TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2017) 47: 1568-1575
© TÜBİTAK
doi:10.3906/sag-1701-174

Investigation of glucocorticoid receptor and calpain-10 gene polymorphisms in Turkish patients with type 2 diabetes mellitus

Naciye Selcen BAYRAMCI[1,*], Leyla AÇIK[2], Çağdaş KALKAN[3], İlhan YETKİN[4]

[1]Department of Bioengineering, Faculty of Engineering and Natural Sciences, Gaziosmanpaşa University, Tokat, Turkey
[2]Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey
[3]Department of Gastroenterology, Faculty of Medicine, Ankara University, Ankara, Turkey
[4]Department of Endocrinology and Metabolism, Faculty of Medicine, Gazi University, Ankara, Turkey

Received: 30.01.2017 • **Accepted/Published Online:** 01.07.2017 • **Final Version:** 13.11.2017

Background/aim: We proposed to investigate the role of calpain-10 (*CAPN10*) gene single nucleotide polymorphism (SNP)-19 and SNP-44 and glucocorticoid receptor (*NR3C1*) gene N363S polymorphisms in Turkish patients with type 2 diabetes mellitus (T2DM).

Materials and methods: Peripheral blood samples were obtained from 125 patients with T2DM and 112 healthy volunteers. Genotyping was carried out by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: There were no statistically significant differences found between the allele and genotype frequencies of *CAPN10* SNP-19, *CAPN10* SNP-44, and *NR3C1* N363S polymorphisms and T2DM development ($P > 0.05$). The *CAPN10* SNP-19 del-allele, *CAPN10* SNP-44 C-allele, and *NR3C1* N363S G-allele were determined to be risk factors for T2DM development. In T2DM patients an association was identified between the *CAPN10* SNP-19 del-allele, homozygous del/del genotype, SNP-44 C-allele, heterozygous TC genotype, *NR3C1* N363S G-allele, heterozygous AG genotype, and increased BMI.

Conclusion: The present study demonstrates that the SNP-44 polymorphism is associated with T2DM susceptibility and contributes to the risk of T2DM.

Key words: Calpain-10 gene, glucocorticoid receptor gene, type 2 diabetes

1. Introduction

Type 2 diabetes mellitus (T2DM) is a long-term metabolic disorder that results from defects in both insulin action and insulin secretion. T2DM is associated with serious micro- and macrovascular complications affecting several organs, including the heart and blood vessels, liver, eyes, muscles, skin, brain, and kidneys, thus resulting in significant mortality and morbidity rates. The prevalence of T2DM is rising rapidly; according to a recent report the total number of people with diabetes is estimated to reach 642 million individuals by 2040 worldwide. In Turkey, diabetes seems to be a serious health problem among the population because the national raw prevalence rate of diabetes in Turkey was about 14.56% in 2013, while the mean raw rate in 23 other European populations in the same year was about 7.06% (in the age group of 20-79 years) (1,2).

The human calpain-10 (*CAPN10*) gene, which encodes 672 amino acid intracellular proteases with 15 exons, is associated with the development of T2DM (3). The human glucocorticoid receptor (*NR3C1*) gene was localized to band q31-32 of chromosome 5. Excess glucocorticoid levels induce glucose intolerance, insulin resistance, and β-cell dysfunction and are associated with incident diabetes (4).

In this study, the SNP-19 and SNP-44 polymorphisms of the *CAPN10* gene and the N363S polymorphisms of the *NR3C1* gene were genotyped in a Turkish population with T2DM to analyze the correlation between the genotypes and the clinicopathological features of the patients.

2. Materials and methods

2.1. Patients

A total of 112 healthy controls and 125 unrelated adult patients who presented to the Endocrinology and Metabolism Clinic of Gazi University Hospital, Ankara, Turkey, with T2DM and other complaints were studied. All the individuals involved in the study were informed

* Correspondence: selcenbayramci@hotmail.com

about the purpose of the study and a written informed consent form was signed by all participants. The study was approved by the Gazi University Faculty of Medicine's Clinical Research Ethics Committee (Acceptance Date: 15/06/2011, Acceptance Number: 228). Demographic and biochemical parameters including age, sex, weight, body mass index (BMI), duration of T2DM, glycated hemoglobin (HbA_{1C}), total cholesterol (TC), postprandial blood glucose (PBG), fasting blood glucose (FBG), low-density lipoprotein (LDL), plasma triglyceride (TG), and high-density lipoprotein (HDL) levels were measured.

2.2. Genotyping

The genomic DNA was extracted from peripheral blood leukocytes using the salting-out method and eluted in Tris-EDTA buffer. A polymerase chain reaction-restriction length polymorphism (PCR-RFLP) method was used to genotype SNP-19 (insertion/deletion) in intron 6 and SNP-44 (T/C) in intron 3 of the *CAPN10* gene and N363S (A/G) in exon 2 of the *NR3C1* gene. PCR amplification was performed in a volume of 50 µL containing 50–100 ng of genomic DNA, 1X PCR buffer (Fermentas, Vilnius, Lithuania), 160 mM deoxynucleoside triphosphates (Fermentas), 2.0 mM $MgCl_2$ (Fermentas), 0.1 U of Taq DNA polymerase (Promega, Madison, WI, USA), and 0.4 µM of each primer for SNP-19 (forward: 5'-GTTTGGTTCTCTTCAGCGTGCAG-3' and reverse: 5'-CATGAACCCTGCAGGGTCTAAG-3'), for SNP-44 (forward: 5'-GCAGGGCGCTCACGCTTGCCG-3' and reverse: 5'-GCATGGCCCCCTCTCTGATTC-3'), and for N363S (forward: 5'-AGTACCTCTGGAGGACAGAT-3' and reverse: 5'-GTCCATTCTTAAGAAACAGG-3') polymorphisms. Amplification was performed as follows: an initial denaturation at 94 °C for 5 min was followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 1 min and 72 °C for 1 min, and a final elongation at 72 °C for 5 min for *CAPN10* SNP-19 and SNP-44, while an initial denaturation at 96 °C for 5 min was followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s and 72 °C for 30 s, and a final elongation at 72 °C for 7 min for NR3C1 *N363S* polymorphisms. After amplification, 10 µL of PCR products of SNP-19 were separated on 3% agarose gel incorporated with ethidium bromide. The *CAPN10* SNP-19 was an insertion/deletion polymorphism consisting of two or three repeats of a 32-bp sequence; 146-bp and 178-bp amplification product bands were identified for del-allele (two repeats) and ins-allele (three repeats), respectively. PCR products (10 µL) of SNP-44 and N363S were separated on 2% agarose gel and visualized by ethidium bromide. A negative control was included in each amplification analysis. The amplified genomic DNA fragments were digested at 37 °C for 1 h with BstUI (Bsh1236I) restriction enzyme (New England Biolabs, Hitchin, UK) for genotyping of SNP-44 and with TasI (Tsp5091) restriction enzyme (New England Biolabs) for genotyping of N363S polymorphisms. The 166-bp amplicon for SNP-44 was restricted by BstUI into 145- and 21-bp fragments for the C-allele. A 249-bp amplicon for the *NR3C1* N363S polymorphism with two TasI restriction sites (135 bp, 95 bp, and 19 bp) for the A-allele and only one restriction site (154 bp and 95 bp) for the G-allele was obtained. Following enzymatic digestion, PCR products were subjected to electrophoresis on 3% agarose gel and stained with ethidium bromide.

2.3. Statistical analysis

All statistical analyses were performed using the SPSS 20.0 (IBM Corp., Armonk, NY, USA). All values are expressed as mean ± standard deviation. Normally distributed variables were compared between T2DM patients and the control group using Student's t-test. The correspondence of the distribution of the genotype and allele frequencies to Hardy–Weinberg equilibrium was assessed using the chi-square (χ^2) test and Fisher's exact test. In all analyses, a $P \leq 0.05$ was considered statistically significant.

3. Results

The demographic and clinical features of the patients are given in Table 1. The patients had a mean age of 55.2 years (range: 20–87 years), BMI of 28.1 kg/m^2 (range: 17.53–43.7 kg/m^2), and average T2DM duration of 107.02 months (range: 1–372 months). The control subjects had a mean age of 38.52 years (range: 6–81 years).

The genotype and allele frequencies of the investigated polymorphisms in the T2DM patients and the control group are presented in Table 2. No statistically significant difference was found between T2DM development and allele frequencies of all studied polymorphisms: SNP-19 ($P = 0.099$; $\chi^2 = 2.728$), SNP-44 ($P = 0.344$; $\chi^2 = 0.896$), and N363S ($P = 0.352$; $\chi^2 = 0.865$). The *CAPN10* gene SNP-19 del-allele and SNP-44 C-allele and the *NR3C1* gene N363S G-allele were determined to be risk factors for T2DM development. The genotype frequencies in the T2DM patient group were consistent with Hardy–Weinberg equilibrium for SNP-19 ($P = 0.127$; $\chi^2 = 2.33$), SNP-44 ($P = 0.075$; $\chi^2 = 3.16$), and N363S ($P = 0.112$; $\chi^2 = 2.52$). There were no statistically significant differences in the genotype frequencies of the *CAPN10* SNP-19, *CAPN10* SNP-44, and *NR3C1* N363S polymorphisms between T2DM patients and the control group.

The demographic and clinical features according to the genotype distributions of all T2DM patients are shown in Table 3, those of female T2DM patients are shown in Table 4, and those of male T2DM patients are shown Table 5. C-allele carriers of the SNP-44 TC genotype were found to be associated with 1.34-fold increased risk of T2DM development in the general T2DM patient group ($P = 0.216$); this was 3-fold in females ($P = 0.527$) and 5-fold

Table 1. Demographic and clinical features of the study group.

Parameters	T2DM patients for SNP-19 (n = 115)	T2DM patients for SNP-44 (n = 105)	T2DM patients for N363S (n = 101)
Sex (M : F)	46 : 69	43 : 62	44 : 57
Age (years)	55.56 ± 11.47	56.13 ± 11.58	56.06 ± 12.53
BMI (kg/m^2)	28.19 ± 3.15	28.18 ± 3.46	28.06 ± 3.68
T2DM duration (months)	111.19 ± 68.45	108.71 ± 65.48	109.93 ± 66.32
HbA_{1c} (%)	8.07 ± 8.47	7.36 ± 1.80	7.35 ± 1.82
FBG (mg/dL)	150.05 ± 57.29	150.50 ± 55.62	151.68 ± 56.21
PBG (mg/dL)	181.68 ± 72.21	178.61 ± 71.69	181.79 ± 74.62
Total cholesterol (mg/dL)	189.93 ± 41.71	191.11 ± 41.13	191.82 ± 41.53
HDL (mg/dL)	41.33 ± 9.58	41.57 ± 9.65	41.02 ± 9.77
LDL (mg/dL)	115.97 ± 42.92	112.89 ± 31.34	113.34 ± 31.53
TG (mg/dL)	183.51 ± 150.99	183.97 ± 141.98	182.78 ± 143.33

in males (P = 0.258). Likewise, the G-allele carriers of the N363S AG genotype were found to be associated with 2.41-fold increased risk of T2DM development in the general T2DM patient group (P = 0.511); this was 2.5-fold in females (P = 0.431) and 2.25-fold in males (P = 0.920). In addition, T2DM patients who were homozygous for the SNP-19 ins-allele had higher BMIs (28.44 ± 3.56 kg/m^2) than those heterozygous or homozygous for del-allele (27.87 ± 2.49 kg/m^2 and 28.40 ± 4.16 kg/m^2, respectively) (P = 0.130), and those who were heterozygous for the SNP-44 C-allele had higher BMIs (30.62 ± 3.98 kg/m^2) than those homozygous for the C- and T-allele (27.68 ± 3.41 kg/m^2 and 28.12 ± 3.36 kg/m^2, respectively) (P = 0.098). Similarly, those who were heterozygous for the N363S G-allele had higher BMIs (28.08 ± 3.25 kg/m^2) than those homozygous for the A-allele (28.01 ± 4.40 kg/m^2) (P = 0.933). No homozygote for the N363S rare G-allele was detected. Sixty-five of the 101 T2DM patients were found to be heterozygous for the N363S of *NR3C1* (AG genotype and G-allele frequency: 0.644).

There were no statistically significant differences in HbA_{1C}, FBG, PBG, TG, TC, HDL, or LDL levels and all investigated genotypes of the SNP-19, SNP-44, and N363S polymorphisms in T2DM patients (P ≥ 0.05). However, a statistically significant difference was observed for the SNP-44 genotypes in male patients with T2DM. Weight (P = 0.001), BMI (P = 0.098), HbA_{1C} (P = 0.329), FBG (P = 0.462), and PBG (P = 0.418) levels in patients heterozygous for SNP-44 were higher than in patients homozygous for the T-allele. Statistically significant differences for SNP-44 genotypes were observed for BMI and weight in male patients with T2DM (P = 0.031 and P = 0.004, respectively).

Hyperglycemia severity determinants (duration of T2DM, treatment of diabetes, FBG and HbA_{1C} levels) were not significantly different in carriers of the N363S AG and AA genotypes. In addition, significantly higher BMI (P = 0.968), TC (P = 0.732), HDL (P = 0.024), and LDL (P = 0.776) levels were observed in female patients with T2DM who had the N363S AG genotype as compared to male patients with T2DM. Statistically significant differences between N363S AA and AG genotypes were observed for HDL levels in female patients with T2DM (P = 0.024).

4. Discussion

We have studied the effects of *CAPN10* and *NR3C1* gene polymorphisms on the risk of

T2DM and on the levels of HbA_{1C}, FBG, PBG, TC, TG, HDL, and LDL in T2DM patients and a control group living in Turkey. We tested 2 polymorphisms in the *CAPN10* gene, SNP-19 and SNP-44, and a polymorphism in the *NR3C1* gene, N363S, for association with T2DM using a case-control design.

In our study, no significant difference was found between the T2DM patients and the control group in terms of the *CAPN10* gene SNP-19 polymorphism allele and genotype frequencies. The odds ratio between del-allele and T2DM development was specified as 1.388 (95% CI = 0.940–2.049). Interestingly, the odds ratio between the del-allele and the development of T2DM in the Turkish population is remarkably similar to that reported in the Scandinavian Caucasians (5), Hispanic Americans (6), the Palestinian population (7), and the Tunisian Arab population (8). As part of our study, the examined demographic details in analysis included higher BMIs for the T2DM patients who were homozygous for the SNP-19 ins-allele than those heterozygous or homozygous for del-allele. However, it was found that TC and TG levels were significantly lower in T2DM patients who were

Table 2. Genotype and allele distribution of the investigated SNPs in T2DM and control groups.

SNP-19						
Genotype	T2DM (n = 115)	Controls (n = 100)	OR	95% CI	χ^2	P
Ins/Ins	18 (15.65%)	26 (26.00%)	1.000			
Ins/Del	45 (39.13%)	34 (34.00%)	1.912	0.905–4.039	2.915	0.088
Del/Del	52 (45.21%)	40 (40.00%)	1.878	0.906–3.892	2.905	0.088
Ins/Del+Del/Del	97 (84.35%)	74 (74.00%)	1.893	0.966–3.711	3.519	0.061
Allele						
Ins	81 (35.22%)	86 (43.00%)	1.000			
Del	149 (64.78%)	114 (57.00%)	1.388	0.940–2.049	2.728	0.099
SNP-44						
Genotype	T2DM (n = 105)	Controls (n = 100)				
TC+CC	39 (37.14%)	29 (29.00%)	1.000			
TT	66 (62.86%)	71 (71.00%)	0.691	0.385–1.242	1.532	0.216
Allele						
C	70 (33.33%)	58 (29.00%)	1.000			
T	140 (66.67%)	142 (71.00%)	0.817	0.537–1.242	0.896	0.344
N363S						
Genotype	T2DM (n = 101)	Controls (n = 46)				
AA	36 (35.64%)	19 (41.30%)	1.000			
AG	65 (64.35%)	27 (58.70%)	1.271	0.622–2.596	0.433	0.511
Allele						
A	72 (35.64%)	38 (41.30%)	1.000			
G	130 (64.35%)	54 (58.70%)	1.271	0.768–2.106	0.865	0.352

OR: Odds ratio, CI: confidence interval.

homozygous for the SNP-19 ins-allele. Our findings are consistent with the results reported in the Tunisian Arab population (8).

A significant association between the *CAPN10* gene SNP-44 TC genotype and T2DM was found in Mexican Americans (3), the British/Irish population (9), and the Palestinian population (7). Indeed, the results of our study suggest that the *CAPN10* gene SNP-44 TC genotype is associated with increased risk of T2DM in the Turkish population.

The *CAPN10* gene SNP-44 C-allele has been reported to increase T2DM development approximately 1.5- to 2.5-fold in the British population (9) and 2.7-fold in the Palestinian population (7). Similarly, it was determined that the *CAPN10* SNP-44 genotype was associated with 1.34-fold increased risk of developing T2DM in the Turkish population, with 3-fold increase in females and 5-fold in males. In addition, the SNP-44 TC genotype was associated with increased BMI in T2DM patients. This conclusion supports the results of a previous study from Turkey, which reported that the *CAPN10* gene SNP-44 TC genotype was associated with T2DM in a Turkish population (10). We have also found that there is a statistically significant difference between BMI and weight with SNP-44 genotypes, especially in male Turkish T2DM patients. Taken together, the *CAPN10* gene SNP-44 TC genotype in Turkish T2DM patients may be a risk factor for obesity development, especially in males.

To our knowledge, the current study is the first to examine the distribution and frequency of *NR3C1* gene

Table 3. Demographic and clinical features according to genotype distributions of *CAPN10* gene SNP-19 and SNP-44 and *NR3C1* gene N363S polymorphisms in all T2DM patients.

	CAPN10 SNP-19			
T2DM patient parameters	Del/Del (n = 52)	Ins/Del (n = 45)	Ins/Ins (n = 18)	P
Age (years)	57.19 ± 11.07	53.57 ± 12.47	55.83 ± 9.66	0.402
Weight (kg)	76.36 ± 9.97	74.94 ± 7.08	77.72 ± 7.33	0.850
BMI (kg/m^2)	28.40 ± 4.16	27.87 ± 2.49	28.44 ± 3.56	0.130
T2DM duration (months)	116.25 ± 67.67	106.33 ± 124.03	136.66 ± 95.21	0.521
HbA_{1c} (%)	7.52 ± 1.95	7.04 ± 1.79	7.35 ± 1.54	0.465
FBG (mg/dL)	151.78 ± 46.13	141.95 ± 64.16	165.27 ± 67.40	0.300
PBG (mg/dL)	184.03 ± 66.35	177.22 ± 78.29	186.05 ± 76.19	0.194
Total cholesterol (mg/dL)	185.11 ± 39.63	190.11 ± 39.91	203.38 ± 50.68	0.077
HDL (mg/dL)	40.94 ± 9.85	41.40 ± 9.01	42.27 ± 10.60	0.061
LDL (mg/dL)	111.25 ± 31.31	112.91 ± 31.95	137.27 ± 10.60	0.075
TG (mg/dL)	162.19 ± 75.02	180.88 ± 113.07	251.66 ± 310.79	0.059
	CAPN10 SNP-44			
T2DM patient parameters	TT (n = 66)	TC (n = 8)	CC (n = 31)	P
Age (years)	57.15 ± 12.33	54.12 ± 10.10	54.48 ± 10.27	0.506
Weight (kg)	75.71 ± 7.60	86.62 ± 11.58	74.25 ± 8.25	0.001
BMI (kg/m^2)	28.12 ± 3.36	30.62 ± 3.98	27.68 ± 3.41	0.098
T2DM duration (months)	111.50 ± 63.02	118.62 ± 79.75	100.22 ± 68.25	0.667
HbA_{1c} (%)	7.46 ± 1.84	7.95 ± 2.48	7.00 ± 1.48	0.329
FBG (mg/dL)	150.80 ± 50.90	171.87 ± 111.31	144.35 ± 45.37	0.462
PBG (mg/dL)	176.46 ± 64.75	210.87 ± 91.50	174.87 ± 80.49	0.418
Total cholesterol (mg/dL)	190.66 ± 41.82	172.87 ± 32.12	196.77 ± 41.36	0.342
HDL (mg/dL)	41.89 ± 10.45	40.62 ± 9.34	41.12 ± 8.07	0.900
LDL (mg/dL)	112.84 ± 30.63	99.12 ± 30.82	116.54 ± 32.96	0.378
TG (mg/dL)	185.01 ± 170.88	163.37 ± 39.08	187.06 ± 78.30	0.912
	NR3C1 N363S			
T2DM patient parameters	AA (n = 36)	AG (n = 65)	P	
Age (years)	54.22 ± 12.25	57.09 ± 12.09	0.272	
Weight (kg)	76.02 ± 11.06	76.40 ± 9.01	0.855	
BMI (kg/m^2)	28.01 ± 4.40	28.08 ± 3.25	0.933	
T2DM duration (months)	98.41 ± 67.07	116.30 ± 65.55	0.196	
HbA_{1c} (%)	7.31 ± 2.03	7.36 ± 1.71	0.901	
FBG (mg/dL)	150.22 ± 51.03	152.30 ± 59.25	0.847	
PBG (mg/dL)	186.27 ± 70.70	179.30 ± 77.13	0.655	
Total cholesterol (mg/dL)	191.66 ± 40.45	191.90 ± 42.43	0.978	
HDL (mg/dL)	38.97 ± 8.19			
LDL (mg/dL)	113.08 ± 30.29			
TG (mg/dL)	180.66 ± 122.63			

Table 4. Demographic and clinical features according to genotype distributions of *CAPN10* gene SNP-19 and SNP-44 and *NR3C1* gene N363S polymorphisms in female T2DM patients.

	CAPN10 SNP-19			
Female T2DM patient parameters	Del/Del (n = 32)	Ins/Del (n = 27)	Ins/Ins (n = 10)	P
Age (years)	55.31 ± 11.85	52.25 ± 10.91	56.80 ± 10.87	0.452
Weight (kg)	75.81 ± 10.31	72.00 ± 5.70	74.70 ± 7.28	0.221
BMI (kg/m^2)	29.60 ± 4.59	28.20 ± 1.96	29.33 ± 3.11	0.320
T2DM duration (months)	105.78 ± 57.64	93.85 ± 52.91	129.60 ± 96.96	0.310
HbA_{1c} (%)	7.18 ± 1.73	6.76 ± 1.28	7.23 ± 1.63	0.528
FBG (mg/dL)	140.09 ± 46.56	133.03 ± 48.01	153.10 ± 62.59	0.548
PBG (mg/dL)	163.53 ± 53.65	177.55 ± 73.13	185.00 ± 92.19	0.596
Total cholesterol (mg/dL)	193.09 ± 39.03	191.88 ± 42.88	203.50 ± 44.48	0.737
HDL (mg/dL)	44.31 ± 9.50	43.37 ± 10.00	45.30 ± 9.87	0.854
LDL (mg/dL)	113.21 ± 32.44	116.59 ± 35.74	122.00 ± 33.40	0.766
TG (mg/dL)	174.18 ± 80.47	163.11 ± 58.49	162.30 ± 77.31	0.810
	CAPN10 SNP-44			
Female T2DM patient parameters	TT (n = 39)	TC (n = 3)	CC (n = 20)	P
Age (years)	55.97 ± 11.90	54.66 ± 4.16	52.95 ± 11.04	0.632
Weight (kg)	75.20 ± 7.70	82.33 ± 13.79	72.10 ± 9.11	0.117
BMI (kg/m^2)	29.17 ± 3.25	31.52 ± 5.52	28.33 ± 4.05	0.335
T2DM duration (months)	107.74 ± 59.36	104.00 ± 45.43	99.60 ± 77.58	0.902
HbA_{1c} (%)	7.04 ± 1.54	7.23 ± 2.05	7.17 ± 1.45	0.943
FBG (mg/dL)	141.30 ± 52.74	101.33 ± 21.07	145.40 ± 49.68	0.381
PBG (mg/dL)	168.23 ± 67.00	138.33 ± 19.60	177.70 ± 79.04	0.647
Total cholesterol (mg/dL)	198.23 ± 36.84	153.66 ± 11.84	201.15 ± 48.24	0.166
HDL (mg/dL)	46.23 ± 9.68	47.00 ± 5.56	41.55 ± 8.83	0.180
LDL (mg/dL)	119.89 ± 31.17	72.00 ± 13.52	118.85 ± 39.12	0.064
TG (mg/dL)	160.84 ± 75.19	171.33 ± 37.09	193.55 ± 68.42	0.264
	NR3C1 N363S			
Female T2DM patient parameters		AA (n = 19)	AG (n = 38)	P
Age (years)		51.94 ± 11.17	56.13 ± 12.70	0.228
Weight (kg)		75.10 ± 11.60	73.86 ± 10.16	0.681
BMI (kg/m^2)		28.81 ± 4.77	28.85 ± 3.79	0.968
T2DM duration (months)		111.21 ± 76.53	108.34 ± 60.40	0.878
HbA_{1c} (%)		6.85 ± 1.36	7.19 ± 1.56	0.423
FBG (mg/dL)		140.78 ± 54.52	141.63 ± 51.70	0.955
PBG (mg/dL)		177.52 ± 59.03	167.94 ± 75.74	0.632
Total cholesterol (mg/dL)		196.63 ± 42.15	200.76 ± 43.08	0.732
HDL (mg/dL)		40.00 ± 5.96	46.00 ± 10.44	0.024
LDL (mg/dL)		117.21 ± 32.57	120.13 ± 38.04	0.776
TG (mg/dL)		174.31 ± 88.66	171.00 ± 66.33	0.875

Table 5. Demographic and clinical features according to genotype distributions of *CAPN10* gene SNP-19 and SNP-44 and *NR3C1* gene N363S polymorphisms in male T2DM patients.

	CAPN10 SNP-19			
Male T2DM patient parameters	Del/Del (n = 20)	Ins/Del (n = 18)	Ins/Ins (n = 8)	p
Age (years)	60.20 ± 9.18	55.55 ± 14.61	55.83 ± 9.66	0.390
Weight (kg)	77.25 ± 9.59	79.36 ± 6.76	77.72 ± 7.33	0.706
BMI (kg/m^2)	26.47 ± 2.41	27.36 ± 3.12	28.44 ± 3.56	0.149
T2DM duration (months)	133.15 ± 79.85	82.72 ± 50.46	136.66 ± 95.21	0.072
HbA_{1c} (%)	8.07 ± 2.19	7.45 ± 2.34	7.35 ± 1.54	0.508
FBG (mg/dL)	170.50 ± 39.70	155.33 ± 82.55	165.27 ± 67.40	0.769
PBG (mg/dL)	216.85 ± 39.70	176.72 ± 87.66	186.05 ± 76.19	0.265
Total cholesterol (mg/dL)	172.35 ± 38.09	187.44 ± 36.02	203.38 ± 50.68	0.084
HDL (mg/dL)	35.55 ± 7.97	38.44 ± 6.46	42.27 ± 10.60	0.060
LDL (mg/dL)	108.10 ± 29.97	107.38 ± 25.21	137.27 ± 78.72	0.130
TG (mg/dL)	143.00 ± 62.54	207.55 ± 163.11	251.66 ± 310.79	0.257
	CAPN10 SNP-44			
Male T2DM patient parameters	TT (n = 27)	TC (n = 5)	CC (n = 11)	P
Age (years)	58.85 ± 12.95	53.80 ± 13.02	57.27 ± 8.46	0.678
Weight (kg)	76.44 ± 7.55	89.20 ± 10.84	78.18 ± 4.49	0.004
BMI (kg/m^2)	26.59 ± 2.95	30.08 ± 3.39	26.50 ± 1.17	0.031
T2DM duration (months)	116.92 ± 68.75	127.40 ± 99.21	101.36 ± 50.36	0.737
HbA_{1c} (%)	8.07 ± 2.08	8.38 ± 2.83	6.71 ± 1.56	0.158
FBG (mg/dL)	164.51 ± 45.62	214.20 ± 124.46	142.45 ± 38.46	0.079
PBG (mg/dL)	188.37 ± 60.58	254.40 ± 90.25	169.72 ± 86.71	0.096
Total cholesterol (mg/dL)	179.74 ± 46.69	184.40 ± 35.96	188.81 ± 24.55	0.824
HDL (mg/dL)	35.62 ± 8.19	36.80 ± 9.41	40.36 ± 6.81	0.266
LDL (mg/dL)	102.66 ± 27.27	115.40 ± 26.23	112.36 ± 17.93	0.405
TG (mg/dL)	219.92 ± 25.20	158.60 ± 43.69	175.27 ± 96.24	0.742
	NR3C1 N363S			
Male T2DM patient parameters		AA (n = 17)	AG (n = 27)	P
Age (years)		56.76 ± 15.20	58.44 ± 11.27	0.676
Weight (kg)		77.05 ± 10.69	79.96 ± 5.50	0.241
BMI (kg/m^2)		27.13 ± 3.90	26.99 ± 1.85	0.875
T2DM duration (months)		84.11 ± 53.30	127.51 ± 71.84	0.038
HbA_{1c} (%)		7.84 ± 3.30	7.61 ± 1.92	0.734
FBG (mg/dL)		160.76 ± 46.14	167.77 ± 66.50	0.706
PBG (mg/dL)		196.05 ± 82.60	195.29 ± 77.64	0.975
Total cholesterol (mg/dL)		186.11 ± 38.97	179.44 ± 38.93	0.583
HDL (mg/dL)		37.82 ± 10.21	36.77 ± 7.80	0.703
LDL (mg/dL)		108.47 ± 27.75	104.14 ± 19.38	0.546
TG (mg/dL)		187.76 ± 154.76	202.18 ± 227.84	0.820

N363S polymorphisms and their association with T2DM risk, and their impact on demographic and clinical parameters in a Turkish population. Numerous publications have shown that N363S polymorphism, which is located in exon 2 of the *NR3C1* gene and expressed as asparagine-to-serine exchange in the transactivation domain of the glucocorticoid receptor, is associated with obesity in nondiabetic individuals. The association between N363S polymorphism and obesity has been demonstrated, initially reported with a higher BMI in G-allele carriers than in A-allele carriers in elderly Dutch individuals (4).

In contrast to studies reporting that N363S polymorphism is associated with obesity in nondiabetic individuals, we found that *NR3C1* gene N363S polymorphism was also associated with obesity in Turkish patients with T2DM. We observed that the N363S G-allele in Turkish T2DM patients was associated with increased weight, BMI, and HDL levels compared to the A-allele. This result of our study is consistent with the results of a study that found many atherosclerotic risk factors in Anglo-Celtic obesity patients without diabetes and hypertension, especially increased TC and TG concentrations, and high TC/HDL ratios are associated with N363S polymorphism (11). Likewise, in the present study, we found that HDL was positively associated with N363S polymorphism of the *NR3C1* gene, especially in Turkish female T2DM patients. Therefore, the relationship between N363S variation and HDL levels should be explored in greater detail.

A correlation was demonstrated between *CAPN10* gene SNP-19 and SNP-44 and *NR3C1* N363S polymorphisms and T2DM development. The *CAPN10* and *NR3C1* genes are fundamentally important in future research on genetic profiles. Further studies have to be supported to determine SNP molecular effects with more examples of analyzed linkage for possible effects of T2DM pathogenicity. Further studies are required with increased numbers of samples in a wider population.

Acknowledgments

We thank the patients and the nurses for their participation in this study. This study was funded by Gazi University Project of Scientific Investigation 05/2013-12.

References

1. Olefsky JM. Prospects for research in diabetes mellitus. J Am Med Assoc 2000; 285: 628-632.
2. Goll DE, Thompson VF, Li H, Wei W, Cong J. The calpain system. Physiol Rev 2003; 83: 731-801.
3. Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PEH et al. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet 2000; 26: 163-175.
4. Huizenga NA, Koper JW, De Lange P, Pols HAP, Stolk RP, Burger H, Grobbee DE, Brinkmann AO, De Jong FH, Lamberts SWJ. A polymorphism in the glucocorticoid receptor gene may be associated with an increased sensitivity to glucocorticoids in vivo. J Clin Endocrinol Metab 1998; 83: 144-151.
5. Rasmussen SK, Urhammer SA, Berglund L, Jensen JN, Hansen L, Echwald SM, Borch-Johnsen K, Horikawa Y, Mashima H, Lithell H et al. Variants within the calpain-10 gene on chromosome 2q37 (NIDDN1) and relationships to type 2 diabetes, insulin resistance, and impaired acute insulin secretion among Scandinavian Caucasians. Diabetes 2002; 51: 3561-3568.
6. Parra EJ, Hoggart CJ, Bonilla C, Dios S, Norris JM, Marshall JA, Hamman RF, Ferrell RE, McKeigue PM, Shriver MD. Relation of type 2 diabetes to individual admixture and candidate gene polymorphisms in the Hispanic American population of San LuisValley, Colorado. J Med Genet 2004; 41: 1-9.
7. Zaharna MM, Abed AA, Sharif FA. Calpain-10 gene polymorphism in type 2 diabetes mellitus patients in the Gaza Strip. Med Pric Proct 2010; 19: 457-462.
8. Ezzidi I, Turki A, Messaoudi S, Chaieb M, Kacem M, Al-Khateeb GM, Mahjoub T, Almawi WY, Mtiraoui N. Common polymorphisms of calpain-10 and the risk of type 2 diabetes in a Tunisian Arab population: a case control study. BMC Genet 2010; 11: 75.
9. Evans JC, Frayling TM, Cassell PG, Saker PJ, Hitman GA, Walker M, Levy JC, O'Rahilly S, Rao PVS, Bennett AJ et al. Studies of association between the gene for calpain-10 and type 2 diabetes mellitus in the United Kingdom. Am J Hum Genet 2001; 69: 554-552.
10. Arslan E, Acik L, Gunaltili G, Ayvaz G, Altinova AE, Arslan M. The effect of calpain-10 gene polymorphism on the development of type 2 diabetes mellitus in a Turkish population. Endokrynologia Polska 2014; 65: 90-95.
11. Lin RCY, Wang XL, Dalziel B, Caterson ID, Morris BJ. Association of obesity, but not diabetes or hypertension, with glucocorticoid receptor N363S variant. Obes Res 2003; 11: 802-808.