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RESEARCH ARTICLE

ASSOCIATION OF SOME CALPAIN-10 GENE POLYMORPHISMS WITH THE INCIDENCE OF TYPE 2 DIABETES MELLITUS IN IRAQ

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ABSTRACT

Calpain10 is a member of a large family of intracellular proteases. It was the first candidate gene for Type 2 diabetes mellitus (T2DM), earlier studies on Mexican-Americans and other populations have shown that polymorphisms, SNP-43, del/ins-19 and SNP-63, of this ubiquitously expressed protein influence susceptibility to T2DM. Polymerase chain reaction (PCR) (for DEL/INS-19) and Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (for SNP-43 and SNP-63) were used to detect the calpain10 variants by using specific primers and restriction enzymes.Enrichment of allele 1(2R) in Del/Ins-19 and 2R/2R genotype were found in T2DM patients. While the alleles and genotypes distribution of SNP-43 and SNP-63 were not significantly different between patient groups and non-diabetic control subjects. The genotype AA in SNP-43 and genotype TT in SNP-63 were not found neither in T2DM nor in control subjects. Of the eight haplotypes detected, enrichment of haplotype 112 defined by variants of, SNP-43, Del/Ins-19, and SNP-63 was seen in patients. The distribution of the other haplotypes was comparable between patients and control subjects. The calpain10 haplotype combinations were also obtained, and the haplotype combinations 111/111 and 111 / 112; created by SNP-43, del/ins-19 and SNP-63 was associated with increasing the risk of T2DM.

INTRODUCTION

Diabetes mellitus is one of the most common metabolic diseases and a health-care challenge of the twenty-first century (Rathmann *et al*., 2003). Globally, the Middle-East is highly affected by T2DM with a prevalence rate varying between 7 and 22% (Mansour *et al*., 2008).Type 2 diabetes mellitus (T2DM) is a multi-factorial metabolic syndrome, characterized by high blood glucose level, mainly caused by insulin resistance and relative insulin deficiency (Bonnefond *et al*., 2010).The two main reasons underlying the pathophysiology of T2DM are impaired insulin secretion due to the dysfunction of pancreatic beta cells and impaired insulin action because of insulin resistance (Kumar and Clark, 2007). Of the diabetes-related genes, fine mapping and positional cloning suggested that the calpain10 (*CAPN10*) gene might serve as an important T2DM susceptibility gene (Cox *et al*., 2004; Weedon *et al*., 2003). Also, several case control and association studies indicated that polymorphisms in *CAPN10* are associated with the development of T2DM and insulin resistance (Horikawa *et al*., 2000; Carlsson *et al*., 2005). Several single nucleotide polymorphisms (SNP) of *CAPN10* are known to increase the risk of type 2 diabetes in many populations. These variants include: SNP-43 (an A/G transition within intron-3), del/ins-19 (two or three repeats of a 32 bp sequence within intron-6), and SNP-63 (a C/T transition within intron-13) (Evans *et al*., 2001; Cassell *et al*., 2002). Intronic variation incalpain10 gene which located on chromosome 2q3,has been associated with type 2 diabetes in Mexican-Americans, Finns, Germans, British, (Horikawa *et al*., 2000; Evans *et al*., 2001), Tunisia (Ezzidi *et al*., 2010), and Europeans (Tsuchiya *et al*., 2006).

Although *CAPN10* variation was not associated with type 2 diabetes in Samson (Tasi *et al*., 2001) and there was no significant difference in genotype distribution betweentype 2 diabetic patients and controls in Polish and Japanese (Malecki *et al*., 2002; Horikawa *et al*., 2003).However, 112/121 haplotype combination of SNP-43,del/ins-19 and SNP-63 is associated with Type 2 Diabetes Mellitus in Mexican-American, German, Gaza Strips populations (Zahran *et al*., 2010) and it appears to increase type 2 Diabetes Mellitus in Samoans also (Tasi *et al*., 2001). But 112/121 haplotype was less common in UK population and was less risk to Type 2 Diabetes Mellitus (Evans *et al*., 2001) and 112/121 haplotype was not associated with type 2 Diabetes Mellitus in Tunisian of Arab descent population (Ezzidi *et al*., 2010) and Polish population (Malecki *et al*., 2002). A well-established study shows that; haplotype combinations, 111/121 susceptible in Koreans and Northern Europeans, 112/221 susceptible in Chinese and 121/121 in Pan-European and Scandinavians. Moreover, these associations to Type 2 Diabetes Mellitus is in

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contrast to Tunisian (South East Tunisia) populations where 121/221 diplotype is with increased risk of Type 2 Diabetes Mellitus (Ezzidi *et al*., 2010). In Samoans of the western pacific, the most common haplotypes are 121, 111, and 112 (Tasi *et al*., 2001). In addition, 121 haplotype were more prevalent in Type 2 Diabetes Mellitus in Polish Population and 121/121 combination is associated with an increased risk of Type 2 Diabetes Mellitus in Polish Population, however the increased risk of Type 2 Diabetes Mellitus in Polish population is only in the presence of111 haplotype (Malecki *et al.* 2002). The analysis of the pooled data sets showed that SNP-43, del/ins-19 and SNP-63 haplogenotypes were associated with type 2 Diabetes Mellitus in Europeans (Tsuchiya *et al*., 2006). The association of the 121/121 haplogenotype with type 2 Diabetes Mellitus was also observed by meta-analysis and the SNP-43 G allele and the 121 and 221 haplotypes showed significant association with type 2 Diabetes Mellitus in both the pooled and meta-analyses. The rare 112 haplotype reached significance in only the pooled analysis (Tsuchiya *et al*., 2006). Finally, the haplogenotypes 112/121 and 121/121 were associated with significantly increased risk and 111/221 with reduced risk in the pooled analysis (Tsuchiya *et al*., 2006). There is no available data in Iraq about this subject and there is a great need to know the variants in this gene which are related to this disease in Iraq, this study aims to assess calpain10 gene polymorphisms in Iraqi patients with type 2 diabetes mellitus.

MATERIALS AND METHODS

Subjects

The study population consisted of 50 subjects with type 2 diabetes mellitus and 50 with normal fasting blood glucose (80–110 mg/dl). The number of males and females in the patient group are 24 and 26, respectively, while in the control group their respective numbers were 31 and 19. The mean age in patients was 53.7 years while in the control group the mean age was 37.86 years. The numbers of smokers were 34 and 40 % in T2DM patients and control subjects, respectively. The mean for the duration of disease in the patients was 5.89 years.

DNA extraction

DNA was extracted from whole blood samples by using Geneaid DNA Extraction kit (bioneer, korea), according to the manufacturer's instructions. Subsequently, the quality of DNA was assessed by agarose gel electrophoresis using 1% agarose gel stained with ethidium bromide. The purity and concentration of DNA was estimated using Nanodrop at 260 and 280 nm. The DNA sample showing the OD 260:280 nm value of 1.60 to 1.90 was considered as good quality.

Genotyping

We genotyped three polymorphisms in calpain10 gene and they are SNP-43 (A/G), Del/Ins-19 (2 repeats of 32 bp sequence / 3 repeats of 32 bp sequence), and SNP-63 (C/T) in both patients and control group samples, and we estimated the odds ratio (OR) and their 95% confidence intervals (CIs) of the alleles study genotypes, haplotypes and haplotype combinations.

SNP-43

The PCR restriction fragment length polymorphism (PCR-RFLP) method was used for SNP-43. It was amplified with primers: forward primer 5` -GCTGGCTGGTGACATCAG TGC- 3` and reverse primer, 5` -ACCAAGTCAAGGCT TAGCCTCACCTTCATA- 3`(Carlsson*et al.*, 2004).PCR was performed in 25 μl of reaction mixture containing 5 μl of genomic DNA, 12.5 μl of master mix, 0.75 μl of each primer and 6 μl of distilled water to make a final volume. Amplification was performed on thermal cycler using 0.2 ml reaction tubes. The PCR program consisted of 5 min denaturation at 95°C, followed by 30 cycles of denaturation (94°C, 30 s), annealing (60°C, 30s and primer extension (72°C, 30 s). The final cycle was followed by extension at 72°C for 10 min. PCR products were digested with *Nde*I restriction enzyme according to the suppliers instructions. Briefly, enzyme-buffer mix was prepared by mixing 1 μl of restriction enzyme with 2 μl of the respective buffer. Reaction mix was prepared by mixing 15 μl PCR products with 3 μl of enzyme buffer mix. Volume was made up to 20 μl with free nuclease distilled water and incubated for 16 hours at 37C. The digested products were separated on 3.0 % agarose gel and were visualized by ethidium bromide staining.

DEL/INS-19

This deletion/insertion polymorphism was amplified by PCR, primers used: forward primer, 5`-GTTTGGTTCTCTTCAGCGTGGAG- 3`and reverse primer, 5` - CATGAACCCTGGCAGGGTCTAAG- 3`(Evans *et al.*, 2001). The PCR products were separated on 2.5% agarose gel and were visualized by ethidium bromide staining.

SNP-63

The PCR restriction fragment length polymorphism (PCR-RFLP) method was used for SNP-63. It was amplified with primers: forward primer 5`-AAGGGGGGCCAGGGCCTGACGGGGGTGGCG- 3` and reverse primer, 5` -AGCACTCCCAGCTCCTGATC- 3` (Evans *et al.*, 2001).PCR was performed in 25 μl of reaction mixture containing 5 μl of genomic DNA, 12.5 μl of master mix, 1 μl of each primer and 5.5 μl of distilled water to make a final volume. Amplification was performed on thermal cycler using 0.2 ml reaction tubes. The PCR program consisted of 5 min denaturation at 96°C, followed by 30 cycles of denaturation (94°C, 30 s), annealing (62°C, 30s and primer extension (72°C, 30 s). The final cycle was followed by extension at 72°C for 10 min. PCR products were digested with *HhaI* restriction enzyme according to the suppliers instructions. Briefly, enzyme-buffer mix was prepared by mixing 1 μl of restriction enzyme with 2 μl of the respective buffer and 1μl of bovine serum albumin (BSA). Reaction mix was prepared by mixing 10 μl PCR products with 4 μl of enzyme buffer mix. Volume was made up to 20 μl with free nuclease distilled water and incubated for 2 hours at 37C. The digested products were separated on 2.5 % agarose gel and were visualized by ethidium bromide staining.

RESULTS AND DISCUSSION

PCR and RFLP Results

SNP-43 in calpain10 gene was detected as 254bp band (Figure 1). In order to diagnose the alleles of SNP-43, PCR products

were subjected to *Ndel* restriction enzymes. After digestion, allele 1 (A) was detected as a 223 bp and 31 bp which not appear in the figure (Figure 2) and allele 2 (G) was detected as a 254 bp band.

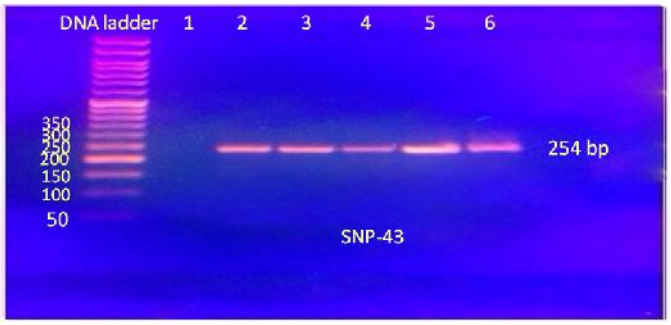

Fig. 1. A photograph of ethidium bromide stained 2.5% agarose gel showing the PCR product of SNP-643. M= 50 bp DNA ladder, lane 1 is negative control; lanes 2 – 6 show the PCR product of SNP-43 (254 bp).

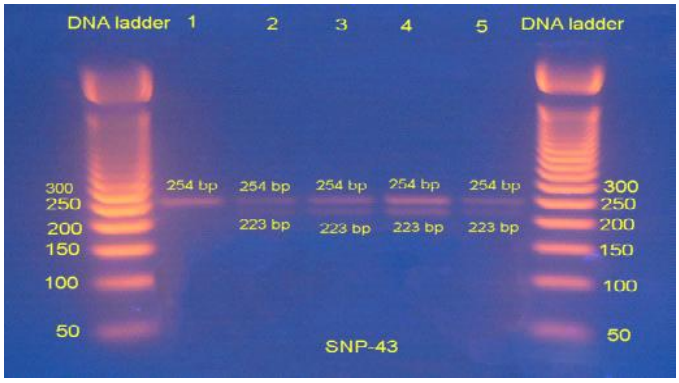

Fig. 2. A photograph of PCR-RFLP products of SNP-43 run on ethidium bromide stained 4% agarose gel. M= 50 bp DNA ladder; lanes 2, 3, 4 & 5 show digested and undigested products (heterozygous), lane 1 show undigested products (homozygous).

The detection of del/ins-19 in calpain10 gene was achieved by using one pair of primers (forward and reverse). Allele 1 (2 repeats of 32 bp sequence) was detected as a 155 bp band, and allele 2 (3 repeats of 32 was detected as a 187 bp band as noted in (Figure 3).

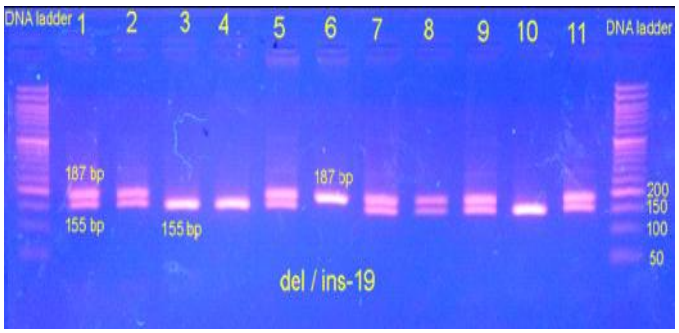

Fig. 3. A photograph ofethidiumbromide stained 3%agarosegel showing the PCRproduct of del/ins-19. M= 50bpDNA ladder, lane 6 show homozygous samples for allele 2 (3 repeats of 32 bp); lanes 3, 4 & 10 show homozygous samples for allele 1 (2 repeats of 32 bp); lanes 1, 2, 5, 7, 8, 9 & 11 show heterozygous samples (2 repeats32 bp/ 3 repeats 32 bp).

SNP-63 incalpain 10 gene was detected as 192 bp band (Figure 4). In order to diagnose the alleles of SNP-63, PCR products were subjected to *Hhal* restriction enzymes. After digestion, allele 1 (C) was detected as a 162 bp band and allele 2 (T) was detected as a 192 bp band (Figure 5).

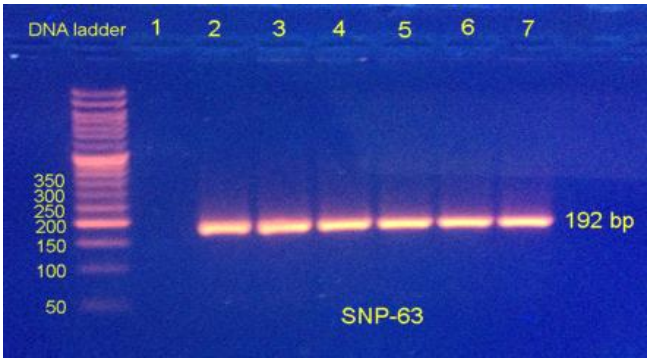

Fig. 4. A photograph of ethidium bromide stained 3% agarose gel showing the PCR product of SNP-63. M= 50 bp DNA ladder, lane 1 is negative control; lanes 2 – 7 show the PCR product of SNP-63 (192 bp).

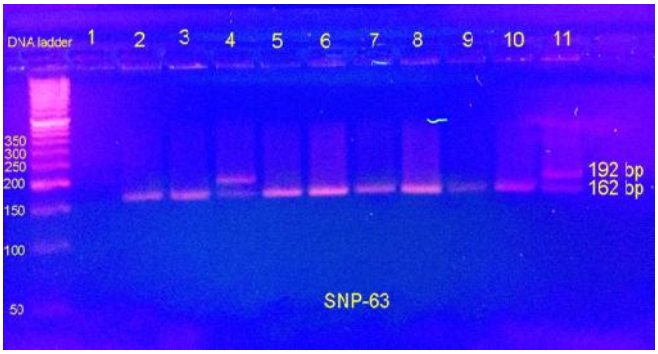

Fig. 5. A photograph of PCR-RFLP products of SNP-63 run on ethidium bromide stained 4% agarose gel. M= 50 bp DNA ladder; lane 1 is negative control ; lanes 2, 3 ,5 ,6, 7, 8, 9 & 10 show digested products (homozygous); lanes 4 & 11 show digested and undigested products (heterozygous).

Genotype and allele frequency of calpain-10 SNPs- 43, -63, and Del/Ins-19

The allele distribution of calpain10 SNP-43, del/ins-19 and SNP-63 were examined in the T2DM patients and control subjects (Table 1). The frequency of allele 1 (A) at SNP-43 was 88% in T2DM and 91% in controls (p=0.94) , also, the frequency of allele 1 (C) at SNP-63 was 77% in T2DM and 76% in controls (p=0.98). In del/ins-19, the allele frequency of the allele 1 (2 repeats of 32 bp sequence) was significantly higher in subjects with diabetes than in control subjects (51 *versus* 38%, p=0.04). The 2X2 contingency chi square test was used to assess the allele frequency comparisons.

Table 1. Allele distribution of calpain 10 SNPs-43, -63and del/ins-19

SNPs	Groups	Alleles		p- value
		1	2	
SNP-43	Control	0.91	0.09	0.94
	T2DM	0.88	0.12	
Del/ins-19	Control	0.38	0.62	0.04 *
	T2DM	0.51	0.49	
SNP-63	Control	0.76	0.24	0.98
	T2DM	0.77	0.23	

SNP-43: allele 1, (A); allele 2, (G) – del/ins-19: allele 1, 2 repeats of 32 bp sequence; allele 2, 3 repeats of 32 bp sequence – SNP-63: allele 1, (C) ; allele, (T).

The genotype distribution of calpain10 SNP-43, del/ins-19 and SNP-63 were examined in the T2DM patients and control subjects (Table 2). There was no significant difference in genotype distribution for all SNPs except del/ins-19. For SNP-43, the frequency of the AA was 76% in T2DM *versus* 82% in control subjects; and the AG was 24% *versus* 18%, while the genotype GG was not found neither in T2DM nor control subjects. For SNP-63, the frequency of the CC was 34% in T2DM *versus* 32% in control subjects and the CT was 66% in T2DM *versus* 68% in control subjects, while, the genotype TT was not found neither in T2DM nor in control subjects. For del/ins-19, the frequency of the homozygous " 2 repeats of 32 bp sequence" was significantly higher in subjects with T2DM than in control subjects (26 % *versus* 0 %, p=0.01), and that of the heterozugous (2 repeats / 3 repeats) was significantly higher in control than in T2DM subjects (76% *versus* 50 %, p=0.04), while the homozygous for (3 repeats of 32 bp sequence) was 24% in both T2DM and control subjects. The 2X3 contingency Chi square tests were used to assess the genotype frequency comparisons. Tests for genetic homogeneity in the allele frequencies of SNP-43, SNP-19 and SNP-63 between the American Samoan and Samoan control subjects showed no significant difference in the allele frequencies at each of the three polymorphic sites between T2DM and control groups (Tsai *et al.*, 2001).

Table 2. Genotype distribution of calpain 10 SNPs -43, -63 and –del/ins-19

SNPs	Genotypes	Groups		p- value
		Control	T2DM	
SNP-43	11	0.82	0.76	0.09
	12	0.18	0.24	0.09
	22	0.00	0.00	1.00
Del/ins-19	11	0.00	0.26	0.01 **
	12	0.76	0.50	0.04 *
	22	0.24	0.24	1.00
SNP-63	11	0.32	0.34	0.97
	12	0.68	0.66	0.97
	22	0.00	0.00	1.00

SNP-43: allele 1, (A); allele 2, (G) – del/ins-19: allele 1, 2 repeats of 32 bp sequence; allele 2, 3 repeats of 32 bp sequence – SNP-63: allele 1, (C) ; allele, (T).

In Poland, Malecki *et al.* (2002) found that both T2DM and control subjects had similar distribution of alleles and genotypes created by variants of SNP-43, SNP-19 and SNP-63. Also, Fingerlin *et al*. (2002) genotyped SNPs -43, -56 and -63 in Finnish subjects with T2DM and control subjects and they found no significant differences in allele and genotype frequencies. Horikawa *et al*. (2003) genotyped three SNPs (SNP-43, and SNP-63) and del/ins-19 in calpain 10 gene and they found that their frequencies are similar in T2DM and controls, suggesting that these genetic variations are not a major factor in the occurrence of T2DM in Japanese. The results of del/ins-19 in this study are disagree with the results of Ezzidi *et al*. (2010) in Tunisia as related with 2R/3R (12) and 3R/3R (22) genotypes which was 53% *versus* 50% for 2R/3R in T2DM and control subjects, respectively, while that of 3R/3R was 27% *versus* 34% in T2DM *versus* control subjects, respectively. As related with 2R/2R genotype, the results of this study are in accordance with the results of Ezzidi *et al*. (2010) who found that percentage of this genotype is 20% in T2DM *versus* 16% in control subjects. In the eastern Indian population, Adak *et al*. (2010) genotyped three calpain 10 variants (SNP-43, del/ins-19 and SNP-63) and they found that the distribution of allele frequencies for these loci did not significantly deviate from Hardy-Weinberg equilibrium for both the diabetic and control groups. In addition, they found no significant differences in genotype frequencies for SNP-43 and SNP-19 between T2DM and non-diabetic controls, whereas, they found a difference in the distribution of genotype frequencies for SNP-63 between T2DM and control subjects, namely, the minor T-allele of SNP-63 was found to present a risk of 3.74 for T2DM. In the southern Idian population, Bodhini*et al*. (2011) found no significant difference in the genotypic distribution of SNP-43, del/ins-19 and SNP-63 among the T2DM and control subjects.

Haplotypes of calpain 10 SNP-43, Del/Ins-19 and SNP-63

The frequencies of haplotypes defined by variants of SNP-43, del/ins-19 and SNP-63 in T2DM patients and controls are shown in (Table 3). A total of six haplotypes were observed in both patients and control groups. The three most common haplotypes are 111, 121 and 122 in T2DM patients which were 42, 21 and 16 %, also, were 38, 25 and 28 % in control subjects, respectively. The haplotype 122 was present in a higher ($p < 0.05$) proportion in control subjects compared to T2DM (OR= 1.37, CI = 0.802) which was 28 *versus* 16 %.
The frequency of 112 haplotype (OR=1.25, CI=0.773) varied significantly between patients and controls. That, the haplotype 112 was absent in the control subjects.

Table 3. Distribution of haplotypes defined by variants of SNP-43, del/ins-19 and SNP-63 inT2DM patients and control group

Haplotypes 43-19-63	T2DM n = 50	Control n = 50	p-value	OR	CI
111	0.42	0.38	0.87 NS	0.08	0.022
112	0.09	0.00	0.04 *	1.25	0.773
121	0.21	0.25	0.87 NS	0.07	0.035
122	0.16	0.28	0.04 *	1.37	0.802
221	0.04	0.03	0.93 NS	0.07	0.005
222	0.08	0.06	0.95 NS	0.06	0.003

An increased risk of diabetes was detected for haplotype 112 in this study . The effect of 112 haplotype probably reflects the contribution of the T- allele of SNP-63 for T2DM patients (Adak *et al*., 2010). Haplotype 112 has been detected as a risk haplotype in many other studies (Horikawa *et al*., 2000; Cassell *et al*., 2002; Adak *et al*, 2010).

Haplotype combinations of calpain 10 SNP-43, Del/Ins-19 and SNP-63

The frequencies of haplotype combinations defined by variants of SNP-43, del/ins-19 and SNP-63 in T2DM patients and controls are shown in (Table 4). A total of ten haplotypes were deserved in both patients and control groups. The frequencies of 111/111 and 111/112 haplotype combinations were significantly higher in T2DM patients in compared with control subjects (8 *versus* 0% and 18 versus 0%, respectively). Also, the haplotype combinations 111/111 and 111/112 were absent in control subjects. Both 111/111 and 111/112 haplotype combinations are associated with increased risk of T2DM in this study.These results are in agreement with the results of

Evans *et al*. (2001) who observed that 111/111 haplotype combination created by SNP-43, del/ins-19 and SNP-63 was associated with increased risk of type 2 diabetes in whites of British/Irish ancestry. In addition, Horikawa *et al*. (2003) indicate that the 111/111 haplotype combination defined by SNP-43, del/ins-19 and SNP-63 polymorphisms was found only in the diabetic patients.As related with 111/112 haplotype combination in this study, the results are in agreement with the results of Adak *et al*. (2010) who detected a combination of haplotype 111/112 conveying in a higher risk to T2DM in eastern India.The haplotype combination 111/112, created by SNP-43, del/ins-19 and SNP-63 was in control group significantly (P<0.05) higher than in T2DM group (22 *versus* 14%, respectively; OR=0.70, CI=0.044). These results are disagree with the results of Kang *et al*. (2006) who found that 111/112 diplotype in calpain10 gene is associated T2DM in Korean population. The 111/122 haplotype combination was significantly higher in control subjects than in T2DM patients (40 *versus* 26%, respectively; OR=2.082, CI=0.833). A 66% of Iraqi subjects studied here in (both control and patient groups) have the haplotype combination 111/122, while this haplotype combination is not found in Samson (Tsai *et al*., 2001), Finnish Cohort (Fingerlin *et al*., 2002), Polish (Malecki *et al*., 2002), Japanese (Horikawa *et al*., 2003), Mexican (Bosque-Plata *et al*., 2004), eastern Indian (Adak *et al*., 2004) and Tunisian (Ezzidi *et al*., 2010).

Table 4. Distribution of haplotypes combinations defined by variants of SNP-43, del/ins-19 and SNP-63 in T2DM patients and control group

Haplotypes combinations	T2DM n = 50	Control n = 50	p-value		OR	CI
111 / 111	0.08	0.00	0.05	*	0.69	0.056
111 / 112	0.18	0.00	0.01	**	1.59	0.272
111 / 121	0.14	0.22	0.05	*	0.70	0.044
111 / 122	0.26	0.40	0.03	*	2.082	0.833
111 / 221	0.02	0.04	0.95	NS	0.03	0.002
111 / 222	0.08	0.10	0.95	NS	0.06	0.003
121 / 121	0.04	0.04	1.00	NS	0.00	0.000
121 / 122	0.06	0.16	0.04	*	1.89	0.732
121 / 221	0.06	0.02	0.87	NS	0.06	0.028
121 / 222	0.08	0.02	0.45	NS	0.03	0.002

In this study there was no significant differences between T2DM and control groups as related with 111/221 haplotype combination, while, studies of Utah Caucasians revealed that the 111/221 genotype showed the highest increased risk of T2DM in this population (Elbein *et al*., 2002). Moreover, there was no significant differences between T2DM and control groups as related with 111/222 haplotype combination. The results of this study found that the homozygotic 121/121 combination of three point haplotypes created by SNP-43, del/ins-19 and SNP-63 is not associated with an increased risk of T2DM in Iraqi patients. Malecki *et al*. (2002) suggest the association of calpain10 121/121 haplotype combination created by SNP-43, del/ins-19 and SNP-63 with T2DM in a Polish population. Tsuchiya *et al*., (2006) indicate to the role of the 121/121 haplotype combination created by calpain10 SNP-43, del/ins-19 and SNP-63 in the susceptibility to T2DM. The results of this study are not able to confirm the association between calpain10 121/121 homozygous combination was significantly higher in control subjects than T2DM patients (16 *versus* 6%, P=0.04, OR=1.89, CI=0.732). There is no significant differences between T2DM and control groups as related with 121/221 haplotype combination in this study, while, Kifagi *et al*. (2008) found an association of 121/221 diolotype with increased risk of T2DM among Tunisian (south of Tunisia). Whereas Ezzidi *et al*. (2010) did not identify specific T2DM at-risk calpain10 haplotype combination in Tunisian population. There is no significant differences between T2DM and control groups as related with 121/222 haplotype combination.

Many haplotype combinations in this study (111/122, 111/222, 121/122 and 121/222) were not found in Samson (Tsai *et al*., 2001), Finnish Cohort (Fingerlin *et al*., 2002), Polish (Malecki *et al*., 2002), Japanese (Horikawa*et al*., 2003), Mexican (Bosque-Plata *et al*., 2004), eastern Indian (Adak *et al*., 2004) and Tunisian (Ezzidi *et al*., 2010). The haplotype combination 112/121 was not found in Iraqi subjects studied here. Whereas this haplotype combination was observed in many studies as a risk factor of T2DM. Horikawa *et al*. (2000) linking specific calpain10 at-risk haplotype combination (112/121) defined by SNP-43, del/ins-19 and SNP-63, with higher risk of T2DM in Mexican-Americans and Europeans. Malecki *et al*. (2002) were not able to confirm the role of the heterozygous 112/121 haplotype combination in susceptibility to T2DM. Horikawa *et al*. (2000) showed that the 112/121 haplotype combination does not represent increased risk of type 2 diabetes in the Japanese population. A similar study of Samson also revealed no significant association between the 112/121 haplotype combination and T2DM (Tasi *et al*., 2001). In the eastern Indian population, Adak *et al*. (2010) could not detect any association of 112/121 haplotype combination with T2DM, also, they found that 112/121 haplotype combination did not differ significantly between T2DM and control groups.

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