

Association of *TCF7L2* gene polymorphisms with susceptibility to type 2 diabetes mellitus in a Chinese Hui population

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ABSTRACT. Diabetes is one of costly chronic diseases. Previous studies across several ethnicities have shown that polymorphisms in the transcription factor 7-like 2 (*TCF7L2*) gene were strongly associated with susceptibility to type 2 diabetes (T2DM). In the present study, the association between the *TCF7L2* gene and the susceptibility to T2DM in a Chinese Hui population was interrogated. Polymerase chain reaction (PCR)- restriction fragment length polymorphism analysis and allelic specific PCR were employed for examining the *TCF7L2* gene rs12255372 (G>T) and rs290487 (C>T), and rs7901695 (T>C) polymorphisms, respectively, in 109 healthy individuals and 111 subjects with T2DM who were of Chinese Hui descent and lived in the Ningxia Hui Autonomous Region of China. The results showed that the genotypic frequency of rs290487 and the allelic frequency distributions of the rs7901695 and rs290487 loci were not significantly different between patients and controls in this population. However, both the genotypic and the allelic frequencies at rs12255372 exhibited statistical

differences between the patients with T2DM and the unaffected cohort ($P < 0.01$). In addition, the frequency of the G allele at the rs12255372 locus in the patients was higher than that in healthy individuals (OR = 1.198, 95%CI = 1.097-1.307). These findings suggest that the *TCF7L2* rs12255372 (G>T) polymorphism might be one of the most important genetic factors associated with T2DM susceptibility, and that individuals in the Chinese Hui population who carry a G allele at this locus might be at risk to develop T2DM.

Key words: TCF7L2; Polymorphism; Type 2 diabetes mellitus; Chinese Hui population

INTRODUCTION

Diabetes mellitus (DM) is a major health problem worldwide. It is estimated that the number of individuals affected by DM will increase to 300 million by the year 2025, with type 2 DM (T2DM) accounting for 90% of the diagnoses (King et al., 1998). The incidence and prevalence of T2DM are predicted to rapidly increase over the next decade owing to human longevity and the surge in obesity in many countries, particularly in China where T2DM will contribute almost 38 million patients to the global burden of DM by 2025 (Dou et al., 2013; Wang et al., 2013b). The global burden in 2010 was 285 million people, and the number of individuals affected by DM is projected to increase by 65% to 438 million by 2030 (Raza et al., 2013).

T2DM is a chronic, complex, and life-long disease with a strong genetic component, characterized by hyperglycemia that can occur through varied mechanisms such as impaired insulin secretion, insulin resistance in peripheral tissues, and increased glucose output by the liver (Tong et al., 2009). Patients with T2DM are at increased risk of other diseases including cardiovascular disease, kidney failure, blindness, neuropathy, and peripheral circulatory disease. T2DM thus has a significant impact on the quality of life of the patient, and increases the morbidity and mortality of other diseases (Liu et al., 2013).

Although it is well-known that insulin resistance plays a major role in the development of T2DM, the pathogenesis of T2DM is generally considered to result from the interactions between multiple susceptibility genes, as well as from interactions between genes and the environment (Edwards et al., 1997; Fu et al., 2013; Li et al., 2013). Transcription factor 7-like 2 (*TCF7L2*) is a Wnt signaling-associated transcription factor which is expressed in a board range of tissues including the gut and the pancreas. The *TCF7L2* gene is located on chromosome 10q25.3 and has been identified as a major T2DM susceptibility gene by a large scale genome-wide association study (GWAS) in 2006 (Grant et al., 2006). This important finding has been subsequently validated by genetic analyses of disease susceptibility in various ethnic groups in recent years (Ip et al., 2012).

Previous genetic analyses have uncovered several genes that showed strong association with T2DM susceptibility, among which the transcription factor *TCF7L2* gene was one of the most important ones (Gloyn et al., 2003). In addition, the impact of polymorphisms in the *TCF7L2* gene on the susceptibility to T2DM has been attributed to their indirect effects on alteration of glucagon-like peptide1 (GLP-1) levels. This notion was supported by the finding in human pancreatic beta cells that overexpression of *TCF7L2* was associated with impaired

insulin secretion (Yi et al., 2005; Cauchi et al., 2006; Shu et al., 2009). Equal importantly, the single nucleotide polymorphisms (SNPs) of *TCF7L2* have been consistently associated with the susceptibility to T2DM in populations of different ethnic descent, making *TCF7L2* one of the most important genes known to date for predisposing to T2DM risks (Bodhini et al., 2007; Tong et al., 2009). The aim of the study is therefore to investigate whether the rs12255372 (G>T), rs290487 (C>T), and rs7901695 (T>C) SNPs of the *TCF7L2* gene locus are associated with the susceptibility to T2DM in a Chinese Hui population from the Ningxia Hui Autonomous Region of China.

MATERIAL AND METHODS

Subjects

Blood samples were taken from 111 patients with T2DM and 109 ethnically matched non-T2DM controls of a Chinese Hui population; patients were consecutively enrolled from the outpatient clinic of the Affiliated Hospital of Ningxia Medical University. Clinical variables were obtained for each patient, including age, alcohol consumption, body mass index, height, weight, cigarette smoking, and family history. Patients with overnight fasting plasma glucose (FPG) levels higher than 126 mg/dL on two consecutive events were included in the T2DM category, while patients with FPG levels below 110 mg/dL and without a family history of diabetes were included in the study as non-T2DM controls. The controls were recruited from the general Hui population and had undergone comprehensive medical screening at the hospital. All subjects were included in this study based on two criteria: pure Hui descent for at least three generations and history of individual ancestors living in the Ningxia Hui Autonomous Region of China for at least three generations. There was no genetic relationship among any individuals involved in the study. All samples were collected under informed consent, and the ethnic committee of Ningxia University approved this study.

SNP screening and genotyping

The genomic DNA of leukocytes isolated from peripheral blood was extracted using a Wizard Genomic DNA purification kit following the manufacturer instructions (Promega, Madison, WI, USA). SNPs at the rs12255372 (*TasI*, G>T), rs290487 (*AccII*, C>T), and rs7901695 (T>C) sites of the *TCF7L2* gene were examined in this study. Genotyping of the rs12255372 (G>T) and rs290487 (C>T) loci was carried out by polymerase chain reaction (PCR)- restriction fragment length polymorphism analysis, and the rs7901695 (T>C) site was genotyped by allelic specific PCR. The primer sets used for PCR and the restriction endonucleases used for digestion are listed in Tables 1 and 2, respectively. PCR products were purified by using a PCR purification kit, followed by digestion with the restriction endonucleases *TasI* or *AccII*, before being resolved on a 2% agarose gel containing ethidium bromide (Table 2). The PCR kit, PCR purification kit, and restriction endonucleases were purchased from TaKaRa (Osaka, Japan). The genotypes were scored blindly, and analysis of all ambiguous samples was repeated. All samples were examined twice to ensure the accuracy of the results.

Table 1. Primer sets used for amplifying the three polymorphisms in the *TCF7L2* gene.

Polymorphisms position	Primer sequence	Annealing (°C)
rs12255372 (G>T)	F: 5'-CTTGAGGTGACTGGAACTAAGGC-3' R: 5'-CTGTCTATTGGCATTCAAATGGA-3'	60 60
rs290487 (C>T)	F: 5'-AGGAGGCTGCCATATTGTTACTT-3' R: 5'-ACACCTTCTCATTTCATTTTCGC-3'	61 61
rs7901695 (T>C)	F1: 5'-AGACAGACACAAAAGCACA-3' F2: 5'-AGACAGACACAAAAGCCCG-3' R: 5'-GAAACCTCCCTTACCCATT-3'	60 60 60

Table 2. Determination of the *TCF7L2* genotypes using PCR-RFLP or AS-PCR assay.

SNPs	Size of PCR product (bp)	Primer set	Restricted enzyme	Fragments of RFLP (bp)	Genotype
PCR-RFLP					
rs12255372(G>T)	346	F/R	TasI	143, 126, 104, 99, 17 143, 104, 99	GT GG TT
rs290487(C>T)	153	F/R	AccII	126, 104, 99, 17 153, 128, 25 128, 25 153	TC CC TT
AS-PCR					
rs7901695(T>C)	449	F1/R F2/R F1, F2/R	N/A N/A N/A	N/A N/A N/A	TT CC CT

PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism. AS-PCR = allele specific PCR.

Statistical analysis

Genotype and allele carrier frequencies were defined as the percentage of individuals carrying the genotype and allele out of the total number of individuals, respectively. The χ^2 and Fisher exact tests of SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA) were used to compare the frequency of discrete variables between patients with T2DM and control individuals. A Bonferroni's corrected P value threshold was employed for correction of type I error. A P value < 0.05 was considered to be statistically significant.

RESULTS

To determine whether the rs12255372 (*TasI*, G>T) (Figure 1A), rs290487 (*AccII*, C>T) (Figure 1B), and rs7901695 (T>C) (Figure 1C) polymorphic loci within *TCF7L2* gene were associated with susceptibility to T2DM in the Chinese Hui ethnic population living in the Ningxia region, we analyzed these SNPs in 109 healthy individuals and 111 patients with T2DM of Hui descent currently living in this region. Polymorphic analysis revealed that all three possible genotypes (GG, GT, and TT at the rs12255372 site; TT, CT, CC at rs290487; and TT, CT, and CC at rs7901695) could be detected for these SNPs. The GG genotype was the major genotype at the rs12255372 locus in the subjects studied. There were no statistical differences in the distributions of either genotypes or alleles at the rs290487 site of the *TCF7L2* gene between patients with T2DM and control cohorts in the Chinese Hui population (Table 3). However, the genotypic frequencies of rs12255372 and rs7901695, and the allelic frequency of the rs12255372 locus showed significant differences between the patients with T2DM and the controls (P < 0.01, Table 3). The frequency of the rs12255372 G allele in the

T2DM group (90.1%) was higher than that in the control group (75.2%), with OR = 1.198, 95%CI = 1.097-1.307. In contrast, the frequency of the rs12255372 T allele was found to be a protective factor for T2DM with a higher frequency in the control group (24.8%) compared to the patient group (9.9%), OR = 0.400, 95%CI = 0.253-0.633 (Table 3). This result suggests that the G allele at the rs12255372 site might be a risk factor for T2DM, and that the T allele at the rs12255372 site might be a protective factor against T2DM in this Chinese Hui population.

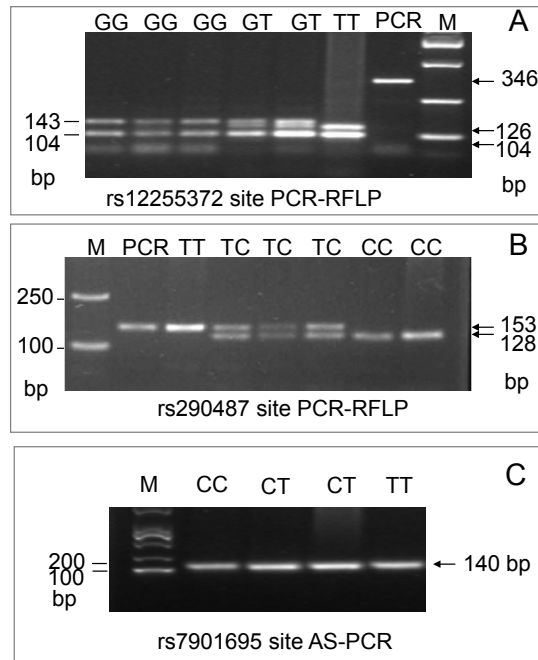


Figure 1. Genotype analysis by PCR-RFLP and AS-PCR assays. **A.** rs12255372 (G>T), **B.** rs290487 (C>T), **C.** rs7901695 (T>C). Lane M = DNA molecular ladders. Other lanes show the corresponding genotypes labeled at the top of each image.

Table 3. Genotype and allele analyses of the polymorphisms of *TCF7L2* gene in T2DM patients and non-T2DM control in a Chinese Hui population.

Position	Genotype/allele	Control [N (%)]	T2DM [N (%)]	χ^2	P	OR (95%CI)			
rs12255372 (G>T)	GG	74 (67.9)	97 (87.4)	12.103	0.002 < 0.01				
	GT	16 (14.7)	6 (5.4)						
	TT	19 (17.4)	8 (7.2)						
	G	164 (75.2)	200 (90.1)				16.999	0.000 < 0.01	1.198 (1.097-1.307)
T	54 (24.8)	22 (9.9)							
rs290487 (C>T)	TT	34 (31.2)	27 (24.3)	5.370	0.068 > 0.05				
	TC	44 (40.4)	62 (55.9)						
	CC	31 (28.4)	22 (19.8)						
	T	112 (51.4)	116 (52.3)				0.034	0.854 > 0.05	1.017 (0.849-1.218)
C	106 (49.1)	106 (47.7)							
rs7901695 (T>C)	TT	22 (20.2)	4 (3.6)	30.708	0.000 < 0.01				
	TC	64 (58.7)	101 (91.0)						
	CC	23 (21.1)	6 (5.4)						
	T	108 (49.5)	109 (49.1)				0.009	0.926	0.991 (0.820-1.198)
	C	110 (50.5)	113 (50.9)						

DISCUSSION

The Chinese Hui minor ethnicity is one of 56 nationalities in China, and has a total population of over 12 million. The Hui are descended from Arabic and Persian merchants who came to China during the 7th century, with most of the group living in the Ningxia Hui Autonomous Region. To retain religious purity and group identity, the Hui population is culturally and religiously conservative; they have always segregated themselves socially from other groups, living in enclaves. The Hui marriage practices tend toward endogamy in all respects, particularly in the rural part of the Ningxia Hui Autonomous Region. Our previous studies have shown that the Ningxia Hui ethnicity has a distinct genetic background and this group has exhibited differences in the susceptibility to rheumatoid arthritis and essential hypertension (Xu et al., 2011, 2012a,b; Yang et al., 2014a,b).

There are currently more than 2.2 million Chinese Hui living in the Ningxia Hui Autonomous Region of China (Ningxia Hui population). The incidence of DM in the Ningxia Hui population has been estimated to be as high as 20%, which is much higher than that in the Chinese Han population in this region, implying that a genetic factor might contribute to this disorder in the Hui population. In support of this hypothesis, we first interrogated the association of *TCF7L2* polymorphisms with the susceptibility to T2DM in the Chinese Hui ethnic group in the present study. We demonstrated significant differences in the genotypic frequencies of rs12255372 and rs7901695, and the allelic frequency at the rs12255372 site was found to differ significantly between the patients with T2DM and the non-T2DM individuals as well. Furthermore, the G allele of the rs12255372 site was suggested to be a likely risk factor for T2DM, whereas the T allele at the rs12255372 locus seemed to behave as a protective factor for T2DM in this population.

Our findings are consistent with other evidence that genetic factors are key contributors to the susceptibility to T2DM in various ethnicities, and the list of risk loci for T2DM is continually expanding. Since the initial report of an association of a common microsatellite (DG10S478) within intron 3 of *TCF7L2* with the susceptibility to T2DM, the strong linkage of SNPs in the *TCF7L2* gene with T2DM has been intensively explored in different populations worldwide. These studies have further confirmed the association of *TCF7L2* gene variants with the susceptibility to T2DM in populations of different ethnic origins, including: British (Groves et al., 2006), Dutch (van Vliet-Ostapchouk et al., 2007), Amish (Damcott et al., 2006), Finnish (Scott et al., 2006), Swedish (Mayans et al., 2007), French (Cauchi et al., 2006), American (United States) (Zhang et al., 2006), Indian (Chandak et al., 2007), and Japanese (Hayashi et al., 2007). A meta-analysis using a total of 33 articles including 42 studies (with 34,076 patients and 36,192 controls) has confirmed that rs12255372 is significantly associated with the susceptibility to T2DM in the global population (Wang et al., 2013a). In agreement with this notion, we also found a strong association of this locus with T2DM in this report. However, no association between the rs290487 and rs7901695 polymorphisms and T2DM was detected in the Chinese Hui population living in the Ningxia region; this result was consistent with those from a report from a meta-analysis using 9 studies of 9422 patients with T2DM and 8585 control subjects in the Han Chinese population (Ren et al., 2013).

In conclusion, this study demonstrated that the rs12255372 (G>T) polymorphism, but not the rs290487 and rs7901695 polymorphic sites, is associated with the susceptibility to T2DM in a Chinese Hui population. Additional research is required to confirm the present findings by using studies encompassing large sample sizes and other populations of various ethnic origins.

Conflicts of interest

The authors declare no conflict of interest.

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