Cloud Publications
International Journal of Advanced Diabetes Research
2013, Volume 1, Issue 1, pp. 16-22, Article ID Med-44

Research Article | Open Access

Calpain 10 Gene Polymorphism and Its Association in Cardiomyopathy and Type 2 Diabetes

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Publication Date: 31 July 2013

Article Link: http://medical.cloud-journals.com/index.php/IJADR/article/view/Med-44

Abstract Type 2 diabetes mellitus (T2DM) is genetically defined as a complex disease caused by the interaction of environment and genes, which are located in different regions from the human genome. Cardiomyopathy, a prominent cardiovascular complication, has been recognized as a micro vascular disease that may lead to heart failure. Calpain 10 (CAPN10) is a susceptibility gene for the disease, located in 2q37.3. Calpains are non-lysosomal calcium dependent cysteine proteases that participate in insulin secretion and action. Polymorphisms in the calpain- 10 gene have been shown to increase the risk for type 2 diabetes and cardiomyopathy. In this research, it has been postulated that SNP- 19 of CAPN10 gene was associated with T2DM and cardiomyopathy. It has been analyzed 30 diabetic patients, 20 cardiomyopathy patients and 10 healthy controls. CAPN10 SNP-19 (Insertion/deletion) polymorphism genotyping was done by PCR. The results showed association of SNP19 with T2DM (χ^2 = 13.19 & p = 0.001) and cardiomyopathy (χ^2 = 5.00 & p = 0.029). In conclusion, results from the present study indicate a significant association of SNP19 in CAPN10 gene to T2DM and cardiomyopathy.

Keywords *CAPN10 Gene, Single Nucleotide Polymorphism 19, Type 2 Diabetes Mellitus*

1. Introduction

Diabetes mellitus (DM), long considered a disease of minor significance to world health, is now taking its place as one of the main threats to human health in the 21st century [1]. Developing countries such as India have had the maximum increases in the last few years. The current prevalence of type 2 diabetes is 2.4% in the rural population and 11.6% in the urban population of India. It has been estimated that by the year 2025, India will have the largest number of diabetic subjects in the world [2]. Cardiomyopathy, a prominent cardiovascular complication, has been recognized as a microvascular disease that may lead to heart failure [3]. Pathogenesis of cardiomyopathy involves vascular endothelial cell dysfunction, as well as myocyte necrosis. Cardiomyopathies are a heterogeneous group of diseases of the myocardium associated with mechanical and/or electrical

dysfunction that usually (but not invariably) exhibit inappropriate ventricular hypertrophy or dilatation and are due to a variety of causes that frequently are genetic [4].

Genome wide association studies have identified a number of genes contributing to type 2 diabetes predisposition [5-8], including calpain10 (CAPN10) [9-12], a ubiquitously expressed protease [13] that serve as an intracellular calcium-dependent cysteine proteases [14]. CAPN10 protein also regulates insulin secretion [12, 15-17] and insulin-mediated glucose metabolism. Calpain-10 (CAPN10) is the first T2DM susceptibility gene to be identified through a genome scan, with polymorphisms being associated with altered CAPN10 expression [18]. The highest expression of CAPN10 mRNA is found in human heart, followed by the pancreas, brain, liver and kidney. CAPN10 is located on chromosome 2q37, consists of 15 exons spanning 31 kb [19].

Recent studies have shown that the variation in CAPN10 (MIM 605286), the gene encoding calpain10, affects susceptibility to type 2 diabetes mellitus in Mexican Americans and in two northern-European populations [20]. SNP-19 was also found to be associated with measures of insulin action in Pima Indians with normal glucose tolerance, suggesting that calpain-10 increases susceptibility to type 2 diabetes through its effects on the oxidation of glucose in skeletal muscle [21]. The goal of this study is to test role of calpain10 gene especially in the insertion/deletion (I/D) polymorphism in cardiomyopathy patients and in type 2 diabetic patients in Indian patients.

2. Materials and Methods

2.1. Participants

The case-control study comprised of 60 participants allocated into 3 groups. The first group was healthy participants (n = 10); the second group was type 2 diabetes patients (n = 30); the third group was cardiomyopathy patient (n=20).

2.2. Genomic DNA Extraction

Peripheral blood leucocytes taken from controls and patients were used for genomic DNA extraction by phenol-chloroform extraction method [22]. Genotypes were determined by a Polymerase Chain Reaction (PCR). For the purposes of this study, information included age, sex and fasting blood glucose, postprandial sugar, HbA1c, serum cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, serum triglycerides, creatinine, blood urea and CRP level.

2.3. Genotyping of the SNP19 of the CAPN10 Gene

DNA fragments of the SNP-19 (CAPN10-g.7920indel32bp) were amplified with the following primers.

(1) Forward 5’-GTTTGGTTCTCTTCAGCGTGGAG-3’
(2) Reverse primers 5’- CATGAACCCTGGCAGGGTCTAAG-3’ [20].

PCR was performed in a 15µl reaction mixture, containing 10x PCR buffer, 10mmol of each dNTP, 250 nmol of each primer/liter, 3 U/µl of Taq polymerase, and 40 ng of genomic DNA. The cycling conditions were 94°C for 12 min; 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and 72°C for 10 min. The PCR products were separated on a 2% agarose gel.

2.4. Statistical Analysis

The Hardy-Weinberg equilibrium for genotype and allele distribution of SNP19 in controls and patients were compared by chi-square test. The allelic frequencies between the different groups were found

out by the JavaStat- 2 way contingency table analysis. One-way ANOVA test were used for comparison of the biochemical data and are presented as mean ± S.E. Chi-square test was used to perform the genotype incidence and Fisher's exact test was used to calculate 95% confidence interval (CI) and odds ratios(OR).

3. Results and Discussion

Biochemical parameters of healthy controls and all groups are listed in Table 1. The biochemical values of all the patients belonging to different groups were analyzed by using one-way ANOVA method. p-values <0.005 were considered significant.

The p-values of HbA1c and blood urea in comparison of control with other groups were found to be significant. P-value for CRP level and HDL cholesterol was found to be significant in comparison of control with cardiomyopathy. Between control and other two groups the p-values of fasting sugar, postprandial sugar, serum cholesterol, serum triglyceride, creatinine, LDL & VLDL were insignificant. According to Table 1.0 CRP level and HDL cholesterol can be determined as sensitive biochemical marker for cardiac dysfunction.

The PCR products were separated on a 2% agarose gel. allele 1 (two repeats of 32-bp sequence) was 155 bp, and allele 2 (three repeats) was 187 bp. Genotype frequency and allelic frequency of calpain10 gene (SNP-19) was described in Table 2. The genotype frequency of cardiomyopathy subjects was found to be 20(100%) for heterozygous (allele 1 and 2), genotype frequency of T2DM subjects was found to be 10(33.3%) for allele 1 homozygous and 20(66.6%) for heterozygous while genotype frequency of control subjects was 4(40%) for heterozygous (allele 1 and 2) and 6(60%) for allele 2 homozygous.

The allele frequency was 20(50%) for allele 1 and 20(50%) for allele 2 in cardiomyopathy subjects and in T2DM subjects allele frequency was 40(66.6%) for allele 1 and 20(33.3%) for allele 2. The allele frequency was 4(20%) for allele 1 and 16(80%) in control subjects.

From the logistic regression analysis for calpain10 gene (SNP19) polymorphism (Table 3), p-value (p<0.005) was found to be significant in both cases when control subjects were compared with T2DM and cardiomyopathy subjects.

We have tested SNP19 polymorphism in CAPN10 to test role of calpain10 gene especially in the cardiomyopathy patients and in type 2 diabetic patients in Indian patients.

We determined by our study that: CAPN10 gene (SNP-19) I/D polymorphism was associated with increased risk of T2DM and cardiomyopathy (p values were 0.001 and 0.029 respectively).

Table 1: *Biochemical Parameters of Diabetes and Cardiomyopathy*

Biochemical Parameter	**Groups**		
	Group-1 Control (n=10)	**Group-2 T2DM (n=30)**	**Group-3 Cardiomyopathy (n=20)**
Fasting sugar (mg/dL)	96.10±3.08	118.9±6.63 NS[a]	110.8±7.22 NS[a]
Postprandial sugar (mg/dL)	139.8±2.63	153.9±10.55 NS[a]	176.3±13.8 NS[a]

HbA1c (%)	4.8±0.17	7.0±0.11 P<0.001[a]	6.88±0.18 P<0.001[a]
Serum cholesterol(mg/dL)	144.4±5.05	161.7±8.81 NS[a]	178.4±8.32 NS[a]
Serum triglyceride(mg/dL)	136.8±4.20	148.4±7.17 NS[a]	127.55±4.1 NS[a]
CRP level (mg/dL)	3.1±0.45	3.40±0.27 NS[a]	13.9±1.21 P<0.001[a]
HDL cholesterol(mg/dL)	24.8±1.58	22.13±1.56 NS[a]	42.9±7.44 P<0.001[a]
LDL cholesterol(mg/dL)	117.05±2.96	114.46±2.23 NS[a]	117.05±9.76 NS[a]
VLDL cholesterol(mg/dL)	27.54±1.08	29.64±1.42 NS[a]	25.8±3.51 NS[a]
Creatinine (mg/dL)	1.02±0.03	1.0±0.04 NS[a]	1.17±0.02 NS[a]
Blood urea (mg/dL)	21±1.26	30.83±1.17 P<0.001[a]	27.55±1.78 P=0.038[a]

Note: In Table 1 values represent the mean ± SE. Statistical significance between different groups were evaluated by one way ANOVA method. P values < 0.05 were considered significant. The mean difference is significant at the 0.05 level.

Where a = comparison of control with group T2DM & Cardiomyopathy

Table 2: *Genotype and Allelic Frequency in all Subjects of Calpain10 Gene (SNP-19)*

Groups	**Genotyping Results**			**Allelic Frequency**	
	1/1	1/2	2/2	Allele-1	Allele-2
Control	0	4(40%)	6(60%)	20(50%)	20(50%)
T2DM	10(33.3%)	20(66.6%)	0	40(66.6%)	20(33.3%)
Cardiomyopathy	0	20(100%)	0	4(20%)	16(80%)

Table 3: *Logistic Regression Analysis for SNP-19 Polymorphism in Cardiomyopathy and T2DM*

Groups	**P- Value**	**CHI Square Value**	**Odd Ratio**	**95% CI Value**
Control v/s T2DM	0.001	13.199	0.125	0.030-0.474
Control v/s Cardiomyopathy	0.029	5.000	0.250	0.058-1.002

Note: p- value < 0.05 Odd ratio > 1 and 95% confidence interval > 1 is significant.

Our CAPN10 SNP19 polymorphism finding was supported by a recent study performed by I. Ezzidi et al., (2010) [23]. They determined positive association of UCSNP-19 polymorphism with increased risk of T2DM as evidenced by homozygous 2/2 genotype frequency in T2DM patients, and by enrichment

of the UCSNP-19 homozygous variant in overweight and obese T2DM patients. UCSNP-19 has also been implicated in Insulin sensitivity in Finnish [24], Northern European [25], Scandinavian [26] & Spanish subjects [27].

In addition to T2DM, the variants in CAPN10 have been associated with polycystic ovary syndrome [26], obesity [28, 25] and the metabolic syndrome [29], which share some pathophysiological traits that also underlie T2DM (e.g. insulin resistance and/or compensatory hyperinsulinemia). Intriguingly, biological data, mostly from in vitro studies, point to a role of CAPN10 in several key pathways for the pathophysiology of T2DM including insulin-mediated glucose metabolism [6, 10], insulin production and release in pancreatic β cell [6, 11-13] and thermogenesis [14].

4. Conclusion

Our results suggested significant association of CAPN10 gene I/D (SNP19) polymorphism in Cardiomyopathy patients and in type 2 diabetic participants. In future large-scale, well designed studies using a prospective design and population-based controls are needed to either reliably confirm or conclusively refute the postulated effect of CAPN10 gene on Type 2 diabetes mellitus and cardiomyopathy.

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