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GSTT1, Calpain 10 SNP 19 and indices of glycaemia in type 2 diabetes

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Type 2 diabetes mellitus (T2DM) is one of the most widely spread chronic diseases in most countries; the number of diabetic patients is continuously increasing because of urbanization, ageing, population growth, reduced physical activity and increasing prevalence of obesity [1]. Glutathione-s-transferases (GSTs) are a family of antioxidant enzymes that play an important role in decreasing reactive oxygen species and so act as antioxidant defence [2]. They may also contribute to the risk of T2DM although their effects on disease susceptibility are gender specific [3]. Of the 22 GST family members, GST-theta-1 is coded for by *GSTT1* on 22q11.23. *CAPN10* on 2q37.3 codes for Calpain 10, a cysteine protease localized to the cytosol and mitochondria, acting on electron transport chain proteins, causing decreased mitochondrial respiration [4]. *CAPN10* SNP-19 del-allele and *CAPN10* SNP-44 C-allele are risk factors for T2DM [5], whilst *CAPN10* SNP 19 is associated with increased glucose levels in gestational diabetes mellitus [6]. HOMA-IR is a very popular, easy and efficient method in routine clinical practice and studies for patients with T2DM and obesity [7]. We hypothesized links between SNPs in *GSTT1* and *CAPN10* with T2DM and indices of glycaemia.

We tested our hypothesis in a case-controlled study of 100 cases (50 men, 50 women, mean [SD] age 49.5 [7.4] years) with T2DM and 50 unrelated control subjects (25 men, 25 women, age 45.4 [6.4] years: p = 0.194). Cases were recruited from the Department of Experimental and Clinical Internal Medicine at the Medical Research Institute, Alexandria University. Diabetes diagnosis was based on WHO criteria of fasting plasma glucose <7 mmo/L. Approval of the local ethics committee, and informed consent from all the subjects, were obtained. A DNA extraction kit, primers and PCR master mix were purchased from Vivantis Subang Jaya, Selangor Darul Ehsan, Malaysia. Blood glucose kit was purchased from Biosystems. Barcelona, Spain. Insulin ELISA kit was purchased from Raybiotech. Norcross, Georgia, USA, blood glucose and HbA1c were determined by standard clinical chemistry techniques.

A polymerase chain reaction (PCR) was used to genotype *CAPN10* SNP-19 in a total volume of 25 µL containing 50 ng genomic DNA, 1X PCR buffer, 400 µmol/L deoxy nucleotide triphosphate solution (dNTPs), 1.5 Taq polymerase (Vivantis) and 4 µmol/L of each primer *SNP-19* forward primer 5′-GTTT GGTTCTCTTCAGCGTGCAG-3′ and reverse primer 5′-CATGAACCCTGGCAGGGTCTAAG-3′. Thermal cycling conditions were an initial denaturation step at 95 °C for 5 min, 35 cycles at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, and a final extension step at 72 °C for 5 min. PCR products were separated on 3% agarose gel and stained with ethidium bromide (Figure 1). *GSTT1* genotypes were determined by the presence and absence (null) of bands of 480 base pair (bp), respectively, with an internal control of 260 bp. Using this genotyping assay of *GSTT1*, the wild and null genotypes can be clearly categorized [8,9].

Calpain-10 SNP-19 is an insertion/deletion polymorphism and consists of two or three repeats of a 32 bp sequence. The allele 2 (three repeats) was detected as a 178 bp band and allele 1 (two repeats) was detected as a 146 bp band [3]. *GSTT1*-null alleles were analysed using a PCR reaction with beta-globin as an internal control. PCR amplifications were performed in a total volume of 25µL containing 50 ng genomic DNA, PCR buffer, 3 µmol/L MgCl2, 400 µmol/L dNTPs, 1.5 Taq polymerase (Vivantis) and 4 µmol/L of each primer as follows: *GSTT1* forward primer 5′-TTCCTTACTGGTCCTCACATCTC-3′, reverse primer 5′-TCACCGGATCATGGCCAGCA-3′ and β-globin forward primer 5′-CAACTTCATCCAC GTTCACC-3′, reverse primer 5′-GAAGAGCCAAGGACAGGTAC-3′. Thermal cycling conditions were as follows: an initial denaturation step at 95 °C for 5 min, 35 cycles at 94 °C for 1min, 60 °C for 1min and 72 °C for 1 min, and a final extension step at 72 °C for 5 min. The amplification products were size separated on 2% agarose gels and visualized by ethidium bromide staining. The homeostatic model assessment for insulin resistance (HOMA-IR) index was calculated as (fasting blood glucose x fasting insulin)/405 [7]. Data are analysed by chi-squared testing (presented as n and percent) and student's t test (presented as mean with standard deviation).

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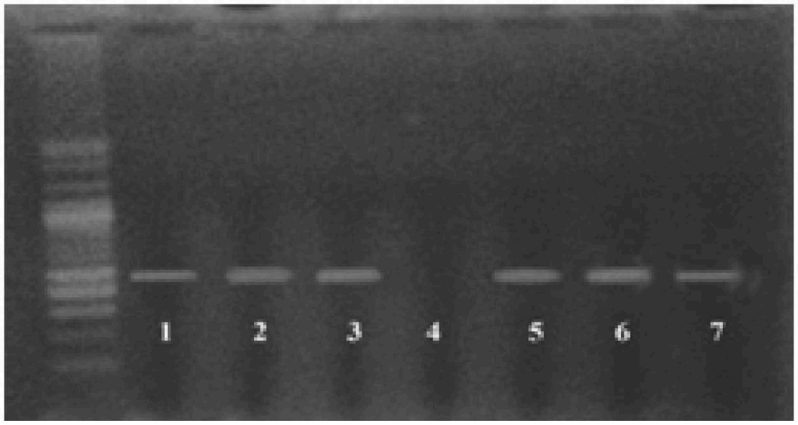

Figure 1. Gel electrophoresis for PCR products of *GSTT1* digestion in patients and controls. Lane 4 is the null genotype: others are positive cases with fragments of 480 bps. Sizing ladder is on the left.

Table 1. *GSTT1* and *CAPN10 SNP19* genotypes, HbA1c and HOMA-IR of controls and T2DM patients.

Item	Controls	T2DM patients	P value
**GSTT1* Null	20% (10)	36% (36)	0.040
GSTT1 Positive	80% (40)	64% (64)	
***CAPN10 SNP19* I	47% (47)	56% (112)	0.141
CAPN10 SNP19 D	53% (53)	44% (88)	
**CAPN10 SNP19* ID	54% (27)	51% (51)	0.348
CAPN10 SNP19 II	20% (10)	30% (30)	
CAPN10 SNP19 DD	26% (13)	19% (19)	
HOMA-IR	1.2 [0.3]	9.4 [2.8]	0.001
HbA1c (%)	5.0 [0.4]	7.3 [0.4]	0.009

*Genotype, **allele. I – insertion, D – deletion. Values between brackets represent number of individuals or alleles.
P value by chi-square or t test.

Table 1 shows genotype, allele, HbA1c and HOMA-IR data. The frequency of the *GSTT1* null genotype (Figure 1) was higher, and the positive genotype lower in the patients with diabetes. There was no difference in the frequency of the *CAPN SNP19* insertion or deletion alleles in the cases or controls. Similarly, there was no difference in the genotype of the insertion/deletion, insertion/insertion or deletion/deletion genotypes. As expected, HOMA-IR and HbA1c of T2DM patients was markedly higher than that of the controls. Within the patient group, HOMA-IR was significantly higher in those with AA *GSTT1* null genotype (7.9 [4.8]) than those with the positive genotype (6.05 [4.4], p = 0.036). Similarly, HbA1c was significantly higher in those with a null genotype (6.9%±1.3) compared to that in patients with a positive genotype (6.4%±1.2, p = 0.043).

There is growing evidence that variation in genes for different isotypes of GST have a role in T2DM. Banerjee et al [10] showed that gene-gene interactions between *GSTM1*, *GSTT1del* and *GSTP1*, and SNPs in genes coding for catalase and superoxide dismutase are very strongly linked with T2DM. Our data add to the literature in that we demonstrate significantly higher frequencies of the *GSTT1* null SNP in T2DM patients compared to control subjects. Furthermore, both indices of glycaemia are higher in the null genotype. Whilst this may have been expected by simple group selection, it nevertheless underlines the link between the null genotype and a major feature of the pathophysiology of diabetes. Wang et al [11] performed a study on Chinese T2DM patients to investigate polymorphism of *GSTT1*, suggesting that the *GSTT1*-null genotype contributes to the T2DM development. They also showed a significant difference in the frequencies of the *GSTT1*-null mutations between the patients and the control subjects. Their results support the notion that *GSTT1* plays a protective role against the development of T2DM. Our results are in agreement with data from Turkish [5], Chinese [11] and Caucasian and Asian [12] populations, despite differences between ethnicity and race.

The relationship between Calpain-10 gene *CAPN10* and the development of T2DM has been investigated in previous studies but findings have varied according to different populations and ethnic groups [5]. Maleki et al found that Calpain-10 SNP-19 is probably associated with T2DM in an Iranian poppulation [13]. Our results showed no significant difference in Calpain-10 SNP-19 polymorphism between T2DM patients and control group which confirm the findings of Picos-Cárdenas et al in a Mexican population [14].

Despite small numbers, our work represents an advance in biomedical science because it shows that the *GSTT1* null genotype frequency is associated with hyperglycaemia in T2DM, whereas there is no difference in the *CAPN10* SNP19 in this condition.

Disclosure statement

No potential conflict of interest was reported by the authors.

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