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# Association of *CAPN10* SNP-19 with Metabolic Traits in Mexican Patients with Type 2 Diabetes

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**ABSTRACT** The calpain-10 SNP-19 has been associated with diabetes as well as diabetes-related metabolic traits. To analyse the relationship between this polymorphism and anthropometric as well as metabolic traits in Mexican patients with type 2 diabetes, 289 cases and 309 controls were recruited into this study. Body mass index, fasting plasma glucose, glycated haemoglobin, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides were assessed. Genotyping was determined by PCR. Apart from HDL-cholesterol, all the metabolic traits were higher in the patients, but the genotype and allele frequencies were similar in both groups. The analysis of the anthropometric and metabolic traits of the patients based on genotype showed differences in waist circumference ($p<0.01$) and triglycerides ($p<0.01$); both were higher in subjects with *del/del* and *del/ins* genotypes, which suggests an association between *CAPN10* SNP-19 and these parameters in Mexican patients with type 2 diabetes. However, additional studies are necessary to clarify the role of this polymorphism in obesity and metabolic traits.

## INTRODUCTION

Type 2 diabetes (T2D) is a metabolic disease characterized by hyperglycemia, and it is the most common form of diabetes (International Diabetes Federation 2017). In 2017, the prevalence of diabetes worldwide was 8.8 percent in adults aged between 20-79 years, accounting for nearly 425 million affected subjects, a figure expected to increase to 629 million by 2045 (International Diabetes Federation 2017). In Mexico, by 2017, the number of patients with diabetes was estimated at about 12 million people, and the country ranked fifth in the world (International Diabetes Federation 2017). The pathogenesis of T2D involves both genetic and environmental causes. Candidate genes comprise those related to β-cell function, secretion, and the action of insulin, including calpain-10 (*CAPN10*) (Stancakova and Laakso 2016). *CAPN10* gene, located on chromosome 2q37, produces a calcium-dependent, non-lysosomal cysteine protease, and it has been suggested that this protein plays a role in facilitating the actin reorganization required for glucose-stimulated insulin secretion in pancreatic INS-1 cells (Turner et al. 2007). Moreover, genetic and functional data indicates that calpain-10 has an important function in insulin resistance and intermediate phenotypes, which could explain its possible participation in the aetiology of T2D (Turner et al. 2005). In this regard, Horikawa et al. (2000) associated some polymorphisms of the *CAPN10* gene with an increased risk of T2D in

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Mexican American and Northern European populations. However, subsequent studies have not been conclusive about the association between the *CAPN10* polymorphisms and T2D risk, and the findings vary considerably according to the ethnic origin of the analysed populations (Picos-Cárdenas et al. 2015). Further, *CAPN10* polymorphisms have been associated with some diabetes-related metabolic traits in several populations around the world. Specifically, the variant named SNP-19 (rs3842570), which is a 32 base pairs (bp) deletion/insertion in intron 6 (Picos-Cárdenas et al. 2015), has been linked with increased glycated haemoglobin (Shima et al. 2003), obesity (Shima et al. 2003; Ezzidi et al. 2010; Paredes et al. 2010; Mendoza-Lorenzo et al. 2013; Sharma et al. 2013; Arslan et al. 2014; Orozco et al. 2014; Loya et al. 2015), and high cholesterol (Zaharna et al. 2010) in T2D patients.

### Aim

The aim of this study is to analyse the possible relationships between the *CAPN10* SNP-19 genotypes and the anthropometric, lipid as well as glycaemic traits in a sample of Mexican patients with T2D.

## MATERIAL AND METHODS

### Subjects

Five hundred ninety-eight individuals were analysed: 289 unrelated subjects diagnosed with T2D according to American Diabetes Association criteria (American Diabetes Association 2019) and 309 unrelated healthy controls without familial history of T2D in first-degree relatives. All of them were Mexican mestizos residing in Reynosa, Tamaulipas, Mexico. Patients and controls were recruited at two clinics of the Jurisdiction 4 of the Health Secretary in Reynosa, Tamaulipas. Controls were individuals older 40 years with normal fasting plasma glycaemia. Body mass index (BMI) was calculated from the weight and height, and waist circumference was measured in all the subjects. Fasting biochemical profiles, such as plasma glucose (FPG), glycated haemoglobin ($HbA_{1c}$), total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides, were determined in both groups in an analyser AutoKem II (Kontro Lab, Roma, Italia). This study was approved by the ethics committee of each participating institution, and informed consent was obtained from patients and controls prior to enrollment. All the analyses were conducted in accordance with the Helsinki Declaration.

### Genotyping

Genomic DNA was isolated from peripheral blood by the CTAB-DTAB method. The alleles were identified by polymerase chain reaction using the following primers: 5'-GTTTGGTTCTCTTCAGCGTGGAG-3' and 5'- CATGAACCCTGGCAGGGTCTAAG-3' according to previously described conditions (Picos-Cárdenas et al. 2015). Briefly, PCR was performed in a volume of 20 μL containing 50 ng of DNA, 10 pM of each oligonucleotide, 1X PCR buffer, 200 μM of each dNTP, 1.5 mM $MgCl_2$, five percent dimethyl sulfoxide, and 2.0 units of *Taq* Platinum DNA polymerase (Invitrogen, USA). The cycling conditions consisted of initial denaturation at 96°C/12min, 35 cycles at 96°C/30sec, 60°C/30sec, and 72°C/30sec, ending at 72°C/10min. The allele with two repeats (*del*) rendered a fragment of 155 bp, and the allele with three repeats (*ins*) rendered a band of 187 bp (not shown). The allele and genotype frequencies were determined from the observed genotypes.

### Statistical Analysis

The comparison of continuous variables between cases and controls was realized by two-tailed t-Student's test (available at http://vassarstats.net/tu.html). The Hardy-Weinberg equilibrium was determined by the Fisher exact test (available at https://ihg.gsf.de/cgi-bin/hw/hwa2.pl). The analysis of allele and genotype frequencies of both groups was carried out by the chi-square test, and the risk of T2D was calculated by odds ratio and ninety-five percent confidence intervals (available at https://ihg.gsf.de/cgi-bin/hw/hwa2.pl). In the patients sample comparison of the metabolic traits' means among the three groups based on genotype was evaluated by the Kruskal-Wallis test (available at http://vassarstats.net/kw3.html). Moreover,

the difference in the frequencies of overweight and obesity for each genotype was analysed by the chi-square test (available at http://vassarstats.net/fisher2x3.html). The *del* allele was taken as reference, and the level of significance was defined as $p<0.05$.

## RESULTS

Majority of the samples consisted of women (67.1% of the patients and 72.2% of the controls). Age, sex, BMI, waist circumference, and metabolic traits are summarized in Table 1; continuous variables are shown as mean ± standard deviation. The comparison between cases and controls showed significant differences for most of the data tested. Apart from HDL cholesterol, all of them were higher in the patients.

**Table 1: Anthropometric and metabolic traits of patients with T2D and controls**

| *Trait* | *T2D (n=289)* | *Controls (n=309)* | *p value** |
|---|---|---|---|
| Age (years) | 52.16± 9.27 | 50.32± 7.84 | ND |
| Sex (female/male) | 194/95 | 223/86 | ND |
| BMI (kg/m$^2$) | 30.65± 5.63 | 27.62± 4.77 | <0.001 |
| Waist circumference (cm) | 99.89± 15.54 | 90.81± 9.65 | <0.001 |
| FPG (mg/dl) | 157.27± 68.34 | 91.19.0±10.45 | <0.001 |
| $HbA_{1c}$ (%) | 8.31± 2.55 | 5.47± 0.54 | <0.001 |
| Total cholesterol (mg/dl) | 195.12± 44.75 | 186.62±29.07 | <0.01 |
| LDL cholesterol (mg/dl) | 114.48± 46.61 | 111.92±36.43 | 0.41 |
| HDL cholesterol (mg/dl) | 46.76± 15.23 | 50.63±13.28 | <0.01 |
| Triglycerides (mg/dl) | 172.50±129.37 | 133.31±75.66 | <0.001 |

Data are expressed as mean ± standard deviation. BMI: Body mass index; FPG: Fasting plasma glucose; $HbA_{1c}$: Glycated haemoglobin; LDL: Low density lipoprotein; HDL: High density lipoprotein; ND: Not determined; *t-student's test (Available at: http://vassarstats.net/tu.html)

The genotype and allele frequencies are shown in Table 2. The *del/ins* genotype and the *ins* allele were most common in both patients and controls, and the allele frequencies were in Hardy-Weinberg equilibrium ($p=0.81$). Moreover, the genotype and allele frequencies did not vary significantly between the two groups.

The analysis of the means of the anthropometric and metabolic traits in the patients group based on genotype is reported in Table 3. Significant differences were detected only for waist circumference ($p<0.01$) and triglycerides level ($p<0.01$); both were higher in subjects with *del/del* and *del/ins* genotypes. Regarding the frequency of overweight and obesity, the values of eighty three percent, eighty five percent, and eighty nine percent were observed for *del/del*, *del/ins*, and *ins/ins* respectively in the group of patients.

## DISCUSSION

As expected, most metabolic and clinical traits showed significant differences between cases and controls, except for LDL cholesterol. Significant differences between both groups were detected neither in genotype distribution nor in allele frequencies, a result similar to those previously described (Picos-Cárdenas et al. 2015 and references cited therein).

Concerning the main objective of this study, namely the analysis of anthropometric features as well as lipid and glycaemic profile in T2D patients according to genotype, no association was

**Table 2: Genotype and allele frequencies for *CAPN10* SNP-19 in patients and controls**

| *SNP-19* | *Controls* | *Patients* | *OR (95%CI)* | *p value** |
|---|---|---|---|---|
| | | *Genotype* | | |
| *del/del* | 43 | 42 | Reference | |
| *del/ins* | 148 | 146 | 1.01 (0.62-1.64) | 0.97 |
| *ins/ins* | 118 | 101 | 0.88 (0.53-1.45) | 0.61 |
| *Allele* | | | | |
| *Del* | 0.38 | 0.40 | Reference | |
| *Ins* | 0.62 | 0.60 | 0.92 (0.86-1.37) | 0.49 |

*Chi-square test (Available at: https://ihg.gsf.de/cgi-bin/hw/hwa2.pl).

**Table 3: Analysis of anthropometric and metabolic traits according to genotype in 289 patients with T2D**

| *Trait* | *del/del* | *del/ins* | *ins/ins* | *p value** |
|---|---|---|---|---|
| N | 42 | 146 | 101 | ND |
| Age (years) | 52.4±10.3 | 51.5±8.7 | 52.8±9.5 | ND |
| Sex (female/male) | 29/18 | 105/49 | 65/23 | ND |
| BMI (kg/m$^2$) | 30.5±5.4 | 30.8±5.7 | 30.3±4.9 | 0.59 |
| Waist circumference (cm) | 102.7±12.6 | 103.2±10.1 | 94.3±14.7 | <**0.01** |
| FPG (mg/dl) | 161.3±68.7 | 155.4±52.1 | 162.4±63.2 | 0.65 |
| $HbA_{1c}$ (%) | 8.4±2.6 | 8.2±2.1 | 8.5±2.6 | 0.59 |
| Total cholesterol (mg/dl) | 197.3±44.1 | 194.6±49.8 | 197.2±42.6 | 0.87 |
| LDL cholesterol (mg/dl) | 117.2±42.5 | 113.5±51.8 | 115.9±39.1 | 0.21 |
| HDL cholesterol (mg/dl) | 47.2±15.7 | 45.8±13.9 | 47.6±14.2 | 0.63 |
| Triglycerides (mg/dl) | 153.1±89.3 | 188.3±143.6 | 142.4±76.1 | <0.01 |

Data are expressed as mean ± standard deviation. BMI: Body mass index; FPG: Fasting plasma glucose; $HbA_{1c}$: Glycated haemoglobin; LDL: Low density lipoprotein; HDL: High density lipoprotein; ND: Not determined; *Kruskal-Wallis test (Available at: http://vassarstats.net/kw3.html).

found between any SNP-19 genotype and BMI ($p$=0.59 for difference in means, and $p$=0.55 for frequency of overweight and obesity). However, Arslan et al. (2014) related the *ins/ins* genotype with a significantly increased BMI, but only in male diabetic patients in a Turkish population. In addition to the *ins/ins* homozygous, Loya et al. (2015) detected an association between the *del/ins* genotype and a higher BMI ($p$=0.037), and Sharma et al. (2013) found that a risk of increase in BMI is associated with the *ins* allele [OR=1.88 (1.19-2.98), $p$=0.007] in T2D patients contrary to the controls in Brahmin Indians. Moreover, the *ins/ins* genotype was significantly associated with obesity in unselected Japanese subjects (Shima et al. 2003), in Mexican children (Mendoza-Lorenzo et al. 2013), and in Colombian youngsters (Orozco et al. 2014). In contrast, Ezzidi et al. (2010) detected a significant association of *del/del* genotype with overweight [OR=2.07 (1.28-3.33); $p$=0.003] and obesity [OR=1.83 (1.10-3.07); $p$=0.021] in a sample of Tunisian Arab T2D patients. They reported the enrichment of this genotype in the overweight and obese groups compared with lean patients (p=0.041). Pihlajamaki et al. (2006) analysed this polymorphism in non-diabetic offspring of T2D patients and detected no association with intra-abdominal fat in a population of Finnish ancestry. Regarding waist circumference, these results agree with those reported by Loya et al. (2015), who also found an association of *del/del* and *del/ins* genotypes with increased waist circumference ($p$=0.01) in a small sample of T2D patients from a Mexican population.

In this study, triglycerides were significantly higher in patients with *del/del* and *del/ins* genotypes. Similarly, Paredes et al. (2010) reported an association between SNP-19 and hypertriglyceridemia, but they did not specify the linkage of any genotype. Moreover, Carlsson et al. (2004) reported that SNP-43 G/G obese individuals had significantly higher triglyceride levels than individuals carrying the A allele ($p$=0.03). Likewise, Jensen et al. (2006) detected an association between this variant and an increase in fasting serum triglycerides in a dominant model ($p$=0.04). Although the polymorphism is different from that of this study, the above shows that *CAPN10* variants have been associated with increased triglycerides. However, Zaharna et al. (2010) detected a higher total cholesterol in T2D patients with *ins/ins* homozygous compared with T2D patients with other genotypes; the findings of this study do not support such an association.

On the other hand, no association between SNP-19 genotypes and FPG, $HbA_{1c}$, as well as lipid parameters was found in this study, which agrees with some results reported previously (Ezzidi et al. 2010; Arslan et al. 2014). However, Mendoza-Lorenzo et al. (2013) detected increased FPG in *ins/ins* homozygous Mexican children ($p$=0.02), and Shima et al. (2003) found higher $HbA_{1c}$ levels in Japanese subjects with this genotype (p=0.024). Furthermore, in a study by Elbein et al. (2002) involving 700 subjects of

63 families of Northern European ancestry with at least two siblings with T2D, SNP-19 showed significant effects on fasting insulin ($p$=0.04) and insulin resistance by homeostasis model assessment ($p$=0.035); however, the effect on fasting insulin was more marked among obese subjects ($p$=0.0061).

In general, the discordances observed among the different studies may be partly explained by the differences in the genetic background of each population, dietary habits, type of sample analysed, sample size, and statistical analysis criteria. It is unclear how SNP-19 polymorphism could influence anthropometric and metabolic traits in humans; however, a reduced lipolytic sensitivity of the β3-adrenoceptor was reported in *del/del* homozygous human fat cells, which suggests that calpain-10 is involved in the regulation of thermogenesis (Hoffstedt et al. 2002). Besides, there is evidence that calpain-10 plays an important role in regulating obesity and diabetes in mice (Cheverud et al. 2010). However, the *ins/ins* genotype has been associated with reduced calpain-10 mRNA expression in the lymphocytes of obese children (Mendoza-Lorenzo et al. 2013), similar to *CAPN10* SNP-43 G/G homozygous in adipose tissue biopsies (Carlsson et al. 2004) and in skeletal muscle (Baier et al. 2000). However, it could be that the detected risks are the consequences of others variants of *CAPN10* (or their combinations) linked to SNP-19 or of different *loci* in linkage disequilibrium with *CAPN10.*

## CONCLUSION

In conclusion, these findings corroborate the assertion that metabolic and clinical traits are significantly different in T2D patients and controls. Moreover, these observations suggest that *CAPN10* SNP-19 is associated with waist circumference and triglycerides level in Mexican patients with T2D, since both parameters were higher in subjects with *del/del* and *del/ins* genotypes.

## RECOMMENDATIONS

Future studies should include larger samples, evaluation of other polymorphisms and analysis of expression to elucidate the relationship between the *CAPN10* gene and the obesity and metabolic traits in humans.

## LIMITATIONS

The principal limitations of this study are the small size of the analysed samples and the underrepresented male population, which precluded the analysis of sex. Moreover, just one polymorphism was analysed.

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