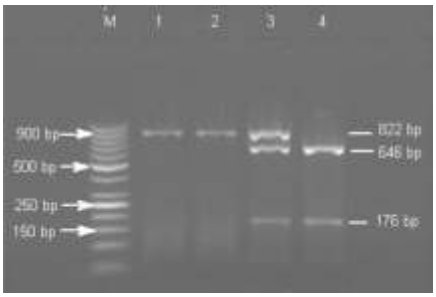

Figure 1: *PCR products of the VDR gene after digestion with BsmI. Lane M: Molecular weight marker (1000-50bp, Fermentas), lane 1: undigested PCR product, lane 2: AA genotype (homozygote), lane 3: GA genotype (heterozygote), lane 4: GG genotype (homozygote)*

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