GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Calpain-10 gene polymorphisms and risk of type 2 diabetes mellitus in Mexican mestizos

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Genet. Mol. Res. 14 (1): 2205-2215 (2015)
Received January 15, 2014
Accepted August 8, 2014
Published March 27, 2015
DOI http://dx.doi.org/10.4238/2015.March.27.6

ABSTRACT. The calpain-10 gene is expressed primarily in tissues important in glucose metabolism; thus, some of its polymorphisms have been associated with type 2 diabetes. In this study, we examined the association between the calpain-10 single-nucleotide polymorphism (SNP)-43, SNP-19, and SNP-63 and type 2 diabetes in Mexican mestizos. We included 211 patients and 152 non-diabetic subjects. Polymerase chain reaction was used

to identify alleles. We compared allele, genotype, haplotype, and diplotype frequencies between both groups and used the chi-square test to calculate the risk. The allele frequency of SNP-43 allele 1 was 70% in controls and 72% in patients; the GG, GA, and AA genotype frequencies were 48.7, 42.8, and 8.5% in controls and 51.2, 41.7, and 7.1% in patients, respectively. For SNP-19, the prevalence of allele 1 (2R) was 32% in controls and 39% in patients. In controls, homozygosity (2R/2R) was 10.5%, heterozygosity was 42.8%, and 3R/3R was 46.7%; in cases, these values were 13.3, 50.7, and 36.0%, respectively. For SNP-63, the frequency of allele 1 was 87% in controls and 83% in patients; genotype frequencies in controls were 75.7% (CC), 23% (CT), and 1.3% (TT), and were 69.7, 27.5, and 2.8%, respectively for the cases. Genotype distributions were consistent with Hardy-Weinberg equilibrium. No significant intergroup differences for allele, genotype, haplotype, or diplotype frequencies were observed. We found no association between these polymorphisms and diabetes. However, our sample size was small, so the role of calpain-10 risk alleles should be further examined.

Key words: *CAPN10* polymorphisms; Mexican mestizo; Type 2 diabetes

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a systemic, chronic, and progressive disease characterized by deficiency in production or action of insulin, which leads to hyperglycemia. The current International Diabetes Federation estimates that approximately 371 million people have diabetes worldwide, and this figure is likely to increase to 500 million by the year 2030. T2DM is among the principal causes of death due to premature cardiovascular disease, vascular-cerebral disorder, and renal failure. In 2012, there were approximately 4.8 million deaths from diabetes worldwide, with nearly 80% occurring in underdeveloped countries (International Diabetes Federation, 2013). In Mexico, the prevalence of T2DM is approximately 14.42%, accounting for 7.3 million patients (Villalpando et al., 2010). The etiology of T2DM is complex and involves both genetic and environmental factors. Among the >40 genes related to T2DM (OMIM, 2013), the calpain-10 (*CAPN10*) gene was initially associated with this disease in Mexican-Americans. This gene consists of 15 exons and produces a 672-amino acid protein (Horikawa et al., 2000). CAPN10 has been implicated in insulin receptor regulation and adipocyte differentiation (Patel and Lane, 1999) and is expressed primarily in tissues important in glucose metabolism, suggesting that it affects insulin secretion and action as well as liver glucose synthesis (Lynn et al., 2002). Single-nucleotide polymorphisms (SNPs) or haplotypes formed by SNP-43 (rs3792267; G4852A), SNP-19 [(rs3842570; 32 base pairs (bp) ins/del], and SNP-63 (rs5030952; C16378T) of this gene have been widely studied in relation to T2DM risk (Horikawa et al., 2000; Evans et al., 2001; Tsai et al., 2001; Fingerlin et al., 2002; Garant et al., 2002; Malecki et al., 2002; Orho-Melander et al., 2002; Rasmussen et al., 2002; Horikawa et al., 2003; del Bosque-Plata et al., 2004; Wu et al., 2005; Kang et al., 2006; Tsuchiya et al., 2006; Chen et al., 2005, 2007; Kifagi et al., 2008; Adak et al., 2010; Ezzidi et al., 2010; Bodhini et al., 2011). In this study, we analyzed whether an association exists between the *CAPN10* SNP-43, SNP-19, and SNP-63 polymorphisms and T2DM in a Mexican mestizo population.

MATERIAL AND METHODS

Subjects

We analyzed 211 unrelated adult T2DM patients, diagnosed according to American Diabetes Association criteria (2013), including 143 women (68%) and 68 men (32%), and 152 unrelated controls with no family history of T2DM in first-degree relatives, including 103 women (68%) and 49 men (32%); all individuals were Mexican mestizos residing in the same region. Patients were enrolled at the Endocrinology Service of the General Hospital and at the Clinic #35 of the Mexican Institute of Social Security in Culiacán, Sinaloa, México. Controls were subjects over 40 years of age with no medical history of T2DM and normal fasting glycemia (80-110 mg/dL). Both patients and controls signed informed consent to participate in this study. The patients' mean age was 58.5 years, while it was 50.4 years in controls. The mean fasting plasma glucose level was 147.77 ± 66.13 mg/dL in patients and 92.85 ± 12.52 mg/dL in controls. Mean body mass index (BMI) of patients and controls was 31.08 ± 5.56 kg/m^2 and 28.47 ± 5.17 kg/m^2, respectively; 46% of the patients had a BMI of 30-40 kg/m^2, whereas 27.9% of the controls were in this range. Mean concentrations of cholesterol and triglycerides in patients were 206.9 mg/dL and 173.04 mg/dL, respectively; in controls, these values were 199.29 mg/dL and 140.4 mg/dL. This study was approved by the ethics committee of each participating institution.

Genotyping

SNP-43 and SNP-63

To identify the alleles of SNP-43 and SNP-63, we carried out real-time polymerase chain reaction (PCR). Primers for the Hybridization Probes (HybProbes) assays were obtained from TIB MOLBIOL, LLC (Adelphia, NJ, USA). For SNP-43, we used the primers 5'-TCCA TCCCAAGGGCTGTT-3' and 5'-TCTGTAGCACCCCAAATCG-3', and the acceptor 5'-GAAGTAAGGCATTTGAAGGTGA-FL-3' and donor 5'-LCR640-GCTAAGCCTTGAC TTGGTGAGGATGAGG-PH-3' probes; for SNP-63, we used the primers 5'-CCTGGTCACT GGATGTTG-3' and 5'-GGCTGGAGTTTGGAGAAGTT-3', and 5'-GGGTGGAGCGAGGG GT-FL-3' as the acceptor and 5'-LCR640-GGCCGCGTCTGTGCAGGC-PH-3' as the donor. Real-time PCR was performed in a LightCycler (Roche; Basel, Switzerland) according to manufacturer instructions. Standard 10-µL reaction mixtures contained 2-4 mM $MgCl_2$, 1X LightCycler FastStart DNA Master Hybridization Probes master mix, 0.25 µM of each primer, 0.2 µM of each probe, 0.01 U/µL *Escherichia coli* uracil N-glycosylase (UNG, Roche), and 100 ng genomic DNA. The reaction was initiated with a 5-min hold at room temperature for contamination control by UNG and 10 min at 95°C to activate the polymerase. Thermal cycling was performed at 95°C for 10 s, 55°C for 20 s, and 72°C for 20 s for 40 cycles. The transition rate between all steps was 20°C/s (Figures 1 and 2).

SNP-19

This polymorphism is a 32-bp insertion/deletion. To identify the polymorphism, we carried out simple PCR using the following primers: 5'-GTTTGGTTCTCTTCAGCGTGGA

G-3' (sense) and 5'- CATGAACCCTGGCAGGGTCTAAG-3' (antisense). The reaction was carried out in a total volume of 20 µL containing 1X PCR buffer, 5% dimethyl sulfoxide, 200 µM of each dNTP, 1.5 mM $MgCl_2$, 2.0 U *Taq* Platinum DNA polymerase (Invitrogen, Carlsbad, CA, USA), 10 pM of each primer, and 50 ng genomic DNA. Amplification conditions were as follows: initial denaturation at 96°C for 12 min, 35 cycles at 96°C for 30 s, 60°C for 30 s, and 72°C for 30 s, followed by final extension at 72°C for 10 min (Haddad et al., 2002). Thus, allele 1, which bears 2 32-bp repeats (2R), yielded a fragment of 155 bp; allele 2, with 3 repeats (3R), was a 187-bp fragment (Figure 3).

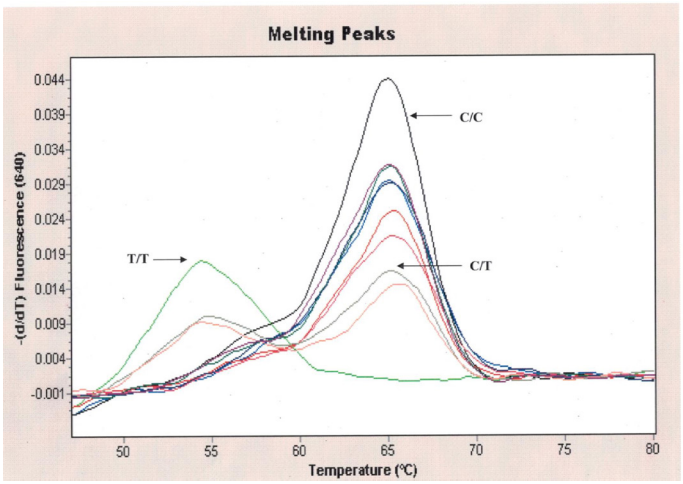

Figure 1. Graphic of a real-time PCR showing results for *CAPN10* SNP-63. C/C: Wild homozygote (only one curve at 65°C), C/T: Heterozygote (two curves: at 54°C and 65°C), T/T: Polymorphic homozygote (one curve at 54°C).

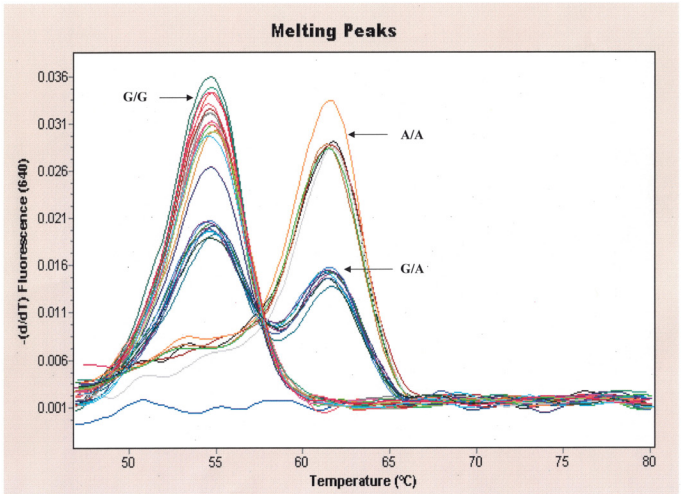

Figure 2. Graphic of a real-time PCR showing results for *CAPN10* SNP-43. G/G: Wild homozygote (only one curve at 55°C), G/A: Heterozygote (two curves; at 55°C and 61°C), A/A: Polymorphic homozygote (one curve at 61°C).

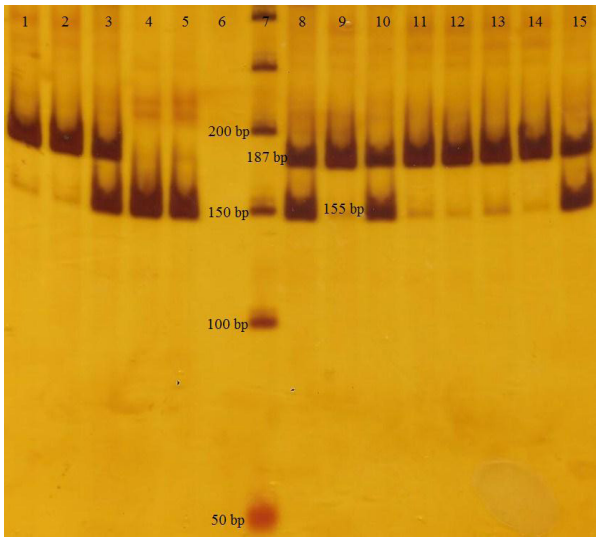

Figure 3. Polyacrylamide gel showing results for *CAPN10* SNP-19 in some individuals; 2R allele is visualized as a fragment of 155 bp and 3R allele is observed as a fragment of 187 bp. *Lanes 1, 2, 9, 11, 12, 13*, and *14*: 3R/3R genotype. *Lanes 3, 8, 10*, and *15*: 2R/3R genotype: *Lanes 4* and *5*: 2R/2R genotype. *Lane 7*: 50-bp marker.

Statistical analysis

Clinical characteristics (BMI, cholesterol, and triglycerides) between cases and controls were analyzed using the Student *t*-test. Hardy-Weinberg equilibrium was evaluated by Fisher's exact test. Comparison of allele and genotype frequencies between groups was carried out using the chi-square test, and risk for T2DM was calculated as the odds ratio and 95% confidence intervals (OR; 95%CI). Moreover, haplotype estimation was conducted using the Hapstat 3.0 software using the genotypes, and expected frequencies of diplotype were estimated according to the Hardy-Weinberg principle. Next, evaluation of risk for haplotypes and diplotypes was calculated (OR; 95%CI). Allele 1 was taken as the reference and the level of significance was defined as $P \leq 0.05$.

RESULTS

BMI and triglycerides were significantly higher in patients than in controls (31.08 ± 5.56 kg/m^2 *vs* 28.47 ± 5.17 kg/m^2; $P < 0.001$ and 173.04 mg/dL *vs* 140.4 mg/dL; $P = 0.012$, respectively), but there were no significant differences in cholesterol concentration ($P = 0.101$). Allele and genotype frequencies of the 3 polymorphisms analyzed are summarized in Table 1. All were consistent with Hardy-Weinberg equilibrium ($P = 1.0$ for SNP-43 and SNP-63, $P = 0.85$ for SNP-19). SNP-43 genotype frequencies were 48.7, 42.8, and 8.5% in controls and 51.2, 41.7, and 7.1% in patients for GG, GA, and AA, respectively. For SNP-19, in controls, the homozygote 2R/2R was observed in 10.5% of subjects, heterozygote 2R/3R was observed in 42.8% of subjects, and the genotype 3R/3R was observed in 46.7% of subjects, while in cases, these genotypes were detected in 13.3, 50.7, and 36.0% of subjects, respectively. For SNP-63, the genotype (CC, CT, and TT) distribution was 75.7, 23, and 1.3% in controls and

69.7, 27.5, and 2.8% in patients, respectively. Overall, allele or genotype frequencies did not vary significantly between the two groups (Table 1). Comparison between patients with BMI < 30 kg/m^2 *vs* patients with BMI ≥ 30 kg/m^2 revealed no significant differences in allele frequencies (P = 0.81 for SNP-43, P = 0.49 for SNP-19, and P = 0.34 for SNP-63). Similarly, there was no evidence of an association between any haplotype or diplotype with T2DM risk (Table 2).

Table 1. Genotype and allele frequencies for SNP-43, SNP-19, and SNP-63 polymorphisms in patients with type 2 diabetes mellitus and controls.

	Controls N = 152	Cases N = 211	OR (95%CI)	*P value
SNP-43				
G/G	74	108	Reference	
G/A	65	88	0.93 (0.60-1.44)	0.74
A/A	13	15	0.79 (0.36-1.78)	0.56
G	0.70	0.72	Reference	
A	0.30	0.28	0.91 (0.66-1.26)	0.56
SNP-19				
2R/2R	16	28	Reference	
2R/3R	65	107	0.94 (0.47-1.87)	0.86
3R/3R	71	76	0.61 (0.31-1.23)	0.16
2R	0.32	0.39	Reference	
3R	0.68	0.61	0.75 (0.55-1.02)	0.06
SNP-63				
C/C	115	147	Reference	
C/T	35	58	1.30 (0.80-2.11)	0.29
T/T	2	6	2.35 (0.42-17.2)	0.25**
C	0.87	0.83	Reference	
T	0.13	0.17	1.35 (0.89-2.06)	0.16

*Chi-square test. **Fisher exact test.

Table 2. Haplotype and diplotype frequencies estimated for SNP-43, SNP 19, and SNP-63 polymorphisms in cases and controls.

Haplotype	Controls (N = 304)	Cases (N = 422)	OR (95%CI)	**P value
121*	0.3536 (107)	0.3511 (148)	Reference	
122	0.0260 (8)	0.0022 (1)	0.09 (0.01-0.73)	0.006$^+$
111	0.2165 (66)	0.2080 (88)	0.96 (0.64-1.45)	0.86
112	0.1076 (33)	0.1608 (68)	1.49 (0.92-2.42)	0.11
221	0.2904 (88)	0.2587 (109)	0.90 (0.62-1.30)	0.56
211	0.0058 (2)	0.0121 (5)	1.81 (0.34-9.49)	0.70$^+$
222	0	0.0071 (3)	ND	ND
Diplotype	(N = 152)	(N = 211)		
121/221*	0.2054 (31)	0.1817 (38)	Reference	
121/121	0.1250 (19)	0.1233 (26)	1.12 (0.52-2.38)	0.78
121/111	0.1531 (23)	0.1461 (31)	1.10 (0.54-2.26)	0.80
121/112	0.0761 (12)	0.1129 (24)	1.63 (0.71-3.78)	0.25
111/221	0.1257 (19)	0.1076 (23)	0.99 (0.46-2.14)	0.97
111/111	0.0469 (7)	0.0433 (9)	1.05 (0.35-3.14)	0.93
111/112	0.0466 (7)	0.0669 (14)	1.63 (0.59-4.54)	0.35
112/221	0.0625 (10)	0.0832 (18)	1.47 (0.59-3.64)	0.41
221/221	0.0843 (13)	0.0669 (14)	0.88 (0.36-2.14)	0.78
Others	0.0744 (11)	0.0681 (14)	1.04 (0.41-2.61)	0.94

*Haplotypes and diplotypes more common were took as reference. **Chi square test. $^+$Fisher Exact test. ND = Not determined.

DISCUSSION

Various studies conducted worldwide have reported an association between the *CAPN10* gene polymorphisms and T2DM risk, but other studies have reported conflicting results (Horikawa et al., 2000; Evans et al., 2001; Tsai et al., 2001; Fingerlin et al., 2002; Garant et al., 2002; Malecki et al., 2002; Orho-Melander et al., 2002; Rasmussen et al., 2002; Horikawa et al., 2003; del Bosque-Plata et al., 2004; Wu et al., 2005; Kang et al., 2006; Tsuchiya et al., 2006; Chen et al., 2005, 2007; Kifagi et al., 2008; Adak et al., 2010; Ezzidi et al., 2010; Bodhini et al., 2011). Allele frequencies of *CAPN10* polymorphisms vary according to the sample analyzed. Lynn et al. (2002) reported a prevalence of 79, 37, and 88% for SNP-43, SNP-19, and SNP-63, respectively, in British subjects without diabetes. Shima et al. (2003) found frequencies of 95.5, 37.6, and 73.4% for these polymorphisms in Japanese T2DM patients, whereas Sáez et al. (2008) observed frequencies of 74, 36, and 95%, respectively, in a random Spanish sample. The frequency of the SNP-43 G allele in our patients was similar to values reported in Mexican (del Bosque-Plata et al., 2004) and European populations (Evans et al., 2001; Fingerlin et al., 2002; Malecki et al., 2002; Orho-Melander et al., 2002; Rasmussen et al., 2002), but differed from those found in Japanese (Horikawa et al., 2003; Shima et al., 2003), Korean (Kang et al., 2006), Chinese (Wu et al., 2005; Chen et al., 2007), Indian (Adak et al., 2010; Bodhini et al., 2011), Samoan (Tsai et al., 2001), and African patients (Garant et al., 2002; Chen et al., 2005; Ezzidi et al., 2010) (Table 3). The genotype and allele frequencies of SNP-43 showed no significant differences between cases and controls in our analysis. This observation agrees with those of previous studies (Evans et al., 2001; Tsai et al., 2001; Fingerlin et al., 2002; Malecki et al., 2002; Rasmussen et al., 2002; Horikawa et al., 2003; del Bosque-Plata et al., 2004; Wu et al., 2005; Kang et al., 2006; Chen et al., 2005, 2007; Adak et al., 2010; Ezzidi et al., 2010; Bodhini et al., 2011), but differs from the results of others. For example, Garant et al. (2002) found an association of the GG genotype to T2DM [OR = 1.38 (1.04-1.83); P = 0.03] in African-Americans, while Orho-Melander et al. (2002) reported an association between the G allele and an increased risk of T2DM [OR = 1.36 (1.07-1.73); P = 0.011] in a Finnish population. In contrast, Kifagi et al. (2008) found an association between the A allele and risk of T2DM in Tunisians (OR = 1.86).

For SNP-19, we did not observe significant intergroup differences in allele and genotype frequencies, which was similar to the results of other studies (Tsai et al., 2001; Fingerlin et al., 2002; Malecki et al., 2002; Orho-Melander et al., 2002; Rasmussen et al., 2002; Horikawa et al., 2003; del Bosque-Plata et al., 2004; Wu et al., 2005; Kang et al., 2006; Chen et al., 2005, 2007; Adak et al., 2010; Bodhini et al., 2011). An association between the 2R allele with T2DM was documented in a single study of Tunisians (Arab) [OR = 1.28 (1.11-1.47); P = 0.0005] (Ezzidi et al., 2010). In our study, the prevalence of the 2R allele in patients was 39%, which was also observed by del Bosque-Plata et al. (2004) in another Mexican sample, and is similar to that reported in Scandinavian (Orho-Melander et al., 2002; Rasmussen et al., 2002), Japanese (Horikawa et al., 2003), Southern Chinese (Wu et al., 2005), and Korean patients (Kang et al., 2006), but differed from that in Samoan (Tsai et al., 2001), Polish (Malecki et al., 2002), Finnish (Fingerlin et al., 2002), West African (Chen et al., 2005), Northern Chinese (Chen et al., 2007), Tunisian (Ezzidi et al., 2010), and Indian patients (Adak et al., 2010; Bodhini et al., 2011). With respect to SNP-63, allele and genotype frequencies showed no differences between groups and were thus concordant with the results of most previous studies (Tsai et al., 2001; Fingerlin et al., 2002; Malecki et al., 2002; Rasmussen et al., 2002; Horikawa et

al., 2003; del Bosque-Plata et al., 2004; Wu et al., 2005; Kang et al., 2006; Chen et al., 2007; Ezzidi et al., 2010; Bodhini et al., 2011), but disagreed with the results of Orho-Melander et al. (2002) and Adak et al. (2010), who reported that the T allele is associated with T2DM risk in Finns [OR = 1.65 (1.12–2.42); P = 0.01] and Eastern Indians [OR = 3.74 (1.44-9.69); P = 0.003], respectively. Although there were significant differences (P = 0.16) in our study, the T allele showed an OR of 1.35 (0.89-2.06).

Table 3. Allele frequencies for SNP-43, SNP-19, and SNP-63 polymorphisms in patients with type 2 diabetes mellitus and controls in several racial groups.

Population	N1 and N2	N1(%)	N2(%)	OR (95%CI)	P	Reference
British	222	73	72	0.95 (0.70-1.28)	0.79+	(Evans et al., 2001)a
	212					
Samoan	172	91.4	91.1	0.92 (0.49-1.73)	0.92+	(Tsai et al., 2002)
	96	32.6	37	1.21 (0.83-1.76)	0.36+	
		85.3	83.7	0.88 (0.54-1.45)	0.72+	
African-American	269	90	87.2	0.76 (0.56-1.03)	0.09+	(Garant et al., 2002)a
	1159					
Polish	229	73.1	69.3	0.83 (0.60-1.14)	0.24*	(Malecki et al., 2002)
	148	34.3	35.1	1.04 (0.76-1.41)	0.81*	
		92.8	90.9	0.77 (0.46-1.32)	0.34*	
Finnish	526	74	75	1.06 (0.86-1.30)	0.65+	(Fingerlin et al., 2002)
	408	45	42	0.89 (0.74-1.07)	0.22+	
		88	88	1.00 (0.75-1.32)	1.0+	
Finnish	395	75.5	69.4	0.73 (0.58-0.94)	0.011+	(Orho-Melander et al., 2002)**
	298	43.5	41.6	0.92 (0.75-1.15)	0.51+	
		88.9	92.9	1.65 (1.13-2.43)	0.01+	
Scandinavian	409	73	72	0.96 (0.74-1.26)	0.79*	(Rasmussen et al., 2002)
Caucasians	200	38	39	1.08 (0.85-1.38)	0.53*	
		93	93	0.98 (0.62-1.55)	0.92*	
Japanese	177	95	95	1.03 (0.52-2.03)	0.92+	(Horikawa et al., 2003)
	172	38	37	0.95 (0.70-1.29)	0.75+	
		74	72	0.91 (0.65-1.27)	0.61+	
Mexican	134	68.8	67.9	0.97 (0.66-1.42)	0.87*	(del Bosque-Plata et al., 2004)
mestizo	114	39	38.4	0.97 (0.68-1.40)	0.88*	
		77.3	74.3	0.85 (0.56-1.29)	0.45*	
West Africa	347	85	86	1.10 (0.74-1.62)	0.72+	(Chen et al., 2005)b
	148	46	45	0.96 (0.73-1.26)	0.82+	
Korean	454	91.3	91.1	0.98 (0.66-1.44)	0.90*	(Kang et al., 2006)
	236	36.5	31.6	0.80 (0.64-1.02)	0.07*	
		71.3	66.9	0.82 (0.64-1.04)	0.10*	
Northern China	493	89.8	89.1	0.93 (0.70-1.23)	0.61*	(Chen et al., 2007)
	553	32.5	34.3	1.09 (0.90-1.30)	0.38*	
		80	77.1	0.84 (0.68-1.04)	0.11*	
Tunisian	917	91.2	89.9	0.86 (0.68-1.08)	0.20*	(Ezzidi et al., 2010)**
	748	46.4	40.4	0.78 (0.68-0.90)	0.001*	
		83.8	83	0.95 (0.79-1.14)	0.54*	
Eastern Indians	200	80.5	81	0.97 (0.63−1.49)	0.83	(Adak et al., 2010)
	100	44.2	40	0.84 (0.59-1.19)	0.34	
		91.2	97.5	3.74 (1.44-9.69)	0.003	
Southern Indians	649	98	98	1.05 (0.62-1.77)	0.94	(Bodhini et al., 2011)
	794	44.4	44.5	1.01 (0.87-1.17)	0.94	
		92.3	90.8	0.86 (0.59-1.25)	0.45	
Mexican mestizo	211	72	70	0.91 (0.66-1.26)	0.56	This study
	152	39	32	0.75 (0.55-1.02)	0.06	
		83	87	1.35 (0.89-2.06)	0.16	

N1 and N2. Cases and controls, respectively. Allele frequencies of SNP-43, SNP-19, and SNP-63 are ordered in row, consecutively. Note: Only allele 1 frequencies are reported. For OR estimation, alleles 1 were taken as reference. *Estimated OR of genotype frequencies reported by the authors. +Estimated OR of allele frequencies reported by the authors. aFrequencies are for SNP-43. bFrequencies correspond to SNP-43 and SNP-19. **These authors considered the allele 3R as allele 1 (To uniform our analysis we here change it, according to Horikawa et al., 2000).

Although the patients had higher BMI than controls in our analysis, the polymorphisms analyzed showed not significant relationship with obesity, as revealed by comparing patients with BMI < 30 kg/m^2 and patients with BMI ≥ 30 kg/m^2. However, SNP-63 TT homozygotes showed an OR of 4.65 (0.53-40.83; P = 0.13), suggesting a strong influence of this genotype on obesity and T2DM. Shima et al. (2003) also did not observe an association between these polymorphisms and higher BMI in a Japanese population using the wild-type genotype as a reference (P > 0.05, P = 0.11, and P = 0.38, for SNP-43, SNP-19, and SNP-63, respectively).

Analysis of a combination of polymorphisms showed that no haplotype was associated with T2DM risk. Because the 122 (G-3R-T) haplotype was predominantly present in the control group (P = 0.006), it is associated with a protective effect against T2DM. However, this combination has not been reported as a protector haplotype in other series (Tsai et al., 2001; Malecki et al. 2002; Rasmussen et al., 2002; Horikawa et al., 2003; del Bosque-Plata et al., 2004; Wu et al., 2005; Kang et al., 2006; Adak et al., 2010; Ezzidi et al., 2010). Instead, in Eastern Indians, the 112 haplotype was related to an increased risk of T2DM [OR = 3.96 (1.34-11.72); P = 0.0079], whereas the 121 haplotype was related to a protector effect when compared with the remaining haplotypes that were pooled into a single group [OR = 0.70 (0.50-0.99); P = 0.05] (Adak et al., 2010). In Tunisia, the 112 haplotype (described by the authors as 122 because they used the 3R allele as allele 1) was not associated with T2DM (P = 0.115), whereas the 121 haplotype (reported by the authors as 111) appeared to increase the risk of T2DM [OR = 1.22 (1.06-1.41); P = 0.006] (Ezzidi et al., 2010). Note that to effectively compare our analysis to previous studies, we considered the 2R allele as allele 1, according to Horikawa et al. (2000). In addition, the 111 haplotype was associated with an increased risk of T2DM in Koreans (P < 0.0042) (Kang et al., 2006).

In discordance with Horikawa et al. (2000), who related the 112/121 diplotype with T2DM in Mexican-American patients, no diplotypes showed an association with T2DM in our study; indeed, this finding is consistent with most studies conducted worldwide (Tsai et al., 2001; Fingerlin et al., 2002; Malecki et al., 2002; Rasmussen et al., 2002; Horikawa et al., 2003; del Bosque-Plata et al., 2004; Wu et al., 2005; Kang et al., 2006; Kifagi et al., 2008; Adak et al., 2010; Ezzidi et al., 2010). Instead, the 112/121 diplotype was associated with lower BMI (P = 0.016), decreased glycated hemoglobin (P = 0.008), and increased high-density lipoprotein levels (P = 0.034) in Japanese T2DM patients (Shima et al., 2003). Furthermore, surveys carried out on diverse racial groups have yielded inconsistent results; diplotypes associated with increased risk of T2DM include combinations 112/121 (Horikawa et al., 2000; Tsuchiya et al., 2006), 121/121 (Malecki et al., 2002; Tsuchiya et al., 2006), 111/121 (Kang et al., 2006), 112/221 (Wu et al., 2005; Adak et al., 2010), 121/221 (Kifagi et al., 2008), and 111/112 (Adak et al., 2010), whereas diplotypes related to a protector effect are 112/221 (Wu et al., 2005), 111/221 (Tsuchiya et al., 2006) and 121/121 (Kang et al., 2006).

Discordances observed for individual polymorphisms, haplotypes, or diplotypes among these diverse studies may be related to differences in sample size, statistical analyses, or selection criteria. However, these discrepancies could also be related to the specific genetic background of the racial groups analyzed. Moreover, as T2DM is a polygenic and multifactorial disease, susceptibility to this disease may be strengthened not only by single polymorphisms, haplotypes, or diplotypes in multiple genes, but also by obesity, lipid profile, poor nutrition, sedentary lifestyle, gender, and other factors (American Diabetes Association, 2013). Hence, future studies should analyze larger samples, consider interracial variations, and

adjust covariates associated with T2DM to obtain more reliable results regarding the role of polymorphisms, haplotypes, or diplotypes of *CAPN10* in T2DM risk.

In conclusion, none of the alleles, genotypes, haplotypes or diplotypes of the 3 polymorphisms analyzed of *CAPN10* were significantly associated with T2DM in our sample of Mexican mestizos, which may be because of the small sample size; thus, further studies should examine the role of *CAPN10* risk alleles in T2DM populations.

ACKNOWLEDGMENTS

Research supported by Universidad Autónoma de Tamaulipas through grant UAT10-SAL-0509 and by Universidad Autónoma de Sinaloa (#grant PROFAPI2010-UAS-100). We thank Dr. H. Rivera for the critical review of the manuscript.

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