

# Study on the correlation between KCNJ11 gene polymorphism and metabolic syndrome in the elderly

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**Abstract.** The aim of the study was to examine the correlation between KCNJ11 gene polymorphism and metabolic syndrome in elderly patients. From January 2014 to January 2015, 54 elderly patients with metabolic syndrome were enrolled in this study as the observation group. During the same period, 46 healthy elderly individuals were enrolled in this study as the control group. KCNJ11 gene polymorphism (rs28502) was analyzed using polymerase chain reaction-restriction fragment length polymorphism. The expression levels of mRNA in different genotypes were detected using FQ-PCR. ELISA was used to evaluate the KCNJ11 protein expression in different genotypes. KCNJ11 gene polymorphism and metabolic syndrome was studied by measuring the blood pressure levels in patients with different genotypes. Three genotypes of *KCNJ11* gene in rs28502 were CC, CT and TT. The CC, CT and TT genotype frequencies in healthy population were 8.5, 9.2 and 82.2%, respectively, while the genotype frequencies in patients with metabolic syndrome were 42.4, 49.8 and 7.8%, respectively. There were significant differences between groups ( $P \leq 0.05$ ). However, the genotype frequencies of C/T in healthy individuals and metabolic syndrome patients were 35.3 and 38.3%, respectively. There were no significant differences between groups ( $P > 0.05$ ). FQ-PCR results showed that the KCNJ11 mRNA expression levels in the control and observation groups had no significant differences ( $P > 0.05$ ). However, the results obtained from ELISA analysis revealed that KCNJ11 protein expression level in the observation group was significantly higher than that in the control group ( $P < 0.05$ ). In conclusion, KCNJ11 gene polymorphism is associated with metabolic syndrome in the elderly. Elderly patients with the CC and TT genotypes are more likely to develop metabolic syndrome.

## Introduction

Metabolic syndrome (MS) is a polygenic inheritable disease caused by the combined effects of environmental factors and genetic factors (1). Obesity, hypertension and diabetes are the main clinical symptoms of metabolic syndrome (2). According to the clinical statistical data, MS has a notable correlation with a variety of diseases, for example, hypertension and heart disease are more common in elderly patients with metabolic syndrome. The deficiency of metabolic function leads to disorder in the body's energy supply, and negatively affects the immune system (3). This can create more important complications in elderly patients (4).

Few studies have been conducted on the pathogenesis of this condition in the elderly, and there is no effective medication for the treatment of metabolic syndrome in the elderly. The symptoms of metabolic syndrome are not significant at the early stage, which makes its diagnosis and treatment more difficult (5). It has been shown that KCNJ11, an 80-kDa protein, can participate in the secretion of insulin (6). A significant correlation between *KCNJ11* gene and type 2 diabetes has been reported (7). KCNJ11 can inhibit the insulin release and increase the blood glucose level (8). At the same time, KCNJ11 can increase the level of lipids in bloodstream and also cause an increase in blood pressure. *KCNJ11* gene contains one exon and one intron (9). The correlation between KCNJ11 gene polymorphism and metabolic syndrome in the elderly has not been studied extensively.

Thus, in the present study, we examined the correlation between KCNJ11 gene polymorphism and metabolic syndrome in the elderly. We provided theoretical and experimental basis for the diagnosis and treatment of metabolic syndrome in the elderly patients.

## Materials and methods

**General data.** From January 2014 to January 2015, 54 elderly patients with metabolic syndrome who were admitted to the Hainan General Hospital (Haikou, China) were enrolled in this study as our observation group. There were 24 males and 30 females with an average age of  $68.34 \pm 7.9$  years. During the same period, 46 healthy elderly individuals were enrolled as the control group. There were 23 males and 23 females in the control group and their average age was  $69.2 \pm 8.4$  years.

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Inclusion criteria were: i) Elderly patients with metabolic syndrome [according to the diagnostic criteria of metabolic syndrome in the elderly in 'Clinical Diagnosis and Treatment of Common Diseases in the Elderly' (10)]; ii) patients without other diseases; and iii) patients aged >80 years. Exclusion criteria were: i) Patients with non-metabolic syndrome; ii) patients with other related diseases, such as heart disease; and iii) patients <60 years of age.

**Methods.** The reagents, such as Ex Taq enzyme, dNTP, 6X buffer and genomic extraction kit were all purchased from Axygen (Union City, CA, USA). Agarose and GodView were both purchased from Solarbio Technology Co., Ltd. (Beijing, China), and other chemical reagents were purchased from Sangon Biotech (Shanghai, China).

**Genomic extraction.** The genomic DNA extraction kit was purchased from Axygen, and we closely followed the instructions provided by the manufacturer.

**Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).** Primers were synthesized by Sangon Biotech, and the primer sequences are shown in Table I. The PCR products were extracted and cloned.

**KCNJ11 gene polymorphism.** For the KCNJ11 polymorphism test, KCNJ11 gene was amplified via PCR, and PCR products were digested with *SepI* endonuclease (11).

**Sequencing.** Plasmids were extracted and submitted to Sangon Biotech for sequencing.

#### FQ-PCR

**RNA extraction.** Blood samples were collected from patients in both groups and used for RNA extraction.

**FQ-PCR.** In order to study the differences in the expression levels of KCNJ11 mRNA in different tissues, FQ-PCR was conducted. The primer sequences are shown in Table II.

**ELISA.** ELISA was conducted by closely following the protocol provided by Li (12).

**Immunohistochemistry.** Immunohistochemical assays were performed according to 'Current Protocols in Molecular Biology' (13).

**Statistical analysis.** The experimental data obtained were analyzed using SPSS 20.0 statistical software (SPSS, Chicago, IL, USA). The correlation between the gene and the differentiated thyroid carcinoma was studied using the ratio (OR) and 95% confidence interval (CI). Unconditional logistic regression was used to calculate the OR value and the results were corrected by sex and other factors.

## Results

**Digestion of different KNSJ11 genotypes.** The results obtained from enzyme digestion of PCR products revealed three bands with the sizes of 659, 426 and 233 bp for the elderly patients with metabolic syndrome after rs28502-site enzyme

Table I. KCNJ11 primer sequence.

Primer name	Sequence
KCNJ11	F: GCCAGGGCCCCCTCCTTCAA
KCNJ11	R: TACCCTCAGACCCACGAGT

F, forward; R, reverse.

Table II. Primers of FQ-PCR.

Primer name	Sequence
qKCNJ11	F: TGCTAGCTGATCGATCGATCGTTCG
qKCNJ11	R: CGTAGCTGATCGATGCTAGCTAGC
GAPDH	F: TGCTAGGCTAGGACGCTAGCTAC
GAPDH	R: CTGGGCTAGATCGACGAGAGCTC

F, forward; R, reverse.

Table III. Alleles between control and observation groups.

Groups	Case (N)	Allele frequency (%)		$\chi^2$	P-value
		T	C		
Control	108	48.4	43.3	14.04	0.026
Observation	92	45.3	40.3		

Table IV. Alleles between control and observation groups.

Groups	Case (N)	Genotype frequency (%)			$\chi^2$	P-value
		CC	CT	TT		
Control	54	8.5	9.2	82.2	16.054	0.000
Observation	46	42.4	49.8	7.8		

digestion (Fig. 1). The results of sequencing showed that base mutation in rs28502 in the observation group was T or C, namely KCNJ11 rs28502T/T and KCNJ11 rs28502 C/C with two bands with the sizes of 384 and 133 bp, while there was only 1 band at 517 bp in the control group. The results of sequencing showed that its base sequence was KCNJ11 rs28502 T/C, and the sequencing is shown in Fig. 2. These results suggested that *KCNJ11* gene in elderly patients with metabolic syndrome was mutated.

**Alleles between control and observation groups.** Results obtained from sequencing of KNSJ11 rs28502 in both groups revealed that the proportion of rs28502 allele C in the elderly patients with metabolic syndrome was 43.3% and that of T

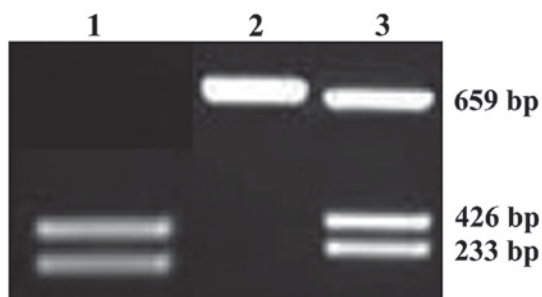


Figure 1. Results of enzyme digestion of different KNSJ11 genotypes. Lane 1, sample of elderly patients with metabolic syndrome; lane 2, sample of healthy individuals; and lane 3, sample of elderly patients with metabolic syndrome.

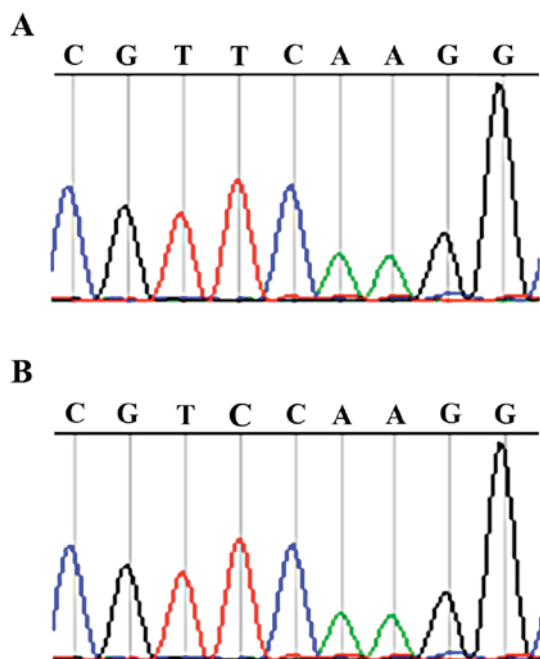


Figure 2. Results of gene sequencing. (A) Sequencing results of the healthy population. (B) Sequencing results of metabolic syndrome in the elderly.

was 48.4%. We detected no significant differences between this proportion and the proportion of C/T in the control group (40.3 and 45.3%, respectively) ( $P>0.05$ ) (Table III).

**KNSJ11 genotypes between control and observation groups.** KNSJ11 gene was amplified in both groups and sequencing results revealed polymorphism distribution in rs28502 base. Sequencing results showed that the proportions of CC, CT and TT in metabolic syndrome patients were 42.4, 49.8 and 7.8%, respectively. These proportions in healthy subjects were 8.5, 9.2 and 82.2%, respectively. These results suggested that the CT genotype was predominant for KNSJ11 in rs28502 site, while CC and CT genotypes were predominant for elderly patients with metabolic syndrome. There were significant differences in CC and TT genotypes between the groups ( $P<0.05$ ) (Table IV).

**Detection of mRNA expression in KNSJ11 gene between the control and observation groups.** KNSJ11 mRNA expression levels in different samples are shown in Fig. 3. It can be seen

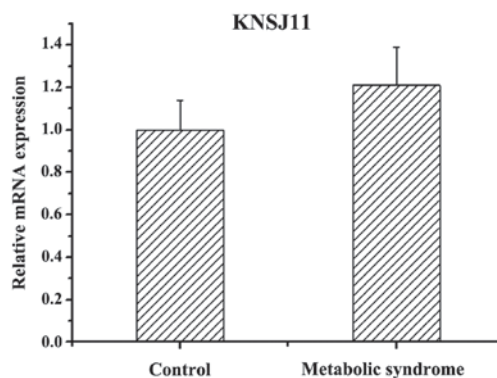


Figure 3. Detection of mRNA expression in KNSJ11 gene between the control and observation groups.  $P<0.05$ .

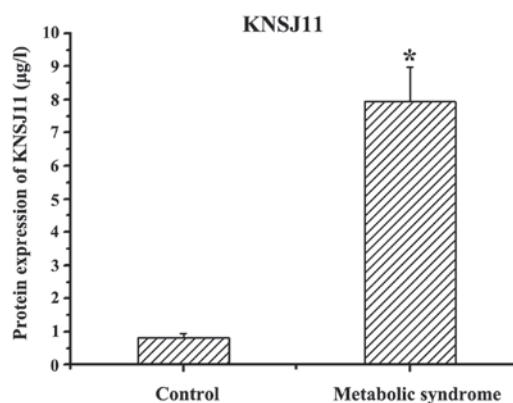


Figure 4. Detection of protein expression in KNSJ11 gene between the control and observation groups.  $P<0.05$ , significant difference between the two groups.

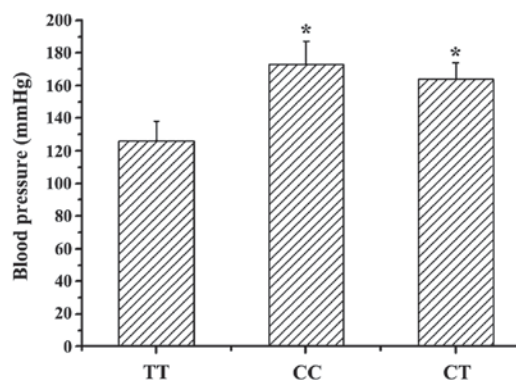


Figure 5. Detection of blood pressure (systolic pressure) in patients with different genotypes.  $P<0.05$ , significant difference between the two groups.

that compared with KNSJ11 mRNA expression level in the control group, the expression level was significantly higher in the metabolic syndrome. The difference was statistically significant ( $P<0.05$ ).

**Detection of protein expression in KNSJ11 gene between the control and observation groups.** KNSJ11 protein expression level measured by ELISA is presented in Fig. 4. KNSJ11 protein expression level in the elderly patients with metabolic syndrome was significantly higher than that in the control

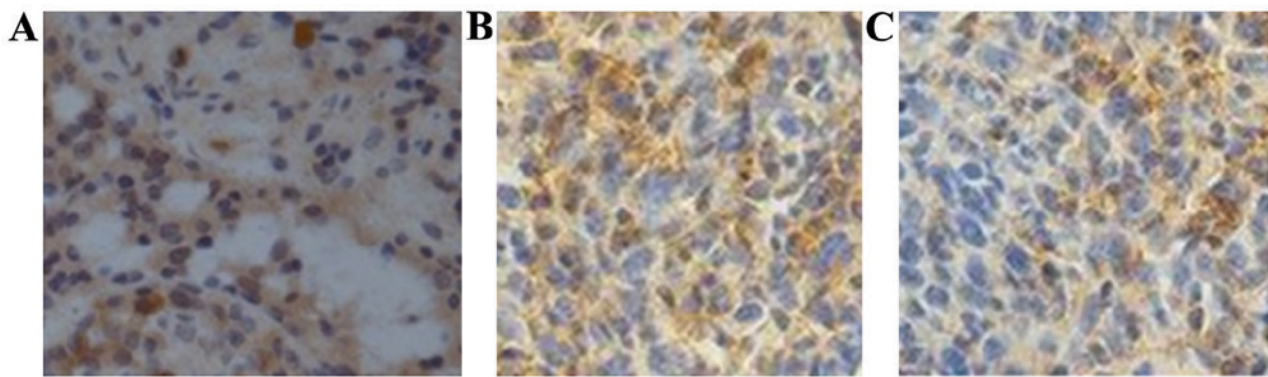


Figure 6. Immunohistochemical determination of patients in different genotypes. (A) Sample of healthy population, (B) sample of metabolic syndrome in the elderly with CT genotype, and (C) sample of metabolic syndrome in the elderly with CC genotype.

group ( $7.94 \pm 1.03 \mu\text{g/l}$  vs.  $0.83 \pm 0.12 \mu\text{g/l}$ , respectively). The difference was statistically significant ( $P < 0.05$ ).

*Detection of blood pressure (systolic pressure) in patients with different genotypes.* To investigate the relationship between KNSJ11 genotype and metabolic syndrome in the elderly patients, blood pressure values in elderly patients with different genotypes were measured and compared. Results showed that the blood pressure levels in elderly patients with metabolic syndrome with CC and CT genotypes were significantly higher than that in TT genotype ( $P < 0.05$ ) (Fig. 5). The differences were statistically significant ( $P < 0.05$ ).

*Immunohistochemical determination of patients in different genotypes.* FQ-PCR results showed that there was no significant difference in the relative expression levels of mRNA in KNSJ11 gene in patients with different genotypes ( $P > 0.05$ ). Results of ELISA showed that there were significant differences in the KNSJ11 protein expression levels in patients with different genotypes ( $P < 0.05$ ). This indicated that different genotypes influenced the KNSJ11 protein expression level at the translational level. Immunohistochemical results showed that KNSJ11-positive cell count in elderly patients with metabolic syndrome was significantly higher than that in the healthy subjects (94.3 vs. 12.5%, respectively) (Fig. 6). There were significant differences between the two groups ( $P < 0.05$ ), which was consistent with results obtained from ELISA.

## Discussion

The increase in the size of the aging population in China has led to a significant increase in elderly-related diseases (14,15). Metabolic syndrome in the elderly is a complex disease with cardiovascular and cerebrovascular diseases with obesity, hypertension and hyperglycemia as the main clinical symptoms (16). Metabolic syndrome in the elderly has a significant correlation with genetic information; however, the pathogenesis of metabolic syndrome in the elderly remains unknown. There is no efficient medication for metabolic syndrome in the elderly at present (17). Clinical data show that approximately 18.4% of elderly patients with metabolic syndrome suffer from varying degrees of fatty liver and abnormal blood

lipid levels (18). Additional findings have shown that the fatty liver is usually accompanied with hypertension, diabetes and hyperlipidemia (19). In elderly patients with metabolic syndrome, the incidence of type 2 diabetes was significantly higher than that of patients with other diseases (20). Compared with healthy population, the KCNJ11 expression level in patients with metabolic syndrome is significantly different. KCNJ11 plays an important role in the process of  $\text{K}^+$  transport.  $\text{K}^+$  is important in maintaining the intracellular and extracellular osmotic pressure stability, maintaining the stability of cell membrane and promoting intracellular and intracellular exchange. It has been shown that there is a close relationship between KCNJ11 gene polymorphism and type 2 diabetes mellitus (21). KCNJ11 gene polymorphism can significantly increase the resistance of patients with type 2 diabetes to sulfonylurea drugs, and the polymorphism of KCNJ11 gene is also found in elderly patients with metabolic syndrome (21). *KCNJ11* gene in patients with type 2 diabetes mellitus also shows a certain polymorphism; at the same time, we found that *KCNJ11* gene in patients in different regions in China showed a certain level of polymorphism. We also found a correlation between the polymorphism and lifestyle habits. It has been shown that in the elderly suffering from metabolic syndrome, KCNJ11 gene polymorphism and type 2 diabetes are closely related. In the present study, we studied the correlation between KCNJ11 gene polymorphism and metabolic syndrome in elderly patients, and the results suggested that rs28502 polymorphism of *KCNJ11* gene had a significant correlation with the metabolic syndrome. The proportion of CC and CT genotypes in *KCNJ11* gene in elderly patients with metabolic syndrome was significantly higher than that in healthy individuals. This result suggested that the elderly with CT and CC genotype are more likely to develop metabolic syndrome (22). FQ-PCR results revealed that KCNJ11 mRNA expression in patients with different genotypes had no significant difference. We also found that KCNJ11 protein expression levels in patients with CC and CT genotypes were significantly higher than those with TT genotype.

We concluded that KCNJ11 gene polymorphism was associated with metabolic syndrome in the elderly. We also concluded that elderly patients with the CC and TT genotypes are more likely to develop metabolic syndrome.

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