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The Association Between the rs2975760 and rs3792267 Single Nucleotide Polymorphisms of Calpain 10 (CAPN10) and Gestational Diabetes Mellitus

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Data Collection B
Statistical Analysis C
Data Interpretation D
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Background:

This study aimed to investigate the association between the rs2975760 and rs3792267 single nucleotide polymorphisms (SNPs) of the calpain 10 (CAPN10) gene and gestational diabetes mellitus.

Material/Methods:

The study included 138 patients with gestational diabetes mellitus and 152 healthy pregnant women. Venous blood was separated, and the DNA was extracted. The rs2975760 and rs3792267 SNP polymorphisms of CAPN10 were detected using polymerase chain reaction (PCR). The frequencies of different genotypes in patients with gestational diabetes mellitus and healthy pregnant women were determined, and the relationship between different SNP genotypes and the risk of gestational diabetes mellitus was analyzed.

Results:

There were no significant differences in the frequencies of the TT, CT and CC genotypes of rs2975760 and the frequencies of the GG, AG and AA genotypes of rs3792267 between the women with gestational diabetes and the controls. Expression of rs2975760 and rs3792267 were not associated with the risk of gestational diabetes in the dominant model, recessive model, and additive model. However, grade B and grade D diabetes in the CC and TC genotypes of rs2975760 were significantly different from those in the TT genotype ($P < 0.05$). Grade B and grade D diabetes in the AA and AG genotypes of rs3792267 were significantly different compared with those in the GG genotype ($P < 0.05$), and allele A was significantly increased compared with allele G ($P < 0.05$).

Conclusions:


The rs2975760 and rs3792267 SNP polymorphisms of CAPN10 showed no significant association with the incidence of gestational diabetes mellitus and only a mild association with the severity.

MeSH Keywords:

Calpain • Diabetes, Gestational • Genotype

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Background

Gestational diabetes mellitus is the condition of increased blood glucose above the normal level before pregnancy or impaired glucose tolerance in women during pregnancy [1,2]. Gestational diabetes mellitus is harmful to the pregnant woman and the fetus and increases the risk of pregnancy-induced hypertension, ketoacidosis, excessive amniotic fluid, and abortion [3,4]. The probability of dystocia, birth canal injury and cesarean section are also increased [3,4]. In gestational diabetes, the prevalence of fetal macrosomia is as high as 25–42%, which inhibits the development of the embryo and leads to reduced fetal development in early pregnancy [5,6]. Gestational diabetes mellitus is a cause of fetal malformation, fetal abortion, premature birth, and fetal death [5,6]. However, the causes of gestational diabetes mellitus remain unclear. In most cases, diabetes mellitus only appears after pregnancy, and is related to the environment, eating habits and hormones, and genetic factors in different populations [7, 8].

Expression of the calpain 10 gene, CAPN10, has been shown to be associated with type 2 diabetes mellitus, and it is closely associated with blood glucose and insulin secretion. The mutation of base pairs in the CAPN10 gene results in amino acid changes that increase in blood glucose [9–11].

This study aimed to investigate the association between the rs2975760 and rs3792267 single nucleotide polymorphisms (SNPs) of CAPN10 and gestational diabetes mellitus.

Material and Methods

Patient data

The study included 138 women with gestational diabetes mellitus who were treated in our hospital from June 2015 to June 2018 and 152 healthy pregnant women with normal blood glucose. The study inclusion criteria included a diagnosis of gestational diabetes mellitus, a single pregnancy from natural conception, a maternal examination performed at our hospital, and agreement to participate in the study. Study exclusion criteria included pregnant women with liver or kidney disease, diabetes, hypertension, or other medical diseases before pregnancy, endocrine diseases that included Cushing's syndrome, hypothyroidism, and hyperthyroidism, and refusal to participate in the study. All women signed informed consent, and the study was approved by the Ethics Committee of Jining No. 1 People's Hospital.

Grading of patients with gestational diabetes mellitus

According to age, the course and the presence of vascular complications, patients with gestational diabetes mellitus

were graded using the White classification for maternal and fetal risk in gestational diabetes mellitus. The following grading categories were used: Grade A, after dietary control, a fasting blood glucose of <5.8 mmol/L and a 2-hour postprandial blood glucose <6.7 mmol/L; Grade B, diabetes mellitus occurring >20 years with a course of <10 years; Grade C, and age of onset of diabetes mellitus that ranged from 10–19 years, or a course of disease that ranged from 10–19 years; Grade D, onset of diabetes mellitus of <10 years, or duration >20 years, or combined diabetic retinopathy.

Extraction of the genomic DNA

All subjects provided 5 mL of whole blood that was collected during the regular prenatal examination at 24–28 weeks' gestation. The genomic DNA was extracted from the blood using Omega Mag-Bind DNA Kit (OmegaBiotek, Doraville, GA, USA), and the concentration and purity of DNAs were measured using a NanoDrop spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). DNA was stored at –20°C.

Single-nucleotide polymorphism (SNP) genotyping using polymerase chain reaction (PCR)

The primer sequence at the SNP site and its TaqMan probe sequence were designed using Oligo 6.0 primer design software (www.oligo.net/) (Table 1). Primers were synthesized by Beijing Genomics Institute (BGI, Beijing, China). Initially, 1 µL of DNA solution and 1.2 µL of primer solution, including 0.4 µL of forward and reverse primers and 0.4 µL of probe primers, were added to the pre-configured 17.8 µL of TransStart Probe quantitative PCR (qPCR) SuperMix (Beijing TransGene Biotech Co., Ltd, Beijing, China) and evenly mixed by slight shaking and placed in a CFX96 fluorescence qPCR instrument (Bio-Rad, Hercules, CA, USA). The PCR reaction conditions were: 94°C for 3 min, 94°C for 10 s and 60°C for 15 s for a total of 40 cycles. The instrument software generated the experimental results. Each sample was analyzed in triplicate. Diethyl pyrocarbonate (DEPC) water was used as the negative control, and a positive plasmid containing the sequence was used as the positive control (Shanghai Sangon Biotech Co., Ltd., Shanghai, China). The C, T, A, G alleles were only analyzed in relation to the grades of gestational diabetes mellitus due to a limitation of funding and time.

Statistical analysis

Data were analyzed using SPSS version 19.0 software (IBM, Armonk, NY, USA). The difference in the distribution of genotypes between the study group and the control group was analyzed using the chi-squared (χ^2) test, and the relationship between each genotype and the risk of gestational diabetes mellitus was determined using logistic regression analysis. A P-value <0.05 was considered to be statistically significant.

Table 1. Primer sequences.

SNP	Primer sequence	Probe sequence
rs2975760	Forward: 5'-CCCTGCTCTGTGCCACAC-3'	HEX: 5'-CGCTTGTGTGAAGTAAGGCGTT-3'
	Reverse: 5'-AAATCGTCCAACCGTCTCCT-3'	FAM: 5'-CGCTTGTGTGCAAGTAAGGCGTT-3'
rs3792267	Forward: 5'-AGCGGAGGACGGGACGAGGA-3'	HEX: 5'-AGGCGTTTGAAGGTGAGGCTA-3'
	Reverse: 5'-TCGTCCAACCGTCTCAAC-3'	FAM: 5'-AGGCATTTGAAGGTGAGGCTA-3'

Table 2. Clinical and demographic data of women in the study group and control group.

Index	Study group (n=138)	Control group (n=152)	P-value
Age (years)	28.2±9.3	27.5±8.3	0.423
Smoking			
Yes	32 (23.19%)	45 (29.61%)	0.303
No	106 (76.81%)	107 (70.39%)	
Drinking			
Yes	67 (48.55%)	74 (48.68%)	1
No	71 (51.45%)	78 (51.32%)	
BMI	24.3±4.7	23.7±4.1	0.401
Parity			
0	75 (54.35%)	83 (54.61%)	0.587
1	34 (24.64%)	41 (26.97%)	
≥2	29 (21.01%)	28 (18.42%)	

Results

Clinical and demographic data

There were no significant differences in age, living habits, body mass index (BMI), and parity between the study group and the control group ($P>0.05$) (Table 2). According to White grading, there were 51 women with Grade A, 39 women with Grade B, 26 women with Grade C, and 22 women with Grade D gestational diabetes mellitus.

TaqMan polymerase chain reaction (PCR) genotyping results of the two single nucleotide polymorphisms (SNPs) of CAPN10, rs2975760, and rs3792267

The 138 patients with gestational diabetes mellitus and 152 healthy pregnant women all had good genotyping results (Figure 1). The distribution conformed to the Hardy-Weinberg equilibrium: $P(rs2975760)=0.31$, and $P(rs3792267)=0.58$.

Association between CAPN10 SNP polymorphism and the occurrence of gestational diabetes mellitus

Differences in the frequencies of TT, CT, and CC genotypes of rs2975760 between the study group and the control group were not significant ($P>0.05$). The frequencies of GG, AG, and AA genotypes of rs3792267 were not significant between the study group and the control group ($P>0.05$) (Table 3).

Analysis results of the risk of gestational diabetes mellitus through different models

There was no risk of gestational diabetes mellitus in the dominant model, the recessive model, and the additive model of the rs2975760 and rs3792267 SNP polymorphisms of CAPN10 ($P>0.05$) (Table 4).

Relationship between gene polymorphism and white grading in patients with gestational diabetes mellitus patients

Grade B and grade D cases of diabetes mellitus in the CC and TC genotypes of rs2975760 were significantly different from those in TT genotype ($P<0.05$), and allele C was higher than

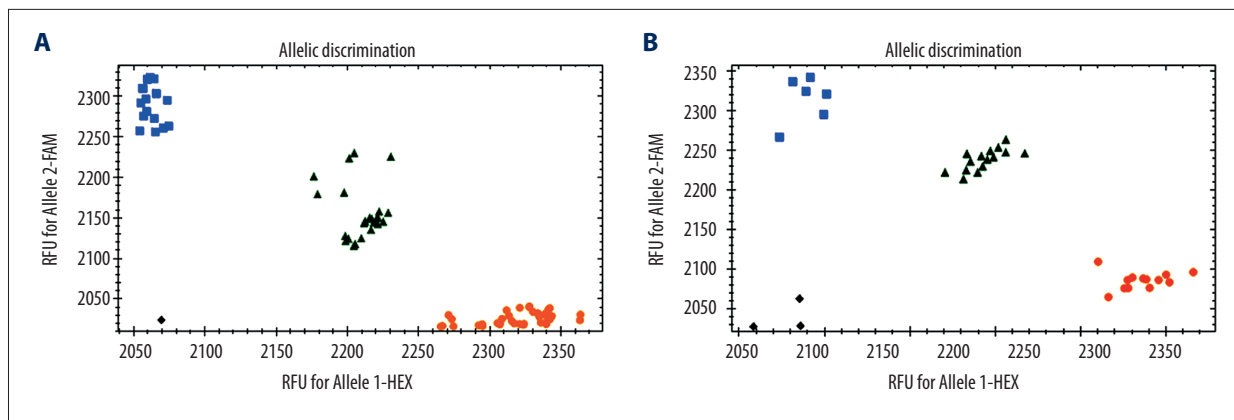


Figure 1. Genotyping results of the rs2975760 and rs3792267 single nucleotide polymorphisms (SNPs) of CAPN10. (A) Genotyping results of rs2975760. (B) Genotyping results of rs3792267. At rs2975760, HEX is marked as TT, FAM is marked as CC, and the middle green part is CT. At rs3792267, HEX is labeled as GG, FAM is labeled as AA, and the middle green part is AG.

Table 3. Association between single nucleotide polymorphisms (SNPs) of the calpain 10 (CAPN10) gene and gestational diabetes mellitus.

Genotype	Study group		Control group		OR (95% CI)	P-value
	n	%	n	%		
rs2975760						
TT	53	38.41	65	42.76	1	0.531
CT	68	49.28	79	51.97	1.056 (0.923–1.332)	0.856
CC	17	12.31	8	5.27	2.606 (2.232–3.014)	0.078
rs3792267						
GG	95	68.84	113	74.34	1	0.388
AG	35	25.36	36	23.68	1.156 (0.822–1.439)	0.783
AA	8	5.80	3	1.97	2.743 (2.213–3.321)	0.161

allele T ($P < 0.05$) (Table 4). Grade B and grade D cases of diabetes mellitus in the AA and AG genotypes of rs3792267 were significantly different from those in GG genotype ($P < 0.05$), and allele A was higher than allele G ($P < 0.05$) (Table 5).

Discussion

The control of blood glucose levels in gestational diabetes mellitus uses diet, drugs, and lifestyle changes. Early detection and control of gestational diabetes mellitus improves the health of both the pregnant woman and the fetus [12,13]. Therefore, early detection and prevention of gestational diabetes mellitus will benefit from the identification of susceptible women. The calpain 10 (CAPN10) gene has a role in the control of blood glucose concentrations and structural changes in the calpain 10 protein encoded by this gene affect the increase in blood glucose [14,15]. Gene polymorphisms are an important manifestation of genetic changes and genetic differences

between people [16]. Previous studies have shown that gene polymorphisms of CAPN10 are correlated with type 2 diabetes mellitus in China, and the waist-hip ratio and body mass index of patients with the GG genotype of SNP43 were relatively high [17]. Other studies have shown that the distribution frequency of single nucleotide polymorphisms (SNPs) of CAPN10 are different between different ethnicities and populations, and so identification of the same change in genotype cannot be used to identify the susceptibility of Europeans and Asians to gestational diabetes mellitus [18,19].

Based on the correlation between SNP polymorphisms of CAPN10 and type 2 diabetes mellitus, in this study on gestational diabetes mellitus the findings showed that the proportions of mutant-type homozygotes of two typical SNPs (rs2975760 and rs3792267) of CAPN10 were less than those of wild-type homozygotes. Although the number of mutations in the study group was higher than that in the control group, the change in the SNP polymorphisms had no significant

Table 4. Risk of gestational diabetes associated with the rs2975760 and rs3792267 single nucleotide polymorphisms (SNPs) of CAPN10.

Model	Genotype	Study group (n=138)	Control group (n=152)	OR (95% CI)	P-value
rs2975760					
Dominant model	TT	53 (38.41)	65 (42.76)	1	0.531
	CT+CC	85 (61.59)	87 (57.24)	1.198 (0.757–1.416)	
Recessive model	TT+TC	121 (87.69)	144 (94.73)	1	0.079
	CC	17 (12.31)	8 (5.27)	2.529 (1.825–4.044)	
Additive model	TT	53 (38.41)	65 (42.76)	1	0.084
	CC	17 (12.31)	8 (5.27)	2.606 (1.923–3.532)	
rs3792267					
Dominant model	GG	95 (68.84)	113 (74.34)	1	0.388
	AG+AA	43 (31.16)	87 (25.66)	1.198 (0.757–1.416)	
Recessive model	GG+AG	130 (94.2)	149 (98.03)	1	0.161
	AA	8 (5.8)	3 (1.97)	2.529 (1.825–4.044)	
Additive model	GG	95 (68.84)	113 (74.34)	1	0.149
	AA	8 (5.8)	3 (1.97)	2.606 (1.923–3.532)	

Table 5. Association between the grade of gestational diabetes mellitus and the rs2975760 and rs3792267 single nucleotide polymorphisms (SNPs) of CAPN10.

Grade	rs2975760					rs3792267				
	TT (n=53)	TC (n=68)	CC (n=17)	T (n=174)	C (n=102)	GG (n=95)	AG (n=35)	AA (n=8)	G (n=225)	A (n=51)
A	4 (7.55)	3 (4.41)	1 (5.88)	11 (6.32)	5 (4.9)	6 (6.32)	3 (8.57)	1 (12.5)	19 (8.44)	5 (9.8)
B	24 (45.28)	21 (30.88)*	5 (29.41)*	69 (39.66)	31 (30.39)	48 (50.53)	10 (28.57)*	2 (25)*	106 (47.11)	14 (27.45)
C	22 (41.51)	30 (44.12)	7 (41.18)	74 (42.53)	44 (43.14)	37 (38.95)	19 (54.29)	3 (37.5)	83 (36.89)	25 (49.02)
D	3 (5.66)	14 (20.59)*	4 (23.53)*	20 (11.49)	22 (22.57)#	4 (4.21)	3 (8.57)	2 (25)*	11 (4.89)	7 (13.73)#

* P<0.05 vs. TT, and # P<0.05 vs. T.

association with gestational diabetes mellitus, even though there were more mutant heterozygotes. If heterozygotes were affected by the external environment or metabolic conditions, the increased probability of mutant-type homozygotes would be increased. Therefore, pregnant women with mutant genotypes of CAPN10 may be identified as having an increased risk of gestational diabetes mellitus, and the impact of base mutations should be appreciated. The findings from previous studies have indicated that the base mutations may reduce the activity of the encoded non-lysosomal cysteine proteases and the ability to degrade blood glucose [20].

In this study, SNP polymorphisms of CAPN10 and different White grades of gestational diabetes mellitus were analyzed. The results showed that genotypes CC and TC of rs2975760 after mutation were significantly different in women with grade B and grade D gestational diabetes mellitus when compared with genotype TT, and allele C was higher than allele T. The AA and AG genotypes of rs3792267 were significantly different in women with grade B and grade D gestational diabetes mellitus compared with genotypes GG, and allele A was higher than allele G. The odds ratio (OR) was 2.6 in both the rs2975760 and rs3792267 polymorphisms. However, further studies are needed to determine whether there are correlations between these two polymorphisms.

The findings from this study indicate that the mutant CAPN10 gene had an effect on the grade of gestational diabetes mellitus in women, and the proportion of women with mutations in the higher grades of the disease was relatively large. These findings may have clinical implications for the identification of women who are at increased risk of gestational diabetes mellitus. However, in the present study, we were only able to evaluate alleles C, T, A and G in relation to the grades of gestational diabetes mellitus, due to the limitations of funding and time. Also, the sample size of this study was small, and future large-scale studies are needed to validate the findings.

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Conclusions

The rs2975760 and rs3792267 single nucleotide polymorphisms (SNPs) of the calpain 10 (CAPN10) gene showed no significant association with the incidence of gestational diabetes mellitus and only a mild association with the severity.

Conflict of interest

None.