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Relationship between *SLC22A1* and *SLC22A4* gene polymorphisms and risks of type 2 diabetes in Chinese Han population.

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Abstract

Objective: This study was conducted to investigate the relationship between *SLC22A1* and *SLC22A4* gene polymorphisms and genetic susceptibility to type 2 diabetes in Chinese Han population.
Methods: The research group comprised 110 patients with type 2 diabetes in Chinese Han population, while and the control group consisted of 110 healthy volunteers. The polymorphisms of *SLC22A1* gene rs628031 and rs2282143 loci and *SLC22A4* gene rs2073838 and rs272893 loci were detected in the subjects in the two groups. Genotype distributions and allele frequencies of the two genes were compared between the research and control groups.
Results: Statistically significant differences were identified in the genotype distributions of *SLC22A1* gene rs628031 and rs2282143 loci between the research and control groups (P<0.05). The A allele frequency of *SLC22A1* gene rs628031 locus and the T allele frequency of rs2282143 locus were higher in the research group than in the control group; these differences were statistically significant (P<0.05). The genotype distributions and allele frequencies of *SLC22A4* gene rs2073838 locus exhibited no significant difference between the research and control groups (P>0.05). However, the genotype distributions of rs272893 locus showed a significant difference between the research and control groups (P<0.05).
Conclusion: The polymorphisms of *SLC22A1* gene rs628031 and rs2282143 loci and *SLC22A4* gene rs272893 locus of patients with type 2 diabetes indicated a significant difference between the research and control groups, thereby suggesting that these genetic locus mutations increase the risk in patients with type 2 diabetes in Chinese Han population.

Keywords: Chinese Han population, Gene polymorphism, Type 2 diabetes, Genetic susceptibility.

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Introduction

Diabetes has become a worldwide public health concern given the increase in the occurrence of this disease [1]. This study showed that *SLC22A1* and *SLC22A4* genes influence the curative effect on patients with type 2 diabetes who took metformin [2]. The polymorphisms of *SLC22A1* gene rs628031 and rs2282143 loci and *SLC22A4* gene rs2073838 and rs272893 loci were closely related to the effectiveness of metformin. However, the correlation between the abovementioned loci polymorphisms of the two genes and the risks in patients with type 2 diabetes mellitus remain to be investigated [3]. The polymorphisms of the above gene loci in 110 cases of healthy volunteers and 110 patients with type 2 diabetes were used in this study considering their conditions. Moreover, the relationship between the gene polymorphisms and genetic susceptibility to type 2 diabetes mellitus was detected in this study. The results were presented as follows.

Materials and Methods

Clinical data

The research group comprised 110 patients with type 2 diabetes who were admitted to our hospital from February 2015 to July 2016. According to the matching principle, the control group consisted of 110 healthy volunteers from the physical examination center. A total of 122 males and 98 females, aged 20-80 y, with an average of 42.8 ± 5.0 y were selected.

Inclusion criteria [4]: the patients in the research group were selected in accordance with the relevant standards in the "prevention and treatment guidelines for diabetes mellitus in

China." The research group was composed of patients who are newly diagnosed with type 2 diabetes, while the control group included volunteers who are healthy. All of the subjects in the two groups were Chinese Han who provided an informed consent and passed the requirements of the hospital ethics committee.

Exclusion criteria [5]: Patients with associated complications of type 2 diabetes, drug abuse and addiction history, viral infection history, severe cardiovascular diseases, mental disorders or cognitive impairment, and those who refused to cooperate in the study were excluded.

Methods

Blood sampling and DNA extraction [6]: 2 ml fasting venous blood was collected from all subjects in the next day. EDTA was added for anticoagulant treatment. Genomic DNA was extracted using a DNA extraction kit (Dalianbao Biotechnology Limited Company) in accordance with Trizol principle. DNA concentration was detected using a spectrophotometer (Backman Kurt Company). Samples were preserved in a refrigerator at -20°C constant temperature.

Synthesis and sequencing of target fragments [7]: primers of polymerase chain reaction (PCR) were designed and synthesized by Shanghai Biotechnology Co., Ltd. The primers were amplified through PCR amplification system (American Bio-Rad Company). The total reaction system was 25 μL, including template DNA 1.5 μL, upstream primer 0.5 μL, downstream primer 0.5 μL, Premix Taq enzyme 12.5 μL, and sterilized ultrapure water 10.5 μL. The reaction conditions were described as follows: pre-degeneration at 68°C for 2 min, degeneration at 94°C for 30 s, extension at 72°C for 45 s, completed after 35 cycles, and annealing at 55°C. A 4 μL mixed liquid that contained exonuclease and alkaline phosphatase was added in a 10 μL amplification product for agarose gel electrophoresis, and the electrophoresis products were analysed and sequenced using a gel imaging analysis system (Versa Doc4000).

Statistical analysis

The data were analysed using SPSS19.0 statistical software. The calculated data were compared using (%) and were tested using the non-parametric rank sum test. $P<0.05$ indicated that the difference was statistically significant.

Results

Baseline data comparison between the two groups

The gender, average age, years of education, and registered permanent residence denoted no significant difference between the research and control groups ($P>0.05$), as summarized in Table 1.

Table 1. *Baseline data comparison between the two groups.*

Baseline data	Research group (n=110)	Control group (n=110)	χ^2/t/Z	P
Gender				
Male	62 (56.36)	60 (54.55)	0.018	0.892
Female	48 (43.64)	50 (45.45)		
Average year (y)	42.4±5.5	43.1±6.1	0.894	0.372
Years of education (y)				
≤ 6 y	15 (13.64)	18 (16.36)	0.026	0.875
6-12 y	83 (75.45)	81 (73.64)		
≥ 12 y	12 (10.91)	11 (10.00)		
Registered permanent residence				
Rural area	61 (55.45)	59 (53.64)	0.018	0.892
Urban area	49 (44.55)	51 (46.36)		

Comparison of genotype distributions of SLC22A1 gene rs628031 and rs2282143 loci

The genotype distributions of *SLC22A1* gene rs628031 and rs2282143 loci indicated a significant difference between the two groups ($P<0.05$), as listed in Table 2.

Table 2. *Genotype distribution comparison of SLC22A1 gene rs628031 and rs2282143 loci between the two groups (%).*

Group	n	rs628031 locus			rs2282143 locus		
		AA	GG	AG	CC	TT	CT
Research	110	32 (29.09)	37 (33.64)	41 (37.27)	33 (30.00)	43 (39.09)	34 (30.91)
Control	110	11 (10.00)	69 (62.73)	30 (27.27)	59 (53.64)	30 (27.27)	21 (19.09)
Z		18.761	10.089				
P		0.000	0.000				

Comparison of allele frequencies of SLC22A1 gene rs628031 and rs2282143 loci

The A allele frequencies of *SLC22A1* gene rs628031 and rs2282143 loci were higher in the research group than in the control group, and the difference was statistically significant between the two groups (P<0.05), as displayed in Table 3.

Table 3. *Comparison of allele frequencies of SLC22A1 gene rs628031 and rs2282143 loci between the two groups.*

Group	n	rs628031 locus		rs2282143 locus	
		A	G	T	C
Research group	220	105 (47.73)	115 (52.27)	120 (54.55)	100 (45.45)
Control group	220	52 (23.64)	168 (76.36)	81 (36.82)	139 (63.18)
χ^2		26.778	13.226		
P		0.000	0.000		

Comparison of genotype distributions of SLC22A4 gene rs2073838 and rs272893 loci

The genotype distributions of *SLC22A4* gene rs2073838 locus showed no significant difference between the research and control groups (P>0.05), whereas the genotype distributions of rs272893 locus demonstrated a significant difference between the two groups (P<0.05), as presented in Table 4.

Table 4. *Comparison of genotype distributions of SLC22A4 gene rs2073838 and rs272893 loci.*

Group	n	rs2073838 locus			rs272893 locus		
		CC	TT	CT	AA	GG	AG
Research	110	28 (25.45)	43 (39.09)	39 (35.45)	52 (47.27)	39 (35.45)	19 (17.27)
Control	110	32 (29.09)	41 (37.27)	37 (24.55)	22 (20.00)	32 (29.09)	56 (50.91)
Z		0.374	5.177				
P		0.602	0.021				

Comparison of allele frequencies of SLC22A4 gene rs2073838 and rs272893 loci

The allele frequencies of *SLC22A4* gene rs2073838 locus showed no statistical difference (P>0.05), and the A allele frequency of *SLC22A4* gene rs272893 locus was much higher in the research group than in the control group (P<0.05), as shown in Table 5.

Table 5. *Comparison of allele frequencies of SLC22A4 gene rs2073838 and rs272893 loci between the two groups (%).*

Group	n	rs2073838 locus		rs272893 locus	
		T	C	A	G
Research group	220	125 (56.82)	95 (43.18)	123 (55.91)	97 (44.09)
Control group	220	119 (54.09)	101 (45.91)	100 (45.45)	120 (54.55)
χ^2		0.230	4.401		
P		0.632	0.036		

Gel electrophoretogram

For example, a 42 y old male with two-year persistence of type 2 diabetes. The gel electrophoresis products of *SLC22A1* gene rs628031 and rs2282143 loci and *SLC22A4* gene rs2073838 and rs272893 loci in type 2 diabetes are depicted in Figure 1.

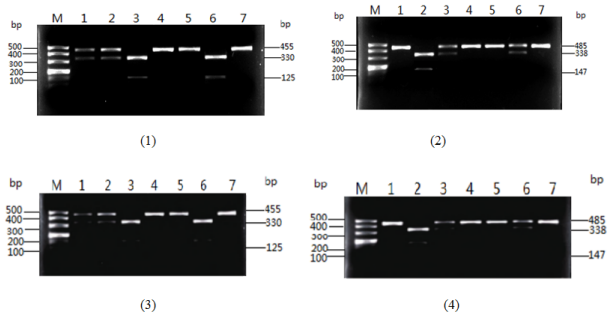

Figure 1. *Gel electrophoresis product of SLC22A1 gene rs628031 (1) and rs2282143 locus (2); gel electrophoresis product of SLC22A4 gene rs2073838 (3) and rs272893 locus (4).*

Discussion

The results of this study showed that the genotype distributions of *SLC22A1* gene rs628031 and rs2282143 loci exhibited a statistical difference between the research and control groups (P<0.05). The A allele frequency of *SLC22A1* gene rs628031 locus and T allele frequency of rs2282143 locus were 47.73% and 54.55%, correspondingly, which were higher than 23.64% and 36.82% in the control group, respectively. The differences were statistically significant (P<0.05), thereby suggesting that the polymorphisms of *SLC22A1* gene rs628031 and rs2282143 loci are closely related to the genetic susceptibility to type 2 diabetes in Chinese Han population [8,9]. The mutation of *SLC22A1* gene rs628031 locus increased the A allele frequency, and the mutation of rs2282143 locus increased the T

allele frequency, thus increasing the risk of type 2 diabetes in Chinese Han population.

In addition, the results of this study showed that the genotype distributions of *SLC22A4* gene rs2073838 locus and allele frequencies exhibited no statistical difference (P>0.05) compared with the control group, whereas the genotype distributions of rs272893 locus demonstrated a statistical difference (P<0.05). Moreover, the A allele frequency of 55.91% was significantly higher than 45.45% in the control group, thereby suggesting that the genotype distribution of *SLC22A4* gene rs2073838 locus and allele frequency may not be associated with the risk of type 2 diabetes in Chinese Han population [10]. However, the abnormal genotype distribution of rs272893 locus may increase the risk of type 2 diabetes, and the increase in A allele frequency positively correlated with the genetic susceptibility to type 2 diabetes.

Conclusion

The analyses presented in this study showed that the polymorphisms of *SLC22A1* gene rs2282143 and rs628031 loci and *SLC22A4* gene rs272893 locus in Chinese Han population with type 2 diabetes indicated a significant difference compared with the control group. Moreover, the increase in A and T allele frequencies may increase the risk of type 2 diabetes in Chinese Han population. This conclusion may guide the clinical prevention and control measures for type 2 diabetes. However, future studies are required to investigate the underlying mechanisms of these gene loci in type 2 diabetes in Chinese Han population.

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