Association of Calpain-10 gene (rs2975760 and rs3792267) Polymorphism with Type 2 Diabetes Mellitus in the Iraqi Population

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Abstract

Background: Type II Diabetes Mellitus (T2DM) can be considered as the main diabetes' type, which is present in all populations worldwide and all regions. Calpain-10 is a part of a vast intracellular protease family. CAPN10 gene polymorphisms have been related to complex types of T2DM.

Objective: the major goal of the presented work is evaluating the relation regarding CAPN10 gene polymorphisms (SNP44 rs2975760 and SNP43 rs3792267) with T2DM in the Iraqi population as well as changes in serum lipid concentration and insulin concentration.

Materials and Methods: Two groups of persons were recruited, 300 patients with type2 diabetes mellitus, and 300 healthy control individuals. Fasting serum glucose and serum lipid concentrations have been evaluated via standard enzymatic approaches, while the concentrations of serum insulin were evaluated via ELISA assay. Genotyping of rs2975760 and rs3792267 SNPs is conducted via PCR-RFLP.

Results: Those of the SNP-44 showed that patients of heterozygous genotype (TC) decreased significantly with respect to the control group. Patients with the homozygous genotype (CC) elevated insignificantly relative to the control group. The minor allele C frequency in patients (12%) is decreased considerably in the patients' group relative to the group of controls (15%). The genotype results of SNP-43 illustrated that patients of heterozygous (GA) genotypes decreased significantly with respect to the control group. Patients of the homozygous genotype (AA) appeared to be insignificantly higher than the controls. The minor allele A frequency in patients (11.5%) is decreased considerably in the patients' group relative to the group of controls (27%). Serum lipid concentrations, insulin, and insulin resistance are distributed in groups of various genotypes of the 2 SNPs deferentially.

Conclusion: SNP-44 and SNP-43 in Iraqi individuals are protective against the development of T2DM. They were implicated in serum lipid changes, insulin and insulin resistance values.

Keywords: *Polymorphism; ELISA; Insulin resistance; RFLP-PCR; CAPN10*

Introduction

T2DM is the major common diabetes form, responsible for approximately 90% of cases in many developed countries and it affects between 10% and 20% of people with age more than 45 [1], T2DM indicates a person with physiological resistance to insulin effect in the peripheral tissues. Above all, insulin generated via the body isn't functional physiologically [2]. There is diabetes in every population in the world and every country, including rural areas of countries with low and middle income. It is considered a global epidemic.

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DM's prevalence has increased globally, with lifestyles changing and obesity growing [3].

Two metabolic disorders have contributed to T2DM: reduced peripheral tissue response to insulin (insulin resistance) as well as incomplete insulin secretion due to β-cell dysfunction [4]. The causes of T2DM are environmental factors with or maybe medical consequences of genetic factors. It is known that more than 36 genes are attributed to the occurrence of DM. They could be involved in about 10 percent of the disease's likelihood of occurrence. Some of these genes in β-cell activity are complicated and might involve insulin genetic defect mutation, hepatocyte nuclear transcription factor-1 alpha, glucokinase enzyme, hepatocyte nuclear transcription factor-4 alpha mutation, and insulin promoter factor [5].

Calpains can be specified as strongly preserved non lysosomal, calcium dependent cysteine protease superfamily, thus as a minimum of fourteen calpain family members were predicted, while their biology and chemistry were thoroughly examined [6]. In vitro data in terms of the inhibition regarding calpain in adipocytes and skeletal muscle cells which lead to decreased glycogen synthesis and decreased insulin-stimulated glucose uptake, indicating that calpains have a role in glucose transport [7]. In addition, CAPN10 can be considered as the first gene with regard to the T2DM sensitivity being identified via genome scan, along with polymorphisms related to altered CAPN10 expression. Extreme CAPN10 mRNA expression occurs in human heart, succeeded by kidney, brain, pancreas and liver [8], located on the chromosome 2q37.3, also includes fifteen exons spanning 31 kb that encode an intracellular protease of 672 amino acids. A lot of case control, as well as interaction researches, indicated that the expression of T2DM and insulin resistance are associated with polymorphisms in CAPN10 [9].

In the production of T2DM, genetic variants such as SNP44 rs2975760 and SNP43 rs3792267, majorly found in intron 3 of CAPN 10 gene, is involved[10]. Allelic frequency differences between cases and controls were shown by SNP44 and SNP43, however, a G>A transition in SNP-43 was correlated with evidence of linkage in the Mexican-American type 2 DM region. G allele frequency is increased relative to controls has been observed in patients[11]. In Mexican Americans, a strong association was identified between the SNP-44 T>C genotype CAPN10 gene and T2DM [12], also in British/ Irish people [13].

Due to a lack of research focusing on this issue, Calpain 10 gene polymorphism, however, is still unclear in Iraqi society. In the current research, the relationship between rs2975760 and rs3792267 SNPs with type 2 diabetes mellitus was explored for gaining insights into genetic history related to the disease in our culture.

Materials and Methods

Subjects

Case control study is used on 600 participants, they have been divided into 2 groups, healthy control group (300) along with group of patients experiencing T2DM (300). The period of the study was from August 2019 till April 2020. The study is conducted in the Postgraduate Laboratory Department of Biochemistry/University of Kufa/Faculty of Medicine. Iraq. The patient group comprised of 300 patients with, T2DM. They were selected from the Diabetes Center in Teaching Hospital (AL-Sadder) in Al Najaf Al-Ashraf, province. They have been observed and recognized by specialist physicians for the measures of inclusion. Patients with heart failure, cardiomyopathy or congenital, heart disease, autoimmune disease, and cancer are excluded.

The control group contained 300 volunteers. They were selected from relatives, friends, and medical staff. Any participant who had a disease for example cardiovascular disease, hypertension, heart disease, the renal disease had been unconcerned from the current study.

Biochemical parameters

Five milliliters of the blood sample were taken from each participant by peripheral vein puncture after overnight fasting. We separated the blood into 2 parts. Part one confined 3ml of blood was placed in a plain tube and left for about15min at 37°C for coagulation then centrifuged for10-15min at 2000 xg. The sera obtained was separated into 3 parts and then stored under -20°C for estimation of fasting serum glucose (FSG),

insulin, and lipid profiles (Low-density lipoprotein cholesterol (LDL-C), total cholesterol TC, very-low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG)). Determination of fasting serum glucose is done by the enzymatic method[14]. The enzyme-linked immune sorbent assay (ELISA) is used for the estimation of serum insulin. For the purpose of calculating insulin resistance, a homeostatic model assessment (HOMA) approach was utilized by means of the next formula:

HOMA= [glucose (in mmole/L) × insulin (in microU/mL)/22.5 [15]. The concentrations of HDL-C, total cholesterol, and triglyceride TG have been evaluated via standard enzymatic methods [16]. The concentrations of VLDL-C and LDL-C are determined indirectly through Friedewald approach [17].

Genotyping of polymorphism

Two milliliters of blood that mixed with EDTA in a tube was used for DNA extraction, using a kit of ReliaPrepTM Blood gDNA [18]. In addition, CAPN10 amplification for SNP44 T/C rs2975760 was carried as described by Evans et al[19]. Forward primer was 5'-GCAGGGCGCTCACGCTTGCCG-3' andthereverse primer was 5'-GCATGGCCCCCTCTCTGATTC-3'. The amplicon size was 166bp. It has been electrophoresed on agarose of 3% and visualized directly with diamond dye under a UV light. The product was digested with *BstUI* restriction enzyme, electrophoresed on agarose of 3% agarose, and visualized directly with diamond dye under a UV light. Wild type genotype (TT) exhibited one band (166pb), homozygous variant (CC) as two bands (145,21bp), and heterozygous genotypes (TC) three bands (166,145and 21bp) (Fig 1).

The amplification of calpain-10 gene for SNP-43 G/A rs3792267 was done as described by Carlsson et al[20]. The forward primer was 5` -GCTGGCTGGTG ACATCAGTGC- 3`. The reverse primer was 5`-ACCAAGTCAAGGCTTA GCCTCACCTTCATA- 3`. The amplicon size was 245bp. It is electrophoresed on agarose of 2% and visualized directly with diamond dye under a UV light, while the product is digested with *Ndel* restriction enzyme, electrophoresed on agarose of 2%, and visualized directly with diamond dye under a UV light. Furthermore, the wild genotype (GG) was manifested as one band (245pb), homozygous variant (AA) as two bands (223,31bp) and the heterozygous genotypes (GA) three bands (245,223and 31bp) (Fig 2).

Statistical Analysis

To determine the variations in means between the healthy and patient groups, the mean ±SD and student t-test are utilized. Student t-test and ANOVA were applied for comparing the mean levels of continuously characteristic through genotype using (SPSS.v.25.0software) SPSS Inc. Chicago, IL. Categorical data (alleles and genotypes) have been evaluated by chi- square test. In terms of all statistical analyses, the significance level has been less than 0.05.

Results

Allele frequencies and genotype of the two SNPs.

The results of SNP-44 T/C(rs2975760) of type 2 diabetes mellitus and control persons with numerous inheritance models were demonstrated in table 1. The codominant model showed that patients of heterozygous genotype (TC) decreased significantly (OR=0.62, CI95%= 0.42-0.92, P=0.02) with respect to the control group. Patients with the homozygous genotype (CC) elevated insignificantly (OR=1.51, CI 95%=0.53-4.31, P=0.43) relative to the control group. The dominant model indicated that patients of TC+CC genotypes declined significantly (OR=0.68, CI 95%=0.47-1.00, P=0.046) with respect to the controls. The additive model showed an insignificant (OR=0.73, CI95%=0.51-1.05, P=0.09) decrease ofT2DM patients than the control group and the recessive model explored an insignificant (OR=1.52, CI95%= 0.53-4.31, P= 0.44) increase than the control group. The minor allele C frequency in patients (12%) was found to be insignificantly (OR=0.77, CI 95% =0.55 – 1.07, P=0.12) reduced in the group of patients relative to the group of controls (15%).

The genotype results of SNP-43 G/A(rs3792267) in T2DM and control persons with various inheritance patterns were mentioned in table 2. Under the codominant model, patients of heterozygous (GA) genotypes seemed to be significantly (OR=0.19, CI95%= 0.13-0.28, P<0.0001) decreased with respect to the control

group. Patients of the homozygous genotype (AA) appeared to be insignificantly (OR=0.81, CI 95%=0.33-1.96, P=0.63) higher than the controls. The dominant model showed that patients of GA+AA genotypes were significantly (OR=0.23, CI95%= 0.16-0.33, P < 0.0001) decreased with respect to the group of controls. Also, the additive model indicated a considerable decrease significantly (OR=0.25, CI95%=0.18-0.36, P<0.0001) regarding patients experiencing T2DM compared to the group of controls. The recessive model pointed out an insignificant (OR=1.35, CI= 0.56-3.25, P=0.51) increase of T2DM patients relative to the control group. The minor allele A frequency in patients (11.5%) was evident to be significantly (OR=0.35, CI 95%=0.25-0.47, P<0.0001) declined in the group of patients relative to the group of controls (27%).

Table 1: Genotype and allele frequency results of SNP -44 T/C (rs2975760) of CAPN10 gene in T2DM and control subjects

SNP-44 T/C	T2DM (N=300)		Control (N=300)		OR (95%CI)	P-value
	No.	%	No.	%		
Co-dominant						
TT	237	79%	216	72%		
TC	54	18%	78	26%	0.62 (0.42 - 0.92)	0.02
CC	9	3 %	6	2%	1.51 (0.53 – 4.31)	0.43
Dominant						
TC+CC	63	21%	84	28%	0.68 (0.47 – 1.00)	0.046
Recessive						
TT+TC	291	97%	294	98%		
CC	9	3%	6	2%	1.52 (0.53 - 4.31)	0.44
Additive						
2CC+TC	72	24%	90	30%	0.73 (0.51 - 1.05)	0.09
MAF	72	12%	90	15%	0.77 (0.55 – 1.07)	0.12

Table 2: Genotype and allele frequency results of SNP-43 G/A (rs3792267) CAPN10 gene in type 2 DM and control subjects

SNP-43 G/A	T2DM (N=300)		Control (N=300)		OR (95%CI)	P-value
	No.	%	No.	%		
Co-dominant						
GG	243	81%	147	49%		
GA	45	15%	144	48%	0.19 (0.13 - 0.28)	< 0.0001
AA	12	4%	9	3%	0.81 (0.33 -1.96)	0.63
Dominant						
GA+AA	57	19%	153	51%	0.23 (0.16 - 0.33)	< 0.0001
Recessive						
GG+GA	288	96%	291	97%		
AA	12	4%	9	3%	1.35 (0.56 -3.25)	0.51
Additive						
2AA+GA	69	23%	162	54%	0.25 (0.18 - 0.36)	< 0.0001
MAF%	69	11.5%	162	27%	0.35 (0.25- 0.47)	< 0.0001

Results of phenotypic parameter analysis in relevance to the genotypes

For the SNP-44 genotype, the co-dominant model revealed a significant association for insulin (P=0.0043), VLDL-C (p=0.05), HOMA-IR (P=0.0087), while other factors did not show significant modifications (Table 3). For the dominant pattern TG, VLDL-C, glucose, insulin, and HOMA-IR show a significant increase in TC+CC model in comparison with TT model (p=0.043, 0.0076,0.036, 0.0034,0.0013 respectively) while LDL-C shows a significant decrease in TC+CC model in comparison with TT model but TC and HDL-C shows an insignificant association.

For the SNP-43 genotype, the co-dominant model revealed a significant association for LDL-C (P=0.0018), TG(P=0.00001), insulin (p=0.009), FBG(P=0.02), and HOMA-IR (P=0.0003) but other factors did not show significant modifications (Table 4).

Under the dominant pattern, glucose, insulin, and HOMA-IR show significant increments (p=0.008,0.0028.0.0002 respectively) in the GA+AA model in comparison to the GG model while other factors did not show significant modifications.

Table 3: Results of phenotypic parameters of diabetic patients analyzed in relevance to the SNP-44 T/C(rs2975760) of calpain10 gene under the co-dominant model

Parameters	TT(N=237) Mean ± SD	TC (N=54) Mean ±SD	CC (N = (9) Mean ±SD	P value
TG(mg/dl)	250.9±37.37	258.3±39.29	271.5±50.22	0.1464
TC (mg/dl)	253.9±32.33	251.5±34.93	231.7±35.07	0.1333
VLDL-C (mg/dl)	49.35±11.58	53.27±10.31	53.39±13.04	0.05
LDL-C (mg/dl)	153.3±34.23	146.8±35.40	128.5±37.84	0.0618
HDL-C (mg/dl)	48.79±7.394	49.54±6.098	48.86±5.690	0.785
FSG(mg/dl)	239.0±33.12	248.9±32.44	238.8±40.57	0.1409
Insulin (µU/ml)	27.67±3.545	28.70±3.794	31.13±1.714	0.0043
HOMA-IR	16.35±3.208	17.62±3.146	18.35±3.155	0.0087

Table 4: Results of phenotypic parameters of diabetic patients analyzed in relevance to the SNP-43G/A (rs3792267) of calpain10 gene under the co-dominant model

Parameters	GG (N=243) Mean ± SD	GA (N=45) Mean ±SD	AA (N= 12) Mean ±SD	P value
TG(mg/dl)	251 ± 38.1	261 ± 27.1	196 ± 89.3	0.00001
TC (mg/dl)	253 ± 32.7	250 ± 38.8	244 ± 30.4	0.5907
VLDL-C (mg/dl)	49.6 ± 11.6	51.1 ± 10.8	55.3 ± 6.51	0.1858
LDL-C (mg/dl)	152 ± 34.7	147 ± 37.8	187 ± 26.6	0.0018
HDL-C (mg/dl)	48.8 ± 7.34	47.5 ± 6.38	46.3± 6.24	0.29885
FSG (mg/dl)	239 ± 33.2	254 ± 32.2	239 ± 26.6	0.02092
Insulin (µU/ml)	27.7± 3.54	29.5 ± 3.36	28.0 ± 1.96	0.0092
HOMA-IR	16.4 ± 3.21	18.5 ± 3.08	16.5 ± 2.35	0.0003

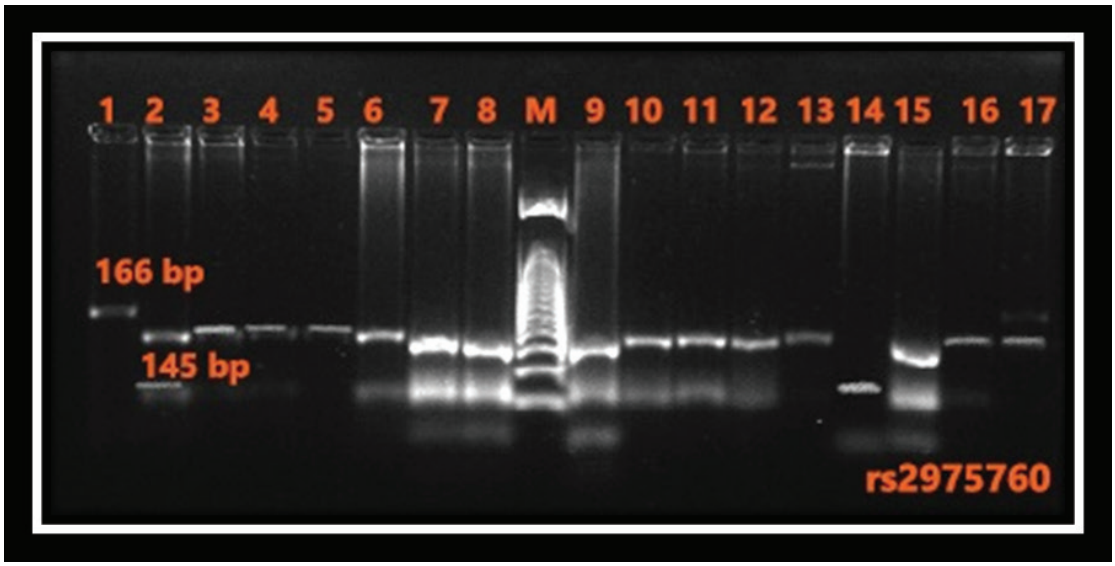

Figure 1: The RFLP product of rs297760 SNP of calpain 10 gene polymorphism after digestion by *BstUI* enzyme. It was electrophoresed on 3% agarose gel for 120min and 75V and immediately visualized under UV light. Lanes 3,4,5,13,16 and 17: (TT) genotype 166bp. Lanes 2, 6, 7, 8,9, 10,11,12 and 15: (TC) genotype 166,145 and 21bp. Lane 14: (CC) genotype 145 and 21bp. M: DNA ladder 50bp

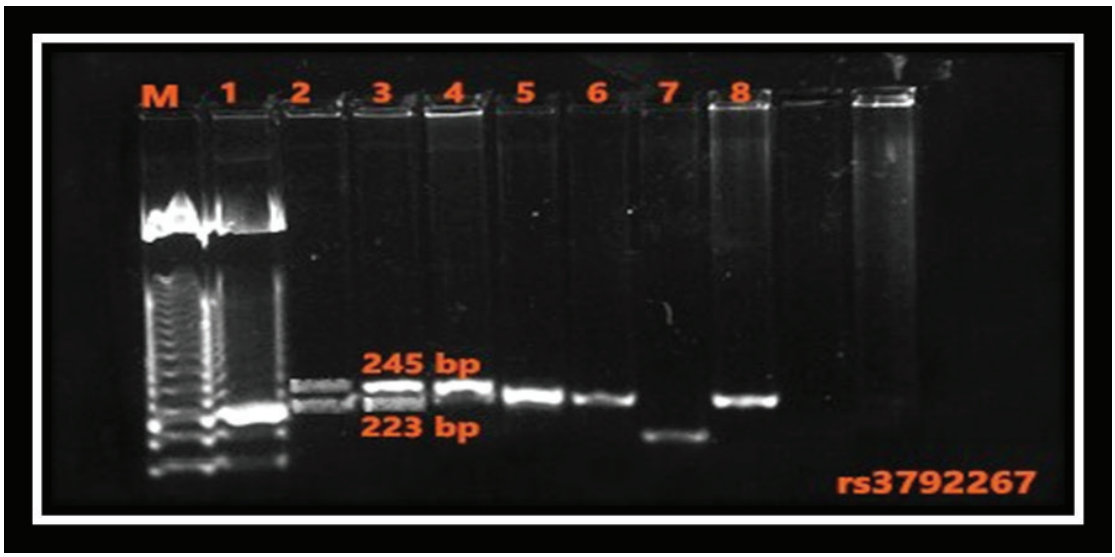

Figure 2: The RFLP product of rs3792267G/A SNP of calpain 10 gene polymorphism after digestion by *Ndel* enzyme. It was electrophoresed on 2% agarose gel (120min and 75V) then visualized under UV light. Lane 4:(GG) genotype 254bp. Lanes 2 and 3:(GA) genotype 254,223and 31bp. Lanes 1,5,6 and 8;(AA) genotype 223 and 31bp. M: DNA ladder 50bp.

Discussion

Today, DM is a major risk factor for many medical conditions, mainly the causes and effects of T2DM in humans have been studied to minimize their impression on the health care system, as it needs huge, financial, efforts and impacts the community's vitality.

The Calpain10 gene has been reported to be a significant T2DM factor, so we have investigated the CAPN10 impact on T2DM risks and lipid profile, insulin as well as insulin resistance levels.

We have evaluated these two polymorphisms for association with T2DM in this work, on the basis

of former researches exploring the relations between combinations regarding the two SNPs (SNP44 and SNP43) and diabetes, using a case-control system. These polymorphisms either affect either T2DM individually or in combination or on triglyceride or cholesterol or other lipid profile levels.

In this study, patients with TC heterozygous of the co-dominant model and the dominant model with TC+CC genotype in SNP-44 are both diminished significantly with respect to the control group. This indicates that SNP-44 may be linked to a protective effect from T2DM incidences or/and development of the disease in the study group.

Regarding SNP-43, patients with GA heterozygous and dominant model GA+AA genotype and minor allele frequency (A) are all showed significant decreases with respect to the control group. Basically, such observations may indicate that SNP-43 has also a protective effect from T2DM occurrence.

The impact of the studied SNPs with T2DM might be due to a combined effect of both gene irregulars with other factors that exacerbate the manifestation of the disease. It may be related to population confirmation and ethnicity [21].

The results of this work are in accordance with the results of (Tsai et al.[22] on Samoans, Evans et al.[23] on British, Horikawa et al.[24] on Japanese; as well as the results of Wu et al.[25] in their researches on Chinese in which they indicated no considerable relations between T2DM as well as the allele frequencies related to SNP43, del/ins19, and SNP63 when individually tested. Our study is well-disposed with Song et al.[26] and Weedon et al. 2003 where they showed that the SNP-44 T allele was protective against diabetes mellitus. The results of this work are in accordance with the results of Jensen et al.[27] , in which they indicated no consistent evidence regarding the relation of T2DM and CAPN10 SNP44.

On the other hand, the results of this work are different from the ones indicated via, in which the frequency related to G-allele (allele-1) in SNP43 indicated a statistically considerable increment in patient group of Mexican-Americans. For the SNP-44 T/C of CAPN10 gene, fasting blood insulin level and insulin resistance were higher in patients with CC genotype of the recessive model, compared to those of the TT genotype. Triglycerides and VLDL-c have increased levels in TC+CC genotype and the level of LDL-c is high in TT genotype compared to the ones of TC+CC genotype. Results of the SNP-43 G/A pointed out that fasting blood glucose, IR and insulin levels have been high in those related to GA genotype, compared to those of the AA and GA genotype. These results are matching the results indicated via previous research indicating that GG genotype being implicated in T2DM development via increasing IR. The current results are not in accordance with the results of Daimon et al.[28] in which they specified that the genotype combinations related to SNP43 G/G and SNP44 T/T had considerably elevated TC level (p=0.02). It is worthy to highlight several restrictions in the present study. Primary, many diabetic patients were taking oral hypoglycemic drugs and anti hypertensive drugs, such drugs might have affected IR and insulin secretory function. A small number of subjects participated in this work. Wide-scale research must be carried out for confirming the relationship between the polymorphism of CAPN10 and T2DM in Iraqi patients and showing a major approach to explain the impact of genetic polymorphisms on diabetes as well as metabolic imbalances.

Conclusions

SNP44 rs2975760 and SNP43 rs3792267 in Iraqi individuals are protective against the development of T2DM. The protective influences varied from one SNP relative to the others. No risk effect for the two SNPs under the studied inheritance models. Both SNPs are implicated in changing lipid metabolism in diabetic patients. Each SNP may be implicated in changing lipid metabolism in a certain style that differed from the other one with varying degrees. There is a high importance in confirming the results of this presented work via including further samples as well as identifying the precise analysis of CAPN10 gene polymorphism in regard to the metabolic complications in T2DM in future studies.

Conflict of Interests: There are no conflict of interests.

Source of Funding: The source of this research costs from self.

Ethical Clearance: Approval from the Ethical Committee (in the faculty of medicine /Kufa university) was taken for the protocol of the study.

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