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Co-existence of risk and protective haplotypes of Calpain 10 gene to type 2 diabetes in the eastern Indian population

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Abstract

Linkage and association studies have detected a role for Calpain-10 *(CAPN10)* polymorphisms in susceptibility to T2DM in many populations. This study aimed to evaluate possible associations between three SNPs in the *CAPN10* (UCSNPs -43, -19 and -63) gene and T2DM in the east Indian population. The distribution of genotype frequency of UCSNP-63 varied significantly between T2DM patients and controls under a dominant model. The uncommon (T) allele (OR = 3.74, 95% CI: 1.44-9.7) of the UCSNP-63 and haplotype 112 (OR = 3.4, 95% CI: 1.17-9.9) were associated with increased risk of T2DM. On the contrary, the most common haplotype 121 (OR = 0.70 95% CI: 0.50-0.99) was associated with a reduced risk for T2DM. In our population a novel 111/112-haplotype combination created by the *CAPN10* UCSNP-43, -19 and –63 was associated with risk of T2DM. Haplotypes 112 and 121 with opposite genetic influences also co-exist in our population.

Keywords

type 2 diabetes, Calpain 10, single nucleotide polymorphisms, association study, haplotypes, eastern India

Introduction

T2DM is a multifactorial metabolic disorder having both genetic and non-genetic determinants with genetic components following an uncertain mode of inheritance.[1] The problem of diabetes is growing in epidemic proportions throughout the world and the greatest increase in prevalence is expected to occur in Asia and Africa.[2] Several lines of evidence suggest that the aetio-pathogenesis of the common form of T2DM includes a strong genetic component contributed by several common genetic variants, each with relatively modest effect, which act in combination with each other and with environmental and lifestyle triggers. Genetic variants that have shown to have an association with type 2 diabetes include polymorphisms in Calpain 10 (*CAPN10*),[3] peroxisome proliferator-activated receptor γ (PPARG-γ), [4] ATP-sensitive inwardly rectifying potassium channel subunit Kir6.2 (KCNJ11),[5] hepatocyte nuclear factor 4-α (HNF4-α/MODY1)[6] and transcription factor-7-like 2 (TCF7L2)[7] genes. Several genes, namely winged helix/forkhead transcription factor (FOXA2),[8] transcription factor-7-like 2 (TCF7L2), insulin-like growth factor binding protein 2 (IGFBP2), peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC-1α Thr394Thr and Gly482Ser variants), fat mass obesity-associated gene (FTO),[9] apolipoprotein E (APOE) (Hha1) and angiotensin-1 converting enzyme (ACE) (I/D),[10] and IL-4 and IL-1RN[11] cytokine genes have been investigated among different Indian ethnic groups.

CAPN10 gene is one of the widely studied loci, initially identified by Horikawa *et al.*[12] as NIDDM1 (non-insulin dependent diabetes mellitus 1), in Mexican Americans from Starr County, TX. It is a non-lysosomal neutral cysteine protease expressed in many tissues, including skeletal muscle, liver and pancreatic islets, and is involved in proinsulin processing, insulin secretion and insulin resistance. Numerous single nucleotide polymorphisms (SNPs) in *CAPN10* have been identified and studies investigating the role of genetic variations in *CAPN10* with T2DM in different populations have yielded variable results. In this study we investigated the role of three *CAPN10* SNPs (UCSNP-43, -19 and -63) at the level of genotype, haplotype and haplotype combination in a sample of 200 eastern Indian patients with T2DM and

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Table 1. Allele frequency, standard deviation and p-value of the Hardy-Weinberg chi-square test (HWE-P) and comparison of allele frequencies of the 3 Calpain-10 SNPs in east Indian T2DM patients and control samples.

CAPN10 SNPs	T2DM (n=200)			Control (n=100)			Comparison of allele frequency	
	Frequency	SD	HWE-P	Frequency	SD	HWE-P	p	OR (95%CI)
UCSNP-43 *(A)*	0.195	0.0012	0.369	0.19	0.002	0.516	0.83	0.97(0.63-1.49)
UCSNP-19 *(Deletion)*	0.442	0.0017	0.112	0.4	0.002	1.0	0.34	0.84(0.59-1.19)
UCSNP-63 *(T)*	0.088	0.0008	0.095	0.025	0.0008	1.0	0.003	3.74(1.44-9.69)

The mean frequency±SD of the minor allele of UCSNP-43, -19 and -63 of *CAPN10* in the eastern Indian study population. 'n' denotes the sample size in each group. The nucleotide written in italics denotes the minor allele whose frequency is given in the table.

100 normoglycemic volunteers collected from a city hospital of Kolkata.

Material and methods

Subjects

A total of 200 T2DM patients (male = 108, female = 92) defined by World Health Organisation criteria participated in this study. Blood samples were also collected from 100 age and sex matched healthy volunteers (male = 54, female = 46) as controls. The 2 h post glucose tolerance test of blood sugar level (after a 75 g glucose load) for diabetic patients is greater than 200 mg/dL (11.11 mmol/L) while the value is less than 140 mg/dL (7.8 mmol/L) for control healthy subjects (without any family history of diabetes or hypertension). The normal glycated haemoglobin (HbA1c) value was less than 6% and that for a diabetic patient was greater than 6%. None of the subjects (both control and patients) were smokers. The individuals belonging to both the groups were unrelated and lived in the eastern Indian province of West Bengal. The study was approved by the Calcutta University Biosafety and Ethics Committee. Informed consent was obtained from all participants.

Genotyping

Genomic DNA isolated from the peripheral blood lymphocyte by the salting out method was used for genotyping of CAPN10 polymorphisms by PCR coupled RFLP method.[13]

Statistical analysis

Hardy-Weinberg equilibrium was tested using the χ^2 goodness-of fit test in T2DM (cases) and healthy subjects (controls) using Haploview (version 3.32).[14] Risk assessment for genotype(s) for all the three loci was performed under co-dominant, dominant and recessive models. Fisher's exact test was applied to examine for differences in allele frequencies in cases and controls. Haplotype frequencies were estimated using Haploview separately in T2DM patients and controls. The frequency of each haplotype was compared between cases and controls against rest of the others pooled as a group as well as with respect to a reference one to estimate the ORs and 95% CIs using a two-way contingency table method. The expected frequencies of diplotype combination were estimated using the Hardy- Weinberg principle. The frequencies of these haplotype combinations in cases and controls were compared with that of the most prevalent diplotype taken as reference. The p-values of at most 0.05 (two-tailed) were considered a statistically significant difference between the two groups.

Results

A total of 200 unrelated patients with T2DM and 100 non-diabetic controls participated in this study. The patients had a mean history of diabetes of 13 years on an average. They had significantly higher levels of post-prandial blood glucose level (12.6 mmol/L) and percentage of glycated haemoglobin (7.32%) compared to those of the control subjects.

Three *CAPN10* SNPs, namely UCSNP-43, -19 and -63, were genotyped in all the 300 study participants. The distribution of allele frequencies for these loci did not significantly deviate from Hardy-Weinberg equilibrium for both the diabetic and control groups (p>0.05) **(**table 1**)**. There were no significant differences in genotype frequencies for UCSNP-43 and -19 between diabetic patients and non-diabetic controls. However, a difference was found in the distribution of genotype frequencies under co-dominant (p-value = 0.025) and dominant (p-value = 0.0079) models for UCSNP-63 between T2DM and normoglycemic individuals, although the former disappeared after BonFerroni correction (table 2). The allele frequencies for UCSNP-43 and -19 did not vary significantly between the two groups while the minor T-allele of UCSNP-63 was found to present

Table 2. Genotype risk assessment under codominant, dominant and recessive models.

CAPN10 SNP	T2DM genotypes			Control genotypes			p-value		
	1/1	½	2/2	1/1	1/2	2/2	Codominant	Dominant	Recessive
UCSNP -43	132	58	10	64	34	2	0.35	0.80	0.35
UCSNP-19	33	111	56	16	48	36	0.35	0.18	1.00
UCSNP-63	169	27	4	95	5	0	0.03	0.008	0.17

The allele-labelling scheme is followed according to Horikawa *et al.*[12]
SNP-43: genotype 1/1, G/G; genotype 1/2, G/A; genotype 2/2, A/A. UCSNP-19: genotype 1/1, two repeats of 32-bp sequence/two repeats of 32-bp sequence (DD); genotype 1/2, two repeats of 32-bp sequence/three repeats of 32-bp sequence (ID); genotype 2/2, three repeats of 32-bp.sequence/three repeats of 32-bp sequence (II). UCSNP-63: genotype 1/1, C/C; genotype 1/2, C/T; genotype 2/2, T/T.

a risk of 3.74 for T2DM (CI: 1.4-9.7; p=0.003) in our sample (table 1**)**.

Haplotypes pertaining to three *CAPN10* SNPs and maximum likelihood estimates of their frequencies were obtained using Haploview (table 3). 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. For UCSNP-63, the C-allele was coded by 1 and the T-allele by 2. A total of eight haplotypes were observed in the patients while six were found in the control samples. The three most common haplotypes are 121, 111 and 221 (table 3). The two haplotypes (122 and 212) that were absent in the control samples were present in very low frequencies in the patients. For association analysis, each *CAPN10* haplotype was compared separately with the most frequent one, which was taken as reference haplotype (haplotype 121), and also with the combined frequency of the remaining haplotypes. The frequency of 112 (OR = 3.4, CI: 1.17-9.9) varied significantly between patients and controls in both comparisons. In contrast, the most common haplotype 121 was present in a higher proportion in control samples compared to cases (OR = 1.42 CI: 1.005-1.99) when compared with the pooled ones.

The haplotypes of *CAPN10* locus were found to be in Hardy-Weinberg equilibrium. We determined the expected frequencies of various haplotype combinations for cases and controls (table 3). Of the 12 different haplotype combinations that were detected in both groups, the most frequent was 111/121. Other notable combinations (frequency $\geq$ 10%) were 111/111, 111/221, 121/121 and 121/221. With respect to the reference combination of 111/121, we found that two haplotype combinations, namely 111/112 and 221/112, were in marginally higher proportion in T2DM patients (table 3).

Discussion

CAPN10 had been identified as a susceptibility gene in T2DM. Since its discovery upon genome-wide scanning followed by positional cloning, a number of studies[12] have attempted to evaluate the genetic association of *CAPN10* with type 2 diabetes in different populations. The present study aimed to investigate the association of genetic variations on the *CAPN10* gene with the development of the disease in individuals inhabiting eastern India. Of the three loci studied, UCSNP-63 presented 3.48 and 3.74 fold increase of the risk of diabetes with respect to the genotype and the allele frequencies, respectively. An increased risk of diabetes was also detected for haplotype 112 in our sample. The effect of 112 haplotype probably reflects the contribution of the T-allele of UCSNP-63 for type 2 diabetes. Haplotype 112 is associated with an increased risk in the presence of the 121- haplotype in Mexican Americans, African Americans and South Indians with type 2 diabetes.[3,12] We could not detect any association of 112/121 combination with diabetes in our population, indicating the diverse ethnic background of Indian populations. However, 112/111 haplotype combination was present in marginally higher frequency in patients when compared to the reference haplotype combination of 111/121. Haplotype 121 is the most prevalent haplotype among eastern Indians and others as well.[15] In our sample, 121 haplotype is present in statistically higher proportion in the normoglycemic group compared to the T2DM patients. Due to the two opposite trends of statistical association of the haplotypes 112 and 121 towards T2DM, the 112/121 haplotype combinations did not differ significantly between the case and control groups in our study.

Haplotype 112 has been detected as a risk haplotype in many other studies.[3,12] Our finding that haplotype 112 in combination with 111 offers a risk of diabetes is a novel one. The heterozygous combinations of haplotype 111 with 121 and 221 have been found to be associated with high risk of T2DM among Koreans, OR = 2.58 (1.6–4.16) and Utah-Caucasian, OR = 1.48 (1.06-1.91),[16,17] while an increased risk of diabetes has been detected for homozygous combination of haplotype 121 in a Polish sample.[15] Such divergence observed among studies can be related to the diverse ethnic background of the population under study or to random chance due to a low population frequency of the variant allele.

Table 3. Haplotype frequencies and association analysis in the east Indian population.

Haplotype UCSNPs -43/19/63	Estimated haplotype frequencies		p [a] OR (95% CI)	p [b] OR (95% CI)
	T2DM	Control		
111	0.339±0.0006	0.324±0.001	0.783 1.06 (0.74-1.53)	0.23 1.27 (0.86-1.88)
112	0.065±0.0001	0.017±0.00008	0.016 3.40 (1.17-9.89)	0.0079 3.96(1.34-11.72)
121	0.386±0.0005	0.468±0.001	0.050 0.70 (0.50-0.99)	–
122	0.015±0.0005	Absent	–	–
211	0.033±0.0008	0.058±0.0002	0.130 0.52 (0.23-1.17)	0.39 0.66(0.28-1.51)
221	0.154±0.0003	0.124±0.0005	0.389 1.284 (0.77-2.11)	0.15 1.51(0.89-2.57)
Others	0.008	0.009	–	–

Reference haplotype combination	Test haplotype combination	Frequency of expected haplotype combination		p-value
		T2DM	Control	
	111/111	0.114	0.105	0.72
	111/112	0.044	0.011	0.05
	111/122	0.010	0	0.23
	111/211	0.022	0.037	0.77
	111/221	0.104	0.080	0.35
111/121	112/121	0.050	0.016	0.10
T2DM: 0.261	112/221	0.020	0.004	0.05
Control: 0.303	121/121	0.149	0.219	0.45
	121/122	0.012	0	0.23
	121/211	0.025	0.054	0.20
	121/221	0.119	0.116	0.73
	211/221	0.010	0.0143	0.69
	221/221	0.024	0.0154	0.73

Results are expressed as mean±SD. Frequencies of haplotypes and diplotypes pertaining to *CAPN10* SNPs in 200 east Indian diabetic subjects and 100 control subjects.

[a] Comparison was made between a haplotype and remaining haplotypes pooled in a group.

[b] Comparison was made between a haplotype and a reference haplotype.

In this work, of the three SNPs studied, only the T-allele of UCSNP-63 was found to exert a dominant effect with respect to susceptibility to diabetes. Our finding that haplotype 112 in combination with haplotype 111 confers a risk of T2DM in our population is expected. However, since UCSNP-63 is located in intron 13 of the *CAPN10* gene, it may be speculated that an extended haplotype harbouring alleles 1, 1 and 2 at UCSNP-43, -19 and -63, respectively, hosts the actual causal variant that increases an individual's risk of diabetes.

The significance of this study lies in detection of the co-existence of two haplotypes with opposite genetic effects in our population. Haplotype 112 has been detected as a risk haplotype in the eastern Indian population and this finding is in agreement with the majority of genetic studies conducted on *CAPN10*.[3] On the other hand, the most prevalent haplotype (121) in our population has a significantly higher presence in normoglycemic controls. The ratios of the frequency of the two haplotypes, namely 121 and 112, are 5.6 and 27.5 in the cases and controls, respectively. A very high frequency of haplotype 121 in our sample suggests the existence of a protective haplotype of *CAPN10* gene in the eastern Indian population. This reinforces the observation of an earlier association study conducted among the Japanese population.[18]

The primary drivers of the epidemic of diabetes are the rapid epidemiological transition associated with changes in dietary patterns and decreased physical activity as evident from the higher prevalence rates of diabetes in the urban

Abbreviations

ATP	adenosine triphosphate
CI	confidence interval
OR	odds ratio
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
SD	standard deviation
SNP	single nucleotide polymorphisms
T2DM	type 2 diabetes mellitus

population. These changes have resulted in escalating rates of both obesity and diabetes across all age groups and socio-economic levels throughout the world.[19] Lifestyle changes and other environmental factors[20] accompanied by urbanisation adversely affect metabolism and thereby contribute to diabetes as is evidenced by the four to six times higher prevalence of T2DM in urban areas compared to rural areas in India.

The high frequency of protective haplotype 121 in all samples, particularly in the control group, is promising indicating the fact that a healthy lifestyle can probably restrain the burden of diabetes, a common risk factor for cardiovascular and many metabolic disorders. However, the present study has been conducted in a sample set collected from eastern India. A further comprehensive study throughout India may enable us to avoid the skewness that may be present in the current data-set.

In summary, our results indicate the pleiotropic roles of *CAPN10* haplotypes with respect to diabetes in our population. The observation of the co-existence of two haplotypes with opposite trends of genetic influence on T2DM in a population is a unique finding. We have not found evidence of the association either of UCSNP-43 and -19 or the haplogenotype 112/121 originally described by Horikawa *et al*.[12] However, we detected a new combination of haplotypes (112/111) conveying a higher risk to type 2 diabetes in eastern India.

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Conflict of interest statement

The authors declare that there is no conflict of interest that would prejudice its impartiality or potential conflict of interest that is fully declared within the text of the article.

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