Family Medicine and Community Health
ORIGINAL RESEARCH

Correlation between KCNQ1 gene polymorphism and type 2 diabetes mellitus in Huaihai region of China

Xing Gao[1], Dongmei Lv[2], Yan Wang[2], Tao Wang[1], Wei Li[3], Yanbo Zhang[1], Yaqin Wei[1], Jiali Zhang[4], Qian Lu[1], Xiaoxing Yin[1]

Abstract

Objective: This article aims to discuss the distribution of KCNQ1 gene polymorphism in the Chinese Han population in the Huaihai region of China and the correlation between KCNQ1 gene polymorphism and incidence of type 2 diabetes (T2DM).

Methods: From December 2010 to July 2011, 200 T2DM inpatients and outpatients in the Endocrinology Department of the Affiliated Hospital of Xuzhou Medical College were selected as the case group and, 200 healthy people identified by the health examination center in the same region were selected as the control group. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) test was used to examine the gene polymorphism of the two groups.

Results: (1) Analysis on the control group showed that at the KCNQ1 rC237892 locus, the genotype frequencies of CC, CT and TT were 36.0% (72/200), 51.0% (102/200) and 13.0% (26/200) respectively, and the allelic frequencies of C and T were 61.5% (246/400) and 38.5% (154/400) respectively. Analysis on the case group showed the genotype frequencies of CC, CT and TT were 47.5% (95/200), 44.0% (88/200) and 8.5% (17/200) respectively, and the allelic frequencies of C and T were 69.5% (278/400) and 30.5% (122/400) respectively. Comparison between the genotype distributions and allelic frequencies of the two tested groups at KCNQ1 rC237892 locus showed differences with statistical significance ($P<0.05$). (2) Comparison between the genotype distributions and allelic C and A frequencies of the control group and the case group showed differences with no statistical significance ($P>0.05$).

Conclusion: Polymorphism at KCNQ1 rs2237892 locus may be correlated to the incidence of T2DM in the Chinese Han population in Huaihai region of China; polymorphism at rsl51290 locus may be irrelevant to the incidence of T2DM in the Chinese Han population in Huaihai region of China.

Keywords: Type 2 diabetes mellitus, KCNQ1, Genotype distribution, Allelic frequency, Polymorphism, Mononucleotide

Introduction

In recent years, correlation between the gene of voltage-gated potassium channel subfamily member 1 (KCNQ1) and the incidence of type 2 diabetes (T2DM) has become a hot area for studies at home and abroad. Japanese scholars Hiroyuki et al. [1] discovered that KCNQ1 gene polymorphism was correlated to the incidence of T2DM in the Japanese population, and this result was also verified in the populations of Singapore, Korea and Pakistan [2–4]. Liu and others [5] made the genotype test in the inland population of China and discovered that the KCNQ1 gene polymorphism

1. Laboratory of New Drugs and Clinical Application, Xuzhou Medical College, Xuzhou 221000, China

2. Pharmacy Department, The affiliated Hospital of Xuzhou Medical College, Xuzhou 221000, China

3. Endocrinology Department, The affiliated Hospital of Xuzhou Medical College, Xuzhou 221000, China

4. School of Pharmacy, Xuzhou Medical College, Xuzhou 221000, China

CORRESPONDING AUTHOR:
Xiaoxing Yin
Laboratory of New Drugs and Clinical Application, Xuzhou Medical College, Xuzhou 221000, China
E-mail: yinxx@vip.sina.com

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did in fact increase the risk of T2DM. Meanwhile, Zhang and others [6] made the similar conclusion while examining the population of Hubei region.

To date there has been no report on the study of KCNQ1 gene polymorphism in the Chinese Han population in the Huaihai region of China. This region is home to one of the important branches of the Chinese population. The morbidity of T2DM in this region is higher, and the lifestyle, culture, diet, economic and living standards, social and health conceptions, and genetic background are different from other regions. Therefore, the Chinese Han population in the Huaihai region of China was selected as the object in this study. A case-control study was used to discuss the correlation between the distribution of KCNQ1 gene polymorphism and the incidence of T2DM in the population of this region so as to further understand the genetic mechanism of the incidence of T2DM.

Data and methods

General data

200 T2DM inpatients and outpatients treated by the Endocrinology Department of the Affiliated Hospital of Xuzhou Medical College during December 2010–July 2011 were selected as case group subjects. Patients all complied with the 1999 T2DM diagnosis standards of the World Health Organization (WHO). The case group included 111 males and 89 females, their average age was 53.7±8.8 years, and they were all Chinese Han residents living in Huaihai region of China. Patients diagnosed with Type 1 diabetes, secondary diabetes, and diabetes combined with other autoimmune diseases were excluded; no subjects had a genetic connection among themselves.

The control group comprised 200 healthy people including 99 males and 101 females who had been selected from the health examination center in the same region, with the average age of 53.7±8.8 years, but without liver, kidney, endocrine or metabolic diseases, and without any family history of hypertension, dyslipidemia, or diabetes. Patients' age and gender distributions between the two tested groups were comparable (t_{age}=1.632, P=0.103; X^2_{gender}=1.444, P=0.230). The authors received consent from all participants.

Main reagents and instruments

DNA extraction reagent kit (Shanghai Saibaisheng Gene Technology Co., Ltd.); primer (Sangon Biotech (Shanghai) Co., Ltd.); dNTP, Buffer and Taq enzyme (Beijing TransGen Biotech Co., Ltd.); restriction enzyme (New England Biolabs, Inc.); DNA marker (Fermentas Corporation); QL-866 vortex mixer (Haimen City Qilin Medical Instrument Factory); 5804R low-temperature and high-speed centrifuges (Eppendorf of Germany); Applied Biosystems 2720 PCR instrument (ABI Corporation); Tanon-1600R gel imaging system (Shanghai Tianneng Technology Co., Ltd.).

Methods

Genome DNA extraction: Two milliliters fasting venous blood was drawn from the elbow of each subject at the sitting posture. EDTA–K2 was used for anticoagulation; the processed blood was saved at –20°C. Then a resin TM genome DNA purification kit was used to extract DNA according to the steps given in the kit instruction.

Sequence of primers: The primers used in this study were all produced by Sangon Biotech (Shanghai) Co., Ltd. (Table 1).

PCR reaction systems and reaction conditions: (1) KCNQ1 rs2237892: 25 μL reaction system, which included 10×Buffer 2.0 μL (with $MgCl_2$), dNTP 1.0 μL (10 mmol/L), upstream and downstream primers 0.5 μL (10 μmol/L) each,

Table 1. Primer sequences of KCNQ1 locus to be detected

Gene locus	Upstream and downstream primers	Annealing temperature (°C)	Product size (bp)
KCNQ1 rs2237892	Upstream-5′-CTTGTGCCCTTGTCACCCAC-3′ Downstream-5′-GGCTTCCAGCCTCCAAGCTG -3′	60.0	354
KCNQ1 rs151290	Upstream-5′-AGCCGTTCCTGCTTGCTACT -3′ Downstream-5′-TGGGAGTGGTTCCAAGGACA-3′	59.5	105

Easy Taq DNA polymerase 0.25 μL (5 U/μL), DNA template 2.0 μL, and double-distilled water for sterilization 18.8 μL. Reaction conditions: Initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extending at 72°C for 30 s, 32 circulations, and final extending at 72°C for 5 min. (2) KCNQ1 rsl51290: 20 μL reaction system included 10×Buffer 2.0 μL (with $MgCl_2$), dNTP 1.0 μL (10 mmol/L), upstream and downstream primers 0.5 μL (10 μmol/L) each, Easy Taq DNA polymerase 0.2 μL (5 U/μL), DNA template 2.0 μL, and double-distilled water for sterilization 13.8 μL. Reaction conditions: Initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 59.5°C for 15 s, extending at 72°C for 30 s, 37 circulations, and final extending at 72°C for 5 min.

Restriction enzyme-digested PCR product: Enzyme digestion system 20 μL, including restriction enzyme 0.5 μL (rs2237892/MspI, rsl51290/BglI), NEBuffer 2.0 μL, PCR product 10.0 μL and double-distilled water for sterilization 7.5 μL. After blending, the PCR product was put at a 37°C drying oven for a 4 h digestion. Enzyme-digested product 5 μL was taken and blended with 1 μL loading buffer. Samples were put in 2.0% agarose gel for electrophoresis for 20 min at the voltage 120 V. DNA Maker was molecular weight standard. Gel imaging system was used to judge the genotype results.

Statistical methods

Gene counting method was used for calculating genotype and allelic frequencies. The compliance of the tested group with Hardy–Weinberg (H–W) equilibrium was examined by x^2 test. The measurement data was expressed in ($x \pm s$) and inspected with t test. SPSS 16.0 software was used for statistical treatment of data. In the case of $P<0.05$, the difference had statistical significance.

Results

Electrophoresis results of enzyme-digested products

At KCNQ1 rs2237892 locus, CC homozygote showed two stripes, 269 bp and 85 bp; TT homozygote showed one stripe, 354 bp; CT heterozygote showed three stripes, 354 bp, 269 bp and 85 bp (Fig. 1). At KCNQ1 rsl51290 locus, AA homozygote showed one stripe, 105 bp; CC homozygote showed one stripe, 81 bp; AC heterozygote showed two stripes, 105 bp and 81 bp (Fig. 2).

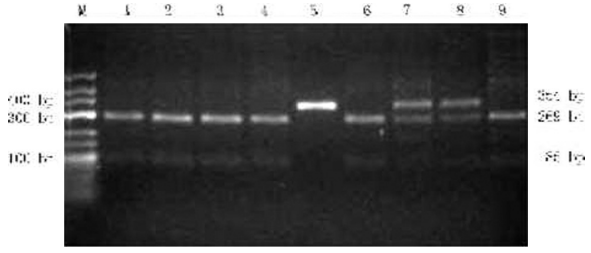

Fig. 1. KCNQ1 gene locus rs2237892 PCR-RFLP electrophoresis results.

Note: M for Marker; Type of M 1, 2, 3, 4, 6 and 9 was CC; type of M 7 and 8 was CT; type of M 5 was TT.

Results of the Hardy–Weinberg equilibrium test

Hardy–Weinberg genetic equilibrium test showed that the distributions of two mentioned genes' genotype and allelic frequencies complied with genetic equilibrium in both groups, and the samples could represent the groups ($P>0.05$).

Genotype distribution and allelic frequency at KCNQ1 rs2237892 locus

Comparison of genotype distributions and allelic frequencies of KCNQ1 rs2237892 between control group and case group showed the differences with statistical significance ($P<0.05$, Table 2).

Genotype distributions and allelic frequencies of KCNQ1 rsl51290

Comparison of genotype distributions and allele frequencies of KCNQ1 rsl51290 between control group and case group

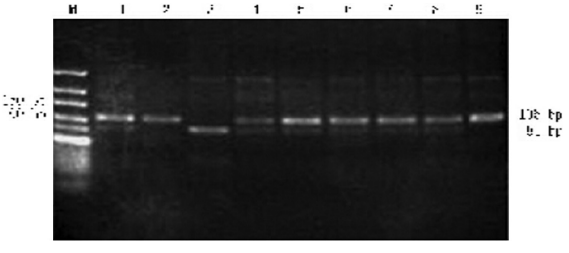

Fig. 2. KCNQ1 gene locus rsl51290 PCR-RFLP electrophoresis results.

Note: M for Marker; 2 and 9 were AA type; 1, 4, 5, 6, 7 and 8 were AC type; 3 was CC type.

Table 2. Comparison of genotype distributions and allelic frequencies of the control group and the case group at KCNQ1 rs2237892 Locus [*n* (%)]

Group	Number of cases	Gene number			Allele	
		CC	CT	TT	C	T
Control group	200	72(36.0)	102(51.0)	26(13.0)	246(61.5)	154(38.5)
Case group	200	95(47.5)	88(44.0)	17(8.5)	278(69.5)	122(30.5)
X^2 value			6.083		5.664	
P-value			0.048		0.017	

showed that the difference had no statistical significance ($P>0.05$, Table 3).

Discussion

T2DM is a kind of complicated polygenetic or multigene disorder and has obvious genetic heterogeneity. It is the result of interactions of multiple genes and genetic and environmental factors. So far, many Chinese and foreign scholars, using the candidate gene screening method and genome-wide scanning technique, have made association analyses for hundreds of T2DM-related susceptible genes. However, studies in different races and regions have shown dissimilar results. In most cases, they could not get positive results that were consistent and repeatable.

KCNQ1 gene is one of the members of voltage-gated potassium channel family. It is located at the 11th chromosome 11 pl 5.5, about 404 kb, and is composed of 17 exons. The exons are in the size range of 47–1122 bp [7]. KCNQ1 will express a kind of voltage-gated potassium channel protein, which is frequently referred to as KvLQTl. KvLQTl is a membrane potential potassium channel protein, which is composed of 676 amino acid residues [8], and expressed in pancreatic islet INS-1β cell, playing an important role in the adjustment of insulin secretion [9].

KCNQ1 gene is the first T2DM susceptible gene detected in Asian population. Scholars of Japan and Korea have verified strong correlation between the polymorphism at rs2237892 locus and T2DM [10, 11]. Compared with other gene loci, there is less study on the correlation between the polymorphism at rs2237892 and T2DM. Scholars of Japan have verified the correlation between the polymorphism of rsl51290 and T2DM [10], but this has not been proven in the German population [12]. The inconsistent study results may be caused by obvious genetic heterogeneity of T2DM, and different genetic backgrounds and environments of difference regions and nations.

This study has shown that significant differences exist in genotype distributions and allelic frequencies at KCNQ1 rs2237892 locus between Han T2DM patients and healthy people in Huaihai region of China, which also suggests that KCNQ1 rs2237892 polymorphism may be correlated to the incidence of T2DM in the Chinese Han population in Huaihai region of China. It has also shown that no significant differences exist in genotype distributions and allelic frequencies at KCNQ1 rsl51290 locus between Han T2DM patients and healthy people in the Huaihai region of China, which may mean that KCNQ1 rsl51290 polymorphism is irrelevant to the incidence of T2DM in the Chinese Han population in the Huaihai region of China. The causes of such results might

Table 3. Comparison of genotype distributions and allelic frequencies at KCNQ1 rsl51290 locus [*n* (%)]

Group	Number of cases	Gene number			Allele	
		CC	CT	TT	C	T
Control group	200	41(20.5)	109(54.5)	50(25.0)	191(47.8)	209(52.2)
Case group	200	47(23.5)	107(53.5)	46(23.0)	201(50.2)	199(49.8)
X^2-value			0.594		0.500	
P-value			0.743		0.479	

include: (1) The sample size of this study was small, which directly affected the statistical results; (2) the gene test methods were restricted, which might have caused the deviation of test results; or (3) the role of the locus KCNQ1 rsl51290 was very weak in causing T2DM, and it is hard for the difference to achieve statistical significance. The study result was negative, but the possibility of correlation between rsl51290 polymorphism and T2DM in the population of the Huaihai region of China cannot be eliminated.

In conclusion, early intervention to the population with the risk of carrying related alleles has great significance for postponing and preventing T2DM, and also provides a basis for the final, clinical realization of gene-oriented, personalized medication. In future studies, we should further increase the number of T2DM patients and control cases, and improve the genotype test technique, to make the results more scientific, accurate and objective; in addition, the mechanism of KCNQ1 gene polymorphism resulting in T2DM remains unclear, which requires further in-depth investigation.

Conflict of interest

The authors declare no conflict of interest.

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