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Association of *TRPC1* Gene Polymorphisms with Type 2 Diabetes and Diabetic Nephropathy in Han Chinese Population

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The recent genome-wide association studies reveal that chromosome 3q resides within the linkage region for diabetic nephropathy (DN) in type 1 and type 2 diabetes mellitus (T1D and T2D). The *TRPC1* gene is on chromosome 3q22-24, and it has been demonstrated that *TRPC1* expression is reduced in the kidney of diabetic animal models. Genetic association of *TRPC1* polymorphism with T1D and DN has been reported in European Americans. However, there are no studies reporting the association of *TRPC1* genetic polymorphism with T2D with and without DN in Chinese population. This study aimed to demonstrate the genetic role of TRPC1 in the development of T2D with and without DN in Chinese Han population. A genetic association study of TRPC1 was performed in T2D cases and in nondiabetic controls from Han population located in Northern Chinese areas. Six tag single nucleotide polymorphism (SNP) markers derived from HapMap data were genotyped. Among the six SNPs, only rs7638459 was suspected as risk factor of T2D without DN, fitting the log-additive model. The adjusted odds ratio (OR) for the CC genotyping was 2.39 (95% confidence interval (CI) = 1.00–5.68), compared with the TT genotyping. In addition, rs953239 was found to be a protective factor of getting DN in T2D, also fitting the log-additive model. When compared with the AA genotyping for SNP rs953239, the adjusted OR for CC genotyping was 0.63 (95% CI = 0.44–0.99). To summarize, this study shows that TRPC1 genetic polymorphisms are associated with T2D and DN in T2D in the Han Chinese population.

Keywords *TRPC1,* Single nucleotide polymorphism, Type 2 diabetes mellitus, Diabetic nephropathy

INTRODUCTION

Type 2 diabetes mellitus (T2D), a common multifactorial metabolic disease, is caused by both environmental and genetic factors. The incidence of T2D continues to rise and increasingly affects individuals of all ages across all ethnic groups (1,2). Genetic studies consistently indicate that diabetes is familial in nature. Although a lot of genome-wide linkage analyses of T2D have been performed, the genes susceptible to T2D remain largely unknown (3–13). Diabetic nephropathy (DN) is a frequent complication of diabetes mellitus, which may lead to end-stage renal disease. DN is the primary cause of morbidity and mortality in patients with Type 1 diabetes mellitus (T1D) and T2D (14).

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The development of DN is likely affected by both the environmental and genetic factors. Several genome-wide scans and linkage analyses indicate that a region of chromosome 3q is linked to DN (15–20). Glomerulosclerosis is the major morphological change in patients with DN (21). Patients with T1D and T2D appear to progress through similar stages of DN, whereas the routes they take may differ (22). Glomerular mesangial cells, located adjacent to glomerular capillaries, appear to play important roles in the physiologic regulation of glomerular hemodynamics. The transient receptor potential channel 1 (*TRPC1*) molecule has been identified in both glomeruli and glomerular mesangial cells (23–26). The *TRPC1* gene is located in chromosome 3q22–24 within the linkage region to DN. Thus, *TRPC1* represents a strong positional and biological candidate for DN.

Recent studies have demonstrated the reduction of *TRPC1* gene expression in diabetic patient (27), db/db mice (28), and kidney and liver of diabetic ZDF- and STZ-administered rats as a result of HNF4 dysfunction (29). Although a study reported the association of *TRPC1* gene with T1D and DN in European Americans (28), the genetic association has not been analyzed in T2D with or without DN in Chinese population. Different ethnic groups have distinct population histories, and in each population, different genes are likely to be responsible for disease susceptibility. The genetic analysis of the Chinese population is likely to make a unique contribution to the understanding of diabetes susceptibility and resistance. This research aims to evaluate the potential role of *TRPC1* gene in the development of T2D and DN in Chinese Han population.

MATERIALS AND METHODS

Subjects

In this work, we conducted a case–control study in Chinese Han population located in the northern region of China. A total of 223 consecutive Chinese patients with T2D were recruited from the Department of Endocrinology and Metabolism, The Second Affiliated Hospital of Harbin Medical University and Department of Endocrine Daqing Oil Field General Hospital. They diagnosed the clinical criterion of T2D as recommended by the Diagnostic Standards of WHO (1999 and 2003) and the International Committee of Experts of Diabetes. The cases were sorted into two groups: (1) T2D with DN Group, which consisted of 134 patients and (2) T2D without DN Group, which consisted of 89 patients. T2D patients older than 8 years and without nephropathy who were age- and sex-matched with the T2D with DN Group were included in the T2D without DN Group. A total of 120 healthy subjects served as controls. These individuals were recruited from a random population sample in Harbin and Daqing from the hospital during the same period when patients with T2D were recruited. All the subjects provided informed consent before participating in the trial.

DNA Extraction

A blood sample of 200 μL was taken from each participant for genotyping. The samples were delivered in a frozen state to the Department of Biotechnology, The Second Affiliated Hospital of Harbin Medical University and stored at −70°C until DNA extraction. Genomic DNA samples were extracted using the TiangenTM Genomic DNA Kit (Tiangen, China).

Single Nucleotide Polymorphism Selection and Genotyping

Six examined single nucleotide polymorphisms (SNPs; rs953239, rs7638459, rs7621642, rs2033912, rs3821647, and rs7610200) were the tag markers in *TRPC1* gene selected from the International HapMap Project database and the information in dbSNP database.

After being diluted quantitatively, DNA of the samples were placed in 384-orifice plate in concentration order by the addition of polymerase chain reaction (PCR) mixture containing 0.1 unit Taq DNA polymerase, 0.5 ng DNA, 2.5 pmol PCR primer each, and 2.5 mmol dNTP. The PCR parameters were as follows: template denaturation for 15 min at 95°C, primer annealing for 30 s at various temperatures depending on the primers used, and primer extension for 30 s at 72°C for 45 cycles. The redundant dNTPs were removed by 0.3 unit alkaline phosphatase. The single-base extended reaction was performed by adding 5.4 pmol extension primer each, 50 μmol dNTP/ddNTP mixture, and 0.5 unit Thermo Sequenase DNA polymerase. The parameters of this reaction were as follows: template denaturation for 2 min at 94°C, for 5 s at 94°C, primer annealing for 30 s at 56°C, and primer extension for 5 s at 72°C for 40 cycles. The reaction products were desalinated in resin for 20 min by addition into SpectroCHIP automatically.

The six SNPs were genotyped using Sequenom MassARRAY matrix-assisted laser desorption ionization-time of flight mass spectrometry platform (MassARRAY, Sequenom, CA, USA), according to the manufacturer's instructions (www.sequenom.com). The call rate for each assay was set at >92.6%. There were no discordances in replicate samples. Primers were designed using Sequenom MassARRAY Assay Design3.1 software, according to the subject sequences and their SNPs in *TRPC1* gene.

Statistical Analysis

The differences in age and gender between patients and controls were compared using test or χ^2 test, whichever was appropriate. A significant departure of genotype frequency from the Hardy–Weinberg equilibrium (HWE) for each SNP was estimated using a HW calculator (Michael H. Court, 2005–2008) downloaded from the Internet (http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls). Significant differences in genotype and allele frequency between cases and controls were assessed by using χ^2 test. Association analysis based on unconditional logistic regression was carried out by calculating the odds ratio (OR) and 95% confidence interval (95% CI) for each SNP in every chosen genetic model. p-Value <0.05 was considered statistically significant. The statistical tests were implemented in the web-based tool SNPstats (http://bioinfo.iconcologia.net/SNPstats) (30).

RESULTS

To analyze the characteristics of the clinical sample, the demographic data of the healthy controls and patients with combined T2D were shown in the Table 1. From the results, it appears that there is no significant correlation difference in gender between the samples in control and in T2D or DN ($p > 0.05$). When compared with control group, T2D or DN groups had significant correlation differences in the patients' age ($p \leq 0.05$).

TABLE 1 Descriptive Characteristics of Study Population

Group	*N*	Mean Age (SD)	*P* value[a]	Sex		*P* value[a]
				Male (%)	Female (%)	
Normal control	120	52.8 (6.4)		61 (50.8)	59 (49.2)	
T2D with DN	134	59.3 (12.4)	$<1 \times 10^{-3}$	75 (56.0)	59 (44.0)	0.413
T2D without DN	89	56.8 (11.8)	0.005	52 (58.4)	37 (41.6)	0.276
Combined T2D	223	58.3 (12.2)	$<1 \times 10^{-3}$	127 (57.0)	96 (43.0)	0.278

Abbreviations: DN, diabetic nephropathy; SD, standard deviation; T2D, Type 2 diabetes mellitus.
[a]p-Value in each group was calculated by comparing each T2D case group with normal control group.

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In the genome-wide association studies (GWAS), HWE test was used to evaluate the quality of genotyping in controls and cases. In this test, the distributions of genotype frequencies of the *TRPC1* SNPs in the cases groups and control group were first figured out, from which their allele frequencies were deduced. Then, the theoretical genotype frequencies were gained based on the HWE. Finally, χ^2 test was used to analyze the calculated and theoretical results. The results of the analysis reveal that if the SNP site in the control cohort was unmatched with Hardy–Weinberg proportion ($p > 0.05$), the quality of the genotyping analysis in this SNP site was suspectable. On the other hand, if SNP loci in the case groups did not conform to HWE, these SNP loci may have association with disease. In this study, the distributions of genotyping and allele frequencies of the *TRPC1* six SNPs obtained from Chinese Han population of the northern region were analyzed. The results are shown in Table 2 that the six SNPs satisfied the HWE assumption ($p > 0.05$).

To identify the relationship between the *TRPC1* and diabetes mellitus, the allele and minor allele frequencies (MAFs) of the *TRPC1* six SNPs loci were analyzed (shown in Table 3). MAF refers to the frequency at which the less common allele occurs in a given population. The mutational sites gained from MAF are always one of the rare mutations in the same gene or the same pathway. It is widely used in GWAS of complex diseases. In the GWAS, lower values of MAF would reduce the statistical performance, which would result in false-negative results. In Table 3, the minor allele of rs7638459 in control group is C, whereas the minor allele of it in the T2D without DN group is T. The differences are significant. The results show that rs7638459 may be associated with the probability of getting T2D.

In order to confirm the association between the genotyping of rs7638459 and the possibility of getting T2D, further multivariate logistic regression analysis was performed. The results show the risk of getting T2D without DN of rs7638459 in these sorts of models (OR and 95% CI values are shown in Table 4). It appears that the optimum genetic model of rs7638459 is the log-additive model ($p = 0.045$). In the rs7638459, CC genotyping could significantly increase the risk of getting T2D without DN, when compared with TT genotyping (OR = 2.39 and 95% CI = 1.00–5.68).

Logistic regression analysis using codominant model was performed for the six SNPs of *TRPC1*, after being adjusted for gender and age. The analysis of the OR values (95% CI), showed no significant differences of SNPs rs7621642, rs2033912, rs3821647, and rs7610200 with diabetes mellitus among T2D without DN and with DN ($p > 0.05$; results not shown). In order to confirm the association between *TRPC1*-specific polymorphism sites and the risk of getting T2D, multivariate logistic regression analysis of rs7638459 and rs953239 was performed (Tables 4 and 5).

Table 5 shows that the most suitable genetic model of rs953239 is the log-additive model ($p = 0.043$, OR = 0.63, and 95% CI = 0.40–0.99). In rs953239, CC genotyping could significantly reduce the risk of getting T2D without DN, when compared with AA genotyping (OR = 2.39 and 95% CI = 1.00–5.68).

DISCUSSION

T2D is a complex genetic disease involving a couple of susceptibility genes. In this study, we have conducted a genetic association study to detect the genetic susceptibility of *TRPC1* for T2D and DN in a Chinese population. In this study, the SNPs rs7638459 and rs953239 of *TRPC1* were found as susceptibility gene sites associated with T2D and T2D without DN. In the rs7638459 polymorphisms, the CC genotyping could significantly increase the risk of getting T2D, when compared with TT

TABLE 2 Genotype Distributions of the Six SNPs in Healthy Control and T2D Patients

		Genotype Frequencies				Hardy–Weinberg Proportions $(p)^a$			
SNP rsID	Genotype	Control	Combined T2D	T2D with DN	T2D without DN	Control	Combined T2D	T2D with DN	T2D without DN
rs953239	A/A	62 (52.5%)	107 (48%)	71 (53%)	36 (40.5%)				
	C/A	45 (38.1%)	102 (45.7%)	57 (42.5%)	45 (50.6%)	0.5	0.14	0.26	0.35
	C/C	11 (9.3%)	14 (6.3%)	6 (4.5%)	8 (9%)				
rs7638459	T/T	42 (35.3%)	52 (26%)	34 (28.3%)	18 (22.5%)				
	C/T	58 (48.7%)	103 (51.5%)	61 (50.8%)	42 (52.5%)	1	0.78	0.86	0.82
	C/C	19 (16%)	45 (22.5%)	25 (20.8%)	20 (25%)				
rs7621642	G/G	46 (38.3%)	72 (32.6%)	45 (34.1%)	27 (30.3%)				
	G/A	56 (46.7%)	119 (53.9%)	71 (53.8%)	48 (53.9%)	0.85	0.095	0.2	0.39
	A/A	18 (15%)	30 (13.6%)	16 (12.1%)	14 (15.7%)				
rs2033912	A/A	40 (33.3%)	79 (35.4%)	50 (37.3%)	29 (32.6%)				
	T/A	63 (52.5%)	113 (50.7%)	68 (50.8%)	45 (50.6%)	0.45	0.4	0.36	0.83
	T/T	17 (14.2%)	31 (13.9%)	16 (11.9%)	15 (16.9%)				
rs3821647	G/G	67 (57.3%)	125 (56.3%)	79 (59%)	46 (52.3%)				
	A/G	43 (36.8%)	86 (38.7%)	49 (36.6%)	37 (42%)	1	0.58	0.81	0.59
	A/A	7 (6%)	11 (5%)	6 (4.5%)	5 (5.7%)				
rs7610200	G/G	53 (44.2%)	81 (42%)	47 (41.2%)	34 (43%)				
	A/G	52 (43.3%)	92 (47.7%)	57 (50%)	35 (44.3%)	0.69	0.52	0.29	0.81
	A/A	15 (12.5%)	20 (10.4%)	10 (8.8%)	10 (12.7%)				

Abbreviations: DN, diabetic nephropathy; SNP, single nucleotide polymorphism; T2D, Type 2 diabetes mellitus.
[a] p-Values for differences between T2D patients and healthy control by χ^2 analysis.

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TABLE 3 Allele Frequencies Distributions and Minor Allele Frequencies of *TRPC1* Polymorthisms

		Allele Frequencies				MAF			
dbSNP rsID	SNP type	Control	Combined T2D	T2D with DN	T2D without DN	Control	Combined T2D	T2D with DN	T2D without DN
rs953239	A	169 (0.72)	316 (0.71)	199 (0.74)	117 (0.66)	C 0.28	C 0.29	C 0.26	C 0.34
	C	67 (0.28)	130 (0.29)	69 (0.26)	61 (0.34)				
rs7638459	T	142 (0.6)	207 (0.52)	129 (0.54)	78 (0.49)	C 0.4	C 0.48	C 0.46	T 0.49
	C	96 (0.4)	193 (0.48)	111 (0.46)	82 (0.51)				
rs7621642	G	148 (0.62)	263 (0.6)	161 (0.61)	102 (0.57)	A 0.38	A 0.4	A 0.39	A 0.43
	A	92 (0.38)	179 (0.4)	103 (0.39)	76 (0.43)				
rs2033912	A	143 (0.6)	271 (0.61)	168 (0.63)	103 (0.58)	T 0.4	T 0.39	T 0.37	T 0.42
	T	97 (0.4)	175 (0.39)	100 (0.37)	75 (0.42)				
rs3821647	G	177 (0.76)	336 (0.76)	207 (0.77)	129 (0.73)	A 0.24	A 0.24	A 0.23	A 0.27
	A	57 (0.24)	108 (0.24)	61 (0.23)	47 (0.27)				
rs7610200	G	158 (0.66)	254 (0.66)	151 (0.66)	103 (0.65)	A 0.34	A 0.34	A 0.34	A 0.35
	A	82 (0.34)	132 (0.34)	7 (0.34)	5 (0.35)				

Abbreviations: DN, diabetic nephropathy; SNP, single nucleotide polymorphism; T2D, Type 2 diabetes mellitus.

TABLE 4 rs7638459 Association with Response Status in Healthy Control and in T2D Patients without DN (Adjusted by Gender and Age)

Model Type	Genotype	Control (%)	T2D without DN (%)	OR (95% CI)	p^a
Codominant	T/T	42 (35.3%)	18 (22.8%)	1.00	0.13
	C/T	58 (48.7%)	42 (53.2%)	1.58 (0.79–3.19)	
	C/C	19 (16%)	19 (24.1%)	2.39 (1.00–5.68)	
Dominant	T/T	42 (35.3%)	18 (22.8%)	1.00	0.086
	C/T-C/C	77 (64.7%)	61 (77.2%)	1.77 (0.91–3.45)	
Recessive	T/T-C/T	100 (84%)	60 (76%)	1.00	0.13
	C/C	19 (16%)	19 (24.1%)	1.78 (0.85–3.71)	
Overdominant	T/T-C/C	61 (51.3%)	37 (46.8%)	1.00	0.73
	C/T	58 (48.7%)	42 (53.2%)	1.11 (0.62–1.99)	
Log-additive	–	–	–	1.55 (1.01–2.38)	0.045*

Abbreviations: DN, diabetic nephropathy; SNP, single nucleotide polymorphism; T2D, Type 2 diabetes mellitus; OR, Odds ratio; 95% CI, 95% confidence interval.
The OR values were counted by the web-based tool SNPstats, adjusted by gender and age.
[a]*p-Values* for the differences between controls and T2D without DN in every chosen model, counted by the web-based tool SNPstats.
*$p < 0.05$

TABLE 5 rs953239 Association with Response Status in T2D Patients without DN and in T2D Patients with DN (Adjusted by Gender and Age)

Model Type	Genotype	T2D without DN (%)	T2D with DN (%)	OR (95% CI)	$^a p$
Codominant	A/A	36 (40.9%)	71 (53%)	1.00	0.13
	C/A	44 (50%)	57 (42.5%)	0.65 (0.37–1.15)	
	C/C	8 (9.1%)	6 (4.5%)	0.37 (0.12–1.16)	
Dominant	A/A	36 (40.9%)	71 (53%)	1.00	0.075
	C/A-C/C	52 (59.1%)	63 (47%)	0.61 (0.35–1.05)	
Recessive	A/A-C/A	80 (90.9%)	128 (95.5%)	1.00	0.16
	C/C	8 (9.1%)	6 (4.5%)	0.46 (0.15–1.38)	
Overdominant	A/A-C/C	44 (50%)	77 (57.5%)	1.00	0.27
	C/A	44 (50%)	57 (42.5%)	0.74 (0.43–1.27)	
Log-additive	–	–	–	0.63 (0.40–0.99)	0.043*

Abbreviations: DN, diabetic nephropathy; T2D, Type 2 diabetes mellitus; OR, Odds ratio; 95% CI, 95% confidence interval.
[a]*p*-Values for the differences between T2D without DN and T2D with DN in every chosen model, counted by the web-based tool SNPstats.
*$p < 0.05$.

genotyping. The SNP rs953239 was significantly associated with DN in T2D. When compared with AA genotyping, CC genotyping could remarkably reduce the risk of getting DN.

TRPC1 belongs to the transient receptor potential (TRP) superfamily of cation channels. TRP cation channels are involved in diverse physiologic processes, including receptor- and store-operated Ca^{2+} signal transduction, ion absorption, and cell death. In addition, the reduction of *TRPC1* gene expression has been found in diabetic patient (27), db/db mice (28), and kidney and liver of diabetic ZDF- and STZ-administered rats (29). Based on these reports, it is hypothesized that *TRPC1* gene may be thought as a disease candidate gene linked to diabetes mellitus. *TRPC1* gene is located on chromosome 3q23. This region of 3q has been reported to have association with T2D (31,11) in Japanese population. The results of this study are in agreement with the report. However, the study of *TRPC1* polymorphisms' association revealed the relationship of *TRPC1* SNPs with T1D with DN and without DN (28). Zhang et al.

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have already analyzed several polymorphisms of *TRPC1* in cases with T1D and DN. Most of the clinical samples are from African European population, and a few of them are from African American, Asian, Hispanic, and Indian populations. The research shows that the MAFs of SNP rs7621642 and rs2033912 have significant association with T1D and DN in the European females, which is opposite to the study results in African American females. Evidence has demonstrated that race and ethnicity are related to difference in the susceptibility of *TRPC1* SNPs with diabetes mellitus and DN (31,11).

As a frequent complication of diabetes mellitus, the development of DN also involved the genetic susceptibility and environmental factors. One genome scan of DN in African American subjects reveals evidence for susceptibility loci on chromosome 3q (17); subsequently, it has been reported that the 3q22 region of the chromosome has association with DN in patients with T1D (16,32). However, among the DN patients with T2D, the association can only be found on chromosome 3q region (17,33) not 3q22. Although no significant association of *TRPC1* DNA polymorphisms with DN was found in GoKinD and African American populations (28), we found, in this study, a genetic association between *TRPC1* polymorphisms with DN in T2D.

In conclusion, this study provides the first evidence that *TRPC1* genetic polymorphisms are associated with T2D and DN in T2D in Chinese subjects. In addition, the association between TRPC1 genetic polymorphisms and diabetes mellitus differs according to the subjects' race and region. As studies using different populations to find the relationship between *TRPC1* and diabetes mellitus would have gotten different results, further research can focus on choosing different populations and increasing the number of samples to find the genetic association between *TRPC1* gene polymorphisms and diabetes mellitus.

Furthermore, genes related to cation channel would be good candidates for evaluating the risk of getting DN. Some of these genes, such as *KCNJ11*, *KCNQ1*, and *SLC12A3*, have been reported to have association with DN. Potassium inwardly rectifying channel, subfamily J, member 11 (*KCNJ11*) gene was identified to be predisposed to the development of T2D in Finnish (34) and Italian (35) patients. Recently, a gene polymorphism analysis of East Asian populations, which consisted of Korean, Japanese, and Chinese, also supports the previous result (36). The allelic and genotypic contrast demonstrated that the association between *KCNJ11* and T2D was significant for rs5210 and rs5219 under different genetic models. The *KCNQ1* gene, which is named potassium voltage-gated channel, KQT-like subfamily, member 1, was originally demonstrated as a susceptibility gene for DN. In the Japanese population, it was found that five SNPs were nominally associated with DN, and the association of rs2237897 was the strongest (37). Moreover, the relationship between SNP of KCNQ1 and T2D was also verified in Korean (38), Chinese (39), Singaporean, and Caucasian patients (40), but not for DN in Chinese population yet. A 10-year longitudinal study for the polymorphism of *SLC12A3*, the solute carrier family 12 (sodium/chloride transporters) member 3, showed that the SLC12A3 +78A(+) genotype may be a factor of protective effect against the development of DN in Japanese patients with T2D (41). In addition, Arg913Gin(G→A) polymorphism of *SLC12A3* was considered to be a predictor of increasing the risk of albuminuria in patients with T2D in Chinese Han population (42).

The aforementioned research suggest that the investigation of the relationship between genes related to cation channel and DN is limited. In addition, the studies to find the SNPs for DN can be broadened to genes encoding proteins or polypeptides, which would activate or inhibit the cation channel. For instance, phospholipase C pathway, activated via coupled GTP-binding proteins and tyrosine receptors, greatly potentiates the activation of TRP nonselective cation channels (43). Thus, it would be considered for our further studies of DN.

Declaration of Interest
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