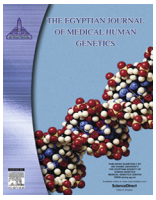
The Egyptian Journal of Medical Human Genetics xxx (2018) xxx–xxx

HOSTED BY

Contents lists available at ScienceDirect

The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com



A study on the association of TCF7L2 rs11196205 (C/G) and CAPN10 rs3792267 (G/A) polymorphisms with type 2 diabetes mellitus in the South Western of Iran

Abdollah Gravand [a], Ali Mohammad Foroughmand [a,*], Mehdi Pourmehdi Boroujeni [b]

[a] Department of Genetics, Faculty of Science, Shahid Chamran University, Ahvaz, Iran
[b] Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

ARTICLE INFO

Article history:
Received 20 October 2017
Accepted 29 January 2018
Available online xxxx

Keywords:
Association study
CAPN10
TCF7L2
Type 2 diabetes mellitus
rs11196205
rs3792267

ABSTRACT

Background: Type 2 diabetes mellitus is a multifactorial and heterogenic disease with a complex etiology. In recent decades the association of a large number of genes has been shown with T2DM. *CAPN10* gene was the first T2DM candidate gene identified through genome-wide screening and positional cloning, and among all identified genes until now, *TCF7L2* gene has shown most association with T2DM. The aim of this study was to investigate the association between *TCF7L2* rs11196205(C/G) and *CAPN10* rs3792267 (G/A) with T2DM in a subset of Iranian population from Khuzestan province. It should be noted that this is the first report of *TCF7L2* polymorphism rs11196205with T2DM in Iran.

Subjects and methods: A case-control association study was performed using 150 T2DM patients and 150 controls. Genotyping for *TCF7L2* rs11196205 was done by Tetra-Primer ARMS-PCR and for *CAPN10* rs3792267 was done by PCR-RFLP Technique.

Results: Statistical analyses were carried out using SPSS version 16. In examining *TCF7L2* rs11196205 based on the genotype GG, results for CG genotype were, 95%CI = (0.5–1.7), OR = 0.92, P-value = 0.79 and for genotype CC were, 95%CI = (0.94–3.92), OR = 1.92, P-value = 0.07. in examining *CAPN10* rs3792267 based on the genotype AA, results for GG genotype were, 95%CI = (0.55–6.8), OR = 1.93, P-value = 0.31 and for genotype GA were, 95%CI = (0.43–5.64), OR = 1.55, P-value = 0.5. So, in both polymorphisms, none of the alleles or genotypes had significant statistical differences between case and control groups (P > 0.05).

Conclusion: Our results showed that *TCF7L2* rs11196205 and *CAPN10* rs3792267 (SNP- 43) polymorphisms are not associated with the risk of T2DM in the studied population.

1. Introduction

One of the most common form of diabetes, constituting ~95% of the diabetic population is Type 2 diabetes mellitus. It is a heterogeneous group of disorders which is associated by hyperglycemia that can occur through mechanisms such as impaired insulin secretion, insulin resistance in peripheral tissues and increased glucose output by the liver [1,2]. Genetic and environmental factors have a strong role in the manifestation of T2DM as a complex genetic disorder. It is becoming an epidemic with increasing prevalence throughout the world [3]. As reported, the prevalence of diabetes has been estimated about 400 million people in the world while >150 million of them seem to be still undiagnosed. It is predicted that this prevalence by 2035 reaches to around 600 million [4]. In Iran, the prevalence of T2DM was about 24% as showed by a systematic review between years 1996 and 2004, and the risk was 1.7% greater for women than for men. According to this report the prevalence of T2DM in Iran seems to be highest amongst developing countries. Previous reports on total urban population of Middle East countries show the prevalence of T2DM as 3.4% in Sudan, 20% in United Arab Emirates, 8.5% in Bahrain, and 12.1% in India [5]. Changing patterns of diet, as well as decrease in physical activity practice

Abbreviations: ADA, American Diabetes Association; ARMS-PCR, Amplification-Refractory Mutation System-Polymerase Chain Reaction; GLP-1, Glucagon-Like Peptide 1; GWAS, Genome-Wide Association Study; PCR-RFLP, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; SNP, Single Nucleotide Polymorphism; T2DM, Type 2 Diabetes Mellitus.

Peer review under responsibility of Ain Shams University.

* Corresponding author.
E-mail address: foroughmand.a@scu.ac.ir (A.M. Foroughmand).

https://doi.org/10.1016/j.ejmhg.2018.01.007
1110-8630/

can be one of the best likely explanations for the growing increase in T2DM prevalence observed over the past decades. However, it is suggested but only in the presence of genetic risk factors, these lifestyle changes may lead to T2DM [6]. Great effort has been made to identify the genes which are associated with T2DM, and so far, several novel susceptibility genes have been uncovered across several ethnic groups, Using GWAS and candidate gene approaches [7,8].

Common variants in *TCF7L2* (transcription factor 7-like 2) gene have been identified as the strongest genetic risk factors for T2DM in multiple ethnic groups [9]. Initially, strong association of *TCF7L2* with T2DM was reported in Icelandic people and then subsequent replication has been found in U.S and Danish populations [3]. *TCF7L2* gene which spans about 215.9 Kb with 17 exons is located on chromosome 10q25.3. This gene encodes a transcription factor which is involved in the wnt signaling pathway. *TCF7L2* gene which affects insulin secretion and glucose production, is expressed in many tissues including fat, liver and pancreatic islets of Langerhans. It has been shown that TCF7L2 gene plays a central role in coordinating the expression of proinsulin and its subsequent processing to form mature insulin [10,11]. rs11196205 at the *TCF7L2* gene is located in intron 4 [12].

Among the >40 genes related to T2DM (OMIM, 2013), the association of *CAPN10* (calpain-10) gene was initially reported with diabetes in Mexican-Americans people [13]. Association of *CAPN10* gene polymorphisms in development of T2DM in multiple ethnic groups in several case-control studies has been indicated. *CAPN10* gene is located on chromosome 2q37.3, and with 15 exons spanning 31 kb, which encodes a 672 amino-acid intracellular protease [14]. Similar to other calpains, *CAPN10* consists of an isoform-specific large subunit and a common small subunit, and was shown to act as intracellular calcium-dependent cysteine proteases in calcium-regulated signaling pathways [15]. Although *CAPN10* is highly expressed in heart, brain, liver, kidney and pancreas, its main role is in tissues such as skeletal muscle. Howbeit insulin secretion, insulin action, insulin stimulated glucose transport and insulin stimulated glycogen synthesis are the significant metabolic activities related to the disease of an individual, evidences from the studies show that there are susceptibility genes controlling these metabolic activities and *CAPN10* and its variants contribute to the metabolic activities and T2DM [16]. rs3792267(G/A) at the *CAPN10* gene is located in the third intron [14].

The aim of this study was investigation of association between rs11196205(C/G) at TCF7L2 gene and rs3792267(G/A) at CAPN10 with T2DM in a population from Khuzestan province, Iran.

2. Subjects and methods

2.1. Subjects

The type of study was case-control and a total of 300 individuals, including 150 unrelated adult T2DM patients (77 men and 73 women; age 52.51 ± 9.15 years) who were selected from among those referred to the Diabetes Clinic and Golestan Hospital in Ahvaz, and 150 unrelated controls (71 men and 79 women; age 56.49 ± 7.18 years) who were selected from among people referred to Valiasr Hospital in Khoramshahr and Golestan Hospital of Ahvaz (Table 1). T2DM criteria were based on the standard of ADA. According to which FPG $\geq$ 126 mg/dl or 2-hPG (2h Plasma Glucose) $\geq$ 200 mg/dl or RBG (Random Blood Sugar) $\geq$ 200 mg/dl to be characterized as diabetic. People with diabetes were chosen as samples that were taking medicines to treat diabetes. For control individuals, lack of history of diabetes in the subjects and among their first-degree relatives was carefully monitored. The case and control subjects were living in Khuzestan province. The project has been approved by especial committee genetic division of Shahid Chamran university for considering the ethical issues of patients and controls who participated in sampling. Before blood sampling, both diabetic patients and healthy subjects signed the consent form to participate in this study. The present study has been carried out in accordance with the Code of Ethics of the world Medical Association (Declaration of Helsinki) for experiments in humans.

2.2. DNA extraction and genotyping

Leukocyte Genomic DNA was extracted from whole blood samples by non-enzymatic salting out method. All participants were genotyped for two SNPs including *TCF7L2* rs11196205 (C/G) and *CAPN10* rs3792267 (G/A).

2.2.1. TCF7L2 rs11196205

Genotyping for rs11196205 was carried out using the TETRA ARMS PCR technique and following primers were used: forward inner primer: 5′-CTGAAAGTTCTCAACATTTATAACTGCC-3′ and reverse inner primer: 5′-CAACCATAACTCTCTTACATACTGGTC-3′ and forward outer primer: 5′-TAGATTGTCTCCTTTTGTTTCTGCTAC-3′ and reverse outer primer: 5′-TAAACATCTGACCTTGA AGCCTACC-3′.

Master Mix PCR was provided from Ampliqon Company. Using Bio-Rad thermal cycler, each PCR was performed in a volume of 25 μl, including 2 μl of genomic DNA, 0.75 μl of each external primer, 2 μl of each internal primer, 5 μl of distilled water, and 12.5 μl of Master mix. The size of DNA fragments amplified with these four primers for *TCF7L2* (434 bp control fragment, 253 bp C allele, 235 bp G allele), was suitable for separation on 3% agarose gel. To determine the size of DNA fragment in gel the 50 bp Ladder was used.

2.2.2. CAPN10 rs3792267

rs3792267 was genotyped using PCR-RFLP Technique. For this purpose, the part of *CAPN10* gene that contains intended polymorphism, was amplified by PCR technique using the primer pair 5′-CACGCTTGCTGTGAAGTAATGC-3′ (forward) and 5′-TGATTCC

Table 1
Demographic and clinical characteristics of the cases and controls.

Characteristics		Cases	Controls	P-value
Male		77(51.3%)	71(47.3%)	P > 0.05
Female		73(48.7%)	79(52.7%)	P > 0.05
Ethnicity	Arab	98(65.3%)	96(%64)	P > 0.05
	non-Arab	52(34.7%)	54(%36)	P > 0.05
Mean Age (years)		52.51 ± 9.15	56.49 ± 7.18	P < 0.01
Diabetes duration (years)		6.32 ± 3.97	–	–
Mean B.M.I (kg/m^2)		28.9 ± 5.02	26.49 ± 4.52	P < 0.001
FPG (mg/dl)		172.15 ± 81.4	91.37 ± 6.29	P < 0.001
HBA1c(%)		8.31 ± 2.33	–	–
TG (mg/dl)		144.76 ± 55.63	122.81 ± 56.96	P < 0.001
TC (mg/dl)		177.9 ± 48.54	184.21 ± 53.18	P > 0.05

Please cite this article in press as: Gravand A et al. . Egypt J Med Hum Genet (2018), https://doi.org/10.1016/j.ejmhg.2018.01.007