Association of calpain-10 polymorphisms with type 2 diabetes in the Tunisian population

C. Kifagi a,1, K. Makni a,1, F. Mniit b, M. Boudawara c, N. Hamza c, N. Rekik b, M. Abid b, A. Rebaï d, C. Granier e, F. Jarraya a,f, H. Ayadi a,*  

a “Targets for Diagnosis and Therapeutic in the Human Pathology” Research Unit, Center of Biotechnology of Sfax and Laboratoire International Associé N°135, Sfax, Tunisia  
b Endocrinology Department, Hédi Chaker Hospital, Sfax, Tunisia  
c Laboratory of Analyses, Social National case of Sfax, Sfax, Tunisia  
d Bioinformatics Research Unit, Center of Biotechnology of Sfax, Sfax, Tunisia  
e CNRS FRE3009 SysDiag and Laboratoire International Associé N°135, Montpellier, France  
f Department of Nephrology, Hédi Chaker Hospital, Sfax, Tunisia  

Received 18 October 2007; received in revised form 9 January 2008; accepted 21 January 2008  
Available online 19 May 2008

Abstract

Background. – Genome-wide analyses of the genetic predisposition to type 2 diabetes mellitus (T2DM) in different isolates and populations have identified regions of interest called non insulin-dependent diabetes mellitus (NIDDM) 1, 2, 3 and 4. At the NIDDM1 locus (2q37.3), calpain-10 (CAPN10) encodes for a ubiquitously expressed protease implicated in the two fundamental pathophysiological aspects of T2DM. This is a report of the results of a study of the association of four CAPN10 polymorphisms with T2DM in the Tunisian population.

Participants and methods. – A total of 222 T2DM patients with a diabetes duration of 10 years or more and 206 healthy controls were enrolled to analyze the frequency distribution of four CAPN10 polymorphisms (UCSNP-43, UCSNP-19, UCSNP-110 and UCSNP-63) using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) in the Tunisian population. We also investigated the association of T2DM with different haplotypes and haplotype combinations.

Results. – Only the A allele of UCSNP-43 showed an association with T2DM (odds ratio, OR = 1.86). We also identified a novel combination of haplotypes (121/221) defined by three polymorphisms (UCNSP-43, -19 and -63) that is associated with an increased risk of T2DM (OR = 2.38).

Conclusion. – In this study involving the Tunisian population, we identified genetic variants within CAPN10 that are linked with T2DM and a novel haplotype combination, 121/221, associated with an increased susceptibility to T2DM.

© 2008 Elsevier Masson SAS. All rights reserved.

Résumé

Étude de l’association du gène de la calpaïne-10 avec le diabète de type 2 dans la population tunisienne.

But. – Le criblage du génome réalisé à partir de différents isolats et populations a permis l’identification de plusieurs loci liés au diabète de type 2 (DT2) dénommés non insulin-dependent diabetes mellitus (NIDDM) 1, 2, 3 et 4. Dans le locus NIDDM1, se trouve la calpaïne-10 (CAPN10) qui est une protéase ubiquitaire, impliquée dans la physiopathologie du DT2. Nous rapportons les résultats de notre étude d’association de quatre polymorphismes du gène CAPN10 avec le DT2 dans la population tunisienne.


Résultats. – Nos résultats ont montré que c’est l’allèle A du polymorphisme UCSNP-43 qui a été associé avec le DT2 (odds ratio, OR = 1.86). Nous avons aussi identifié une nouvelle combinaison d’haplotypes (121/221) associée au DT2 (OR = 2.38) définie par les trois polymorphismes UCSNP-43, -19 et -63.

* Corresponding author.

E-mail address: directeur.general@CBS.RNRT.TN (H. Ayadi).

1 The first two authors contributed equally to the elaboration of this work.
1. Introduction

Type 2 diabetes mellitus (T2DM) is a multifactorial disease, influenced by both genetic and environmental factors, that affects approximately 135 million people worldwide. Two major pathophysiological pathways coexist in the disease: impaired insulin secretion and decreased insulin sensitivity in peripheral tissues [1].

The prevalence of T2DM is higher in some racial/ethnic groups such as Pima Indians and Mexican-Americans [2]. Moreover, the concordance in monozygotic twin pairs ranges from 63 to 90%, suggesting that a genetic component plays a crucial role in the development of the disease [3].

Hanis et al. reported a link to a region on chromosome 2q37.3 among Mexican-Americans [4]. They identified a major susceptibility locus, the noninsulin-dependent diabetes mellitus 1 (NIDDM1) region (MIM 601283), located in the 12cM interval between the two markers D2S125 and D2S140. Subsequently, Horikawa et al. identified, by fine mapping and sequencing of the NIDDM1 locus, calpain-10 (CAPN10) as a putative T2DM susceptibility gene [5]. They were also the first to report that homozygosity of the G allele (allele 1) of UCSNP-43, a CAPN10 polymorphism, is associated with T2DM [5].

Moreover, the haplotype combination 112/121, which is defined by UCSNP-43, -19 and -63 (dbSNP ID rs3792267, rs3842570 and rs5030952, respectively) and which conveys the G/G genotype at UCSNP-43, is associated with T2DM [5].

Calpains are ubiquitous calcium-activated proteases that are implicated in many cellular activities, including intracellular signal transduction, neuronal functions and cytoskeletal rearrangements [8]. Over the past decade, it has been suggested that CAPN10 may be implicated in the two fundamental pathophysiological features of T2DM: insulin resistance and impaired insulin secretion [9]. Calpains are thought to play a role in insulin receptor substrate-1 down-regulation [10]. Paul et al. showed that the translocation of insulin-stimulated glucose transporter-4 in adipocytes is decreased by specific inhibition of CAPN10 [11]. In addition, an isoform of CAPN10 has been reported to regulate exocytosis in pancreatic beta cells [12].

To study the association of CAPN10 with T2DM in the Tunisian population, we undertook a case-control study to analyze the frequency distribution of four CAPN10 polymorphisms — UCSNP-43 (G/A), UCSNP-19 (two versus three repeats of 32 bp), UCSNP-110 (A/G) and UCSNP-63 (C/T) — which are located at intron 3, intron 6, exon 10 and intron 13, respectively. We also calculated the frequency of the different haplotypes and combinations of haplotypes, as defined by the four CAPN10 polymorphisms, in T2DM patients and controls.

Our results show that, in the Tunisian population, the 221 haplotype is an at-risk haplotype and 121/221 is a risky haplotype combination with a 2.4- and 2.38-times higher risk, respectively, of developing T2DM.

2. Participants and methods

2.1. Participants

A total of 222 T2DM patients and 206 healthy controls with normal fasting blood glucose levels (<5.5 mmol/L) were recruited at the Hédi Chaker Hospital and the clinique de la Caisse nationale de Sécurité sociale hospital (Sfax, Tunisia). T2DM patients were diagnosed according to World Health Organization criteria [13], although a diabetes duration of 10 years or more was also required to avoid misclassifications. The healthy controls were randomly selected among subjects aged above 45 years old, among whom none had either a personal or family history of diabetes (type 1 or type 2) in first-degree relatives. The controls also presented with neither an impaired fasting glucose nor high glycemia (3.3–6.1 mmol/L) within the last five years (beginning of the blood-sample collection). All subjects gave their informed consent for the collection of DNA samples and the study was approved by the local ethics committee (Hedi Cheker Hospital). The clinical characteristics of the participants are summarized in Table 1.

2.2. CAPN10 SNP genotyping

We selected single nucleotide polymorphisms (SNPs) at four different regions within CAPN10 namely UCSNP-43, -19, -110 and -63.
features of the four UCSNPs associated with three repeats, 187 bp. Names, accession numbers and digestion manner, using an internal control for each digestion procedure. 

All PCR products obtained for UCSNP-43, -110 and -63 were digested at 37°C with the appropriate enzyme and then separated by electrophoresis in 3% agarose gels, stained with ethidium bromide and, finally, scored under ultraviolet light in a blinded manner, using an internal control for each digestion procedure. The UCSNP-19 was scored as followed: two repeats, 155 bp and three repeats, 187 bp. Names, accession numbers and digestion features of the four UCSNPs associated with CAPN10 are shown in Table 2.

### 2.3. Statistical analysis

Statistical associations were made using a chi-square ($\chi^2$) test that compared the distribution of alleles and genotypes in the T2DM patients (cases) and healthy subjects (controls). A $P$ value smaller or equal to 0.05 was considered a statistically significant difference between the two groups. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated according to Woolf’s formula [16].

Inference of haplotypes from genotype population data was performed using the PHASE programme, version 2.0.2 [17,18]. Haploviev (version 3.32) was used to analyze both the linkage disequilibrium (LD) and the Hardy-Weinberg equilibrium of all genotype polymorphisms.

### 3. Results

#### 3.1. Clinical characteristics of the participants

Using the $\chi^2$ test, we compared gender ratio, age, body mass index (BMI), glycated hemoglobin (HbA1C), and the presence of retinopathy and hypertension between T2DM patients and controls. Except for gender ratio and age, we found significant differences in all of the clinical parameters between the two groups ($P<0.05$) (Table 1).

### 3.2. Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD)

To test the equilibrium status, we tested the HWE for UCSNP-43, -19, -110 and -63 in our control-data set and found all markers to be in equilibrium ($P>0.05$) (data not shown). Analysis of the LD for the four SNPs revealed the presence of none. Consequently, all the $r^2$ coefficients were smaller or equal to 0.038 ($P=0.00007$)

#### 3.3. Genotype data

#### 3.3.1. Allelic and genotypic frequencies

To determine the association of T2DM with the four CAPN10 polymorphisms, we analyzed the distribution of their allelic and genotypic frequencies between patients and healthy controls. Only the A allele of UCSNP-43 showed a risk of 1.86 for T2DM (CI: 1.12–3.09; $P=0.01$). The allelic distributions of the three other SNPs did not significantly differ between the two groups ($P>0.05$).

In the population under study, the GA genotype of UCSNP-43 presented a risk of 1.93 for T2DM (CI: 1.09–3.42; $P=0.01$). None of the other genotypes were found to be associated with T2DM in our study.

#### 3.3.2. Haplotype construction

Using the PHASE programme, haplotypes defined by the four different CAPN10 polymorphisms were inferred (UCSNP-43, -19, -110 and -63). For UCSNP-43, the G allele was coded by 1 and the A allele by 2. For UCSNP-19, 1 represented two repeats of the 32 bp allele and 2 represented three repeats. Similarly, the A allele of UCSNP-110 was coded by 1 and the G allele by 2. For UCSNP-63, the C allele was coded by 1 and the T allele by 2.

We identified four haplotypes comprising three SNPs (UCSNP-43, -19 and -63) and five haplotypes made up of four SNPs (UCSNP-43, -19, -110 and -63) that showed a frequency greater or equal to 0.05 in the two groups (Tables 3 and 4). The 121 haplotype was the most common, with a frequency of 0.4. However, only the 221 haplotype (the A allele for UCSNP-43; three repeats of 32 bp for UCSNP-19; C for UCSNP-63) was associated with T2DM (OR = 2.4) (Table 3).

As for the five haplotypes defined by four polymorphisms, the 1211 haplotype (G allele for UCSNP-43, three repeats of...
Table 4
Frequency of haplotypes defined by UCSNP-43, -19, -110 and -63

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency in T2DM</th>
<th>Frequency in controls</th>
<th>( \chi^2 )</th>
<th>( P )</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1111</td>
<td>0.259465</td>
<td>0.295241</td>
<td>1.47</td>
<td>0.22</td>
<td>0.83 (0.61–1.13)</td>
</tr>
<tr>
<td>1112</td>
<td>0.088982</td>
<td>0.080413</td>
<td>0.27</td>
<td>0.6</td>
<td>1.14 (0.68–1.89)</td>
</tr>
<tr>
<td>1121</td>
<td>0.062511</td>
<td>0.068691</td>
<td>0.08</td>
<td>0.77</td>
<td>0.92 (0.52–1.64)</td>
</tr>
<tr>
<td>1211</td>
<td>0.445196</td>
<td>0.454833</td>
<td>0.05</td>
<td>0.81</td>
<td>0.97 (0.73–1.28)</td>
</tr>
<tr>
<td>2211</td>
<td>0.117552</td>
<td>0.058179</td>
<td>9.15</td>
<td>2.0 \times 10^{-3}</td>
<td>2.14 (1.26–3.66)</td>
</tr>
</tbody>
</table>

Table 5
Frequency of haplotype combinations with UCSNP-43, -19 and -63

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency in T2DM</th>
<th>Frequency in controls</th>
<th>( \chi^2 )</th>
<th>( P )</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>111/111</td>
<td>0.1</td>
<td>0.13</td>
<td>1.06</td>
<td>0.3</td>
<td>0.73 (0.39–1.37)</td>
</tr>
<tr>
<td>111/112</td>
<td>0.056</td>
<td>0.053</td>
<td>0</td>
<td>0.97</td>
<td>1.01 (0.41–2.53)</td>
</tr>
<tr>
<td>111/121</td>
<td>0.296</td>
<td>0.348</td>
<td>1.33</td>
<td>0.24</td>
<td>0.79 (0.51–1.21)</td>
</tr>
<tr>
<td>111/221</td>
<td>0.077</td>
<td>0.04</td>
<td>2.77</td>
<td>0.09</td>
<td>2.05 (0.81–5.32)</td>
</tr>
<tr>
<td>112/112</td>
<td>0.007</td>
<td>0.005</td>
<td>0.26</td>
<td>0.6</td>
<td>0.86 (0.13–52.28)</td>
</tr>
<tr>
<td>112/121</td>
<td>0.08</td>
<td>0.063</td>
<td>0.27</td>
<td>0.6</td>
<td>1.21 (0.55–2.65)</td>
</tr>
<tr>
<td>112/221</td>
<td>0.02</td>
<td>0.007</td>
<td>0.26</td>
<td>0.53</td>
<td>1.87 (0.29–14.86)</td>
</tr>
<tr>
<td>121/121</td>
<td>0.209</td>
<td>0.223</td>
<td>0.16</td>
<td>0.68</td>
<td>0.91 (0.56–1.48)</td>
</tr>
<tr>
<td>121/221</td>
<td>0.108</td>
<td>0.05</td>
<td>5.18</td>
<td>0.02</td>
<td>2.38 (1.05–5.48)</td>
</tr>
<tr>
<td>221/221</td>
<td>0.014</td>
<td>0.003</td>
<td>0.87</td>
<td>0.35</td>
<td>2.81 (0.26–70.6)</td>
</tr>
</tbody>
</table>

32 bp for UCSNP-19, A for UCSNP-110 and C for UCSNP-63) was the most frequent. The 2211 haplotype (A for UCSNP-43, three repeats of 32 bp for UCSNP-19, A for UCSNP-110 and C for UCSNP-63) was the only one associated with T2DM (OR = 2.14) (Table 4).

Table 5 shows that, among the 10 haplotype combinations created, only 121/221 was significantly associated with T2DM (OR = 2.38). Haplotype combinations defined by four SNPs showed no significant differences in T2DM patients compared to controls.

4. Discussion

T2DM is a complex disease that involves interaction between genetic and environmental factors. Linkage studies in Mexican-American affected sib pairs identified a major susceptibility locus for T2DM, dubbed noninsulin-dependent diabetes mellitus 1 (NIDDM1), located at 2q37.3 [4]. Within this locus, the \( \textit{CAPN10} \) gene exhibits genetic variations that are associated with T2DM.

Horikawa et al. were the first to report that homozygosity of the G allele of UCSNP-43 was associated with a 1.54-fold higher risk of T2DM in Mexican-American patients [5]. Although similar findings have been reported for other populations [6,7], the present study found the A allele to be associated with T2DM (OR = 1.85), an observation that is in agreement with the results of the Ng et al. study, which reported an association of the A allele of UCSNP-43 with T2DM in the Chinese population (OR = 2.85) [19]. These conflicting results may be due to the different ethnic origins of the populations under study.

In the Tunisian population, the most frequent haplotype is 121 (frequency = 0.45), whereas haplotype 112 is rare (frequency = 0.08). A recent study on \( \textit{CAPN10} \) polymorphisms in 561 individuals from 11 different ethnic groups showed considerable degrees of variability in \( \textit{CAPN10} \) haplotype frequencies across the various populations [20]. Indeed, the 112 haplotype (defined by UCSNP-43, -19 and -63) is common in African (frequency: 0.59–0.82) and Asian populations (frequency up to 0.29), but unusual in white Europeans (frequency: 0.05). On the other hand, the 121 haplotype is not found in African populations, but is relatively common in other ethnic groups (frequency: 0.38–0.74) [21]. In the present study of the Tunisian population, the haplotype frequencies were more similar to those observed for European and Asian groups than to those more commonly found in African populations. This finding may be explained by the fact that the Tunisian population is a mix of ethnic groups of African, Asian and European origins. In fact, the Tunisian population is genetically heterogeneous because, for many centuries, the country was subjected to successive waves of invasions and migrations. The greatest hereditary influence came from the populations that settled in Tunisia during the last few centuries, mainly Muslims from Arabia and other Asian ethnic groups such as Persians, Yemenites (Beni Hilal) and Turks. After their arrival, these groups commingled with the indigenous inhabitants (Berbers).

Horikawa et al. were the first to show that the haplotype combination 112/121, defined by UCSNP-43, -19 and -63, is a “high risk” combination, associated with a 2.8- to 2.55- and 4.97-fold higher risk of T2DM in the Mexican-American, Finnish and German populations, respectively [5]. We did not find this high-risk haplotype combination in our Tunisian population, whereby findings were more similar to those reported for the Caucasian [15], Scandinavian [22,7], Asian [23–25] and Oji-Cree Indian populations [26].

On the other hand, we identified a novel haplotype combination, 121/221, associated with a 2.38-fold higher risk of
T2DM in the Tunisian population. Chen et al. showed that the 221 haplotype, as defined by the UCSNP-43, -56 and -63 polymorphisms, and which contains the A allele of UCSNP-43, is associated with T2DM in West African populations (OR = 3.7) [27]. In a recent study, subjects with the 1221 haplotype, defined by UCSNP-44, -43, -19 and -63, had higher levels of two-hour insulin in the oral glucose tolerance test (OGTT), higher levels of fasting triglycerides, lower rates of whole-body glucose uptake and higher amount of intra-abdominal fat [28]. The haplotypes 221 (UCSNP-43, -56 and -63) and 1221 (UCSNP-44, -43, -19 and -63), which both contain the A allele of UCSNP-43, may be comparable to the 221 (UCSNP-43, -19 and -63) haplotype associated with T2DM in our study. Moreover, a meta-analysis of 21 population-based studies (5013 cases and 5876 controls) and five family-based studies (487 parent–offspring threeomes) showed that the association of the 112/121 haplotype combination with T2DM is found in the former studies, but not the latter [29]. This meta-analysis suggested that carriers of the G/G genotype for UCSNP-43 have a modest risk (OR = 1.19) of T2DM. In addition, Ioannidis et al. observed that the initial findings of Horikawa et al. could not be well replicated in later, larger studies and, therefore, suggested that the Horikawa et al. results may have overestimated the genetic effects [30].

In conclusion, we show that, in the Tunisian population, the A allele of UCSNP-43 is associated with T2DM. We also demonstrate that 121/221 is an at-risk haplotype combination for the A allele of UCSNP-43 is associated with T2DM. We also may have overestimated the genetic effects [30].

Acknowledgement

This work was supported by the Ministry of Higher Education, Scientific Research and Technology of Tunisia.

References

[4] Hanis CL, Boerwinkle E, Chakraborty R, Ellsworth DL, Concannon P. Concordance for type 2 diabetes mellitus in five family-based studies (487 parent–offspring threeomes) and five family-based studies (487 parent–offspring threesomes) associated with T2DM in our study. Moreover, a meta-analysis of 21 population-based studies (5013 cases and 5876 controls) and five family-based studies (487 parent–offspring threeomes) showed that the association of the 112/121 haplotype combination with T2DM is found in the former studies, but not the latter [29]. This meta-analysis suggested that carriers of the G/G genotype for UCSNP-43 have a modest risk (OR = 1.19) of T2DM. In addition, Ioannidis et al. observed that the initial findings of Horikawa et al. could not be well replicated in later, larger studies and, therefore, suggested that the Horikawa et al. results may have overestimated the genetic effects [30].
