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Associations of *TCF7L2* gene polymorphisms with the risk of diabetic nephropathy

A case–control study

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Abstract

The aim of the study was to explore the correlation between rs7903146 and rs290487 polymorphisms in transcription factor 7-like 2 (*TCF7L2*) gene and diabetic nephropathy (DN) in Chinese Han population.

Polymerase chain reaction–restriction fragment length polymorphism was used to determine genotypes of *TCF7L2* polymorphisms in 90 patients with DN and 96 diabetes patients without DN. The linkage disequilibrium (LD) and haplotype analysis were performed with haploview software. Hardy–Weinberg equilibrium was assessed in the control group based on the genotype distributions of *TCF7L2* polymorphisms. The genotype, allele, and haplotype distribution differences between the case and control groups were analyzed by chi-squared test, and odds ratio (OR) and 95% confidence interval (CI) were used to indicate the relative risk of DN.

People carrying TT genotype of rs7903146 were more easily to be attacked by DN than CC genotype carriers ($P = .02$, OR = 4.26, 95% CI = 1.12–16.24). Meanwhile, T allele also showed 1.85 times risk to suffer from DN compared with C allele (OR = 1.85, 95% CI = 1.02–3.10). However, there was no significant difference in genotypes and alleles frequencies of rs290487 between 2 groups. The strong LD existed between the 2 single nucleotide polymorphisms and haplotype T–T (rs7903146–rs290487) increased the susceptibility to DN (OR = 2.63, 95% CI = 1.31–5.25).

TCF7L2 rs7903146 polymorphism may be associated with the susceptibility to DN in Chinese Han population, but rs290487 is not. Additionally, haplotype is also a risk factor for DN.

Abbreviations: ACR = albumin-to-creatinine ratio, AGE = agarose gel electrophoresis, BUN = blood urea nitrogen, CI = confidence interval, DM = diabetes mellitus, DN = diabetic nephropathy, ESRD = end-stage renal disease, HWE = Hardy–Weinberg equilibrium, LD = linkage disequilibrium, OR = odds ratio, PCR = polymerase chain reaction, T1DM = type 1 diabetes mellitus, T2DM = type 2 diabetes mellitus, TCF7L2 = transcription factor 7-like 2.

Keywords: diabetic nephropathy, haplotype, polymorphism, *TCF7L2* gene

1. Introduction

Diabetic nephropathy (DN) is the most common and serious microvascular complications caused by diabetes. The thickening of the glomerular basement membrane and substance hyperplasia of a large number of basement membrane in mesangial region are the basic pathological change. With the increasing incidence of diabetes mellitus (DM), the morbidity of DN is increased year by year, and it has become the main cause of end-stage renal disease (ESRD).[1] DM can be divided into 2 groups of type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), which all can develop into DN. The US report has revealed that about 30% of T1DM and 20% of T2DM have developed into DN, in which about 50% die of ESRD.[2] Obviously, DN has been the main cause of death in chronic kidney disease.[3] DN not only brings body's illness and the life pressure to patients, but also brings serious economic burden to patients' family. A large number of studies have indicated the role of genetic factors on the susceptibility of DN, but the etiology is still not clear.[4–6] So the research on the etiology of DM and DN will arouse more and more attentions.

Transcription factor 7-like 2 (T-cell specific, HMG-box), also known as TCF7L2 or TCF4, is a highly variable transcription factor, which is a key component of the Wnt-signaling pathway and plays an important role in the regulation of insulin secretion by pancreatic beta cells and the maintenance of glucose homeostasis.[7,8] It is also involved in vascular remodeling through the regulation of smooth muscle cell proliferation and endothelial cell growth.[9,10] In humans, this protein is encoded by the *TCF7L2* gene.[11] Human *TCF7L2* gene spans a 215,863 bases region on chromosome 10q25.3, containing 17 exons and encoding 596 amino acids. *TCF7L2* gene is first identified as a diabetes risk conferring gene in 2006,[12] and a number of polymorphic loci have been identified.

Previous studies have indicated that *TCF7L2* gene polymorphisms are associated with diabetic coronary atherosclerosis and chronic kidney disease.[13,14] Recently, some studies have

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indicated that there is a correlation between *TCF7L2* gene polymorphism and DN.[15] But in China, there are few studies on the relationship between *TCF7L2* gene mutations and DN risk.

Therefore, the aim of our study was to investigate the effect of the rs7903146 and rs290487 polymorphisms of the *TCF7L2* gene on DN in Chinese Han population.

2. Materials and methods

2.1. The case and control groups

A case–control study was conducted in present. There were 186 patients with T2DM in our study who were diagnosed according to the 2003 American Diabetes Association diagnostic criteria for diabetes. The consecutive patients were recruited from Linyi Central Hospital during May, 2013 to January, 2015, who were diagnosed with diabetes for more than 5 years, aged 30 to 75 years old. We further classified all subjects into 2 groups: case group with 90 patients with DN and control group with 96 DM patients without DN, according to their 24-hour urinary albumin-to-creatinine ratio (ACR), blood urea nitrogen (BUN), and serum creatinine.[16–18] Patients who were classified as the case group fulfilled any of the following 3 criteria: the average ACR was >300 μg/mg; the BUN was >20 mg/dL; and the serum creatinine was >1.7 mg/dL. The rest of the patients were classified as the control group without DN.

All subjects knew about this research process and signed the informed consent. Blood samples were collected and the basic information of the objectives was detailedly recorded by specially trained epidemiological investigators. This research was authorized by the Ethics Committee of Linyi Central Hospital. The process of sample collection was conducted according to the national ethics criteria of human genome research.

2.2. DNA extraction

First, 3 mL peripheral venous blood were collected from each patient, and then placed in anticoagulant tubes with ethylenediaminetetraacetic acid and stored at −80°C. Next, the peripheral blood leukocyte genome DNA of all samples were extracted by using Beijing TIANGEN biochemical blood genome DNA extraction kit according to the manufacturer's instructions and then stored in −20°C refrigerator for standby application.

2.3. TCF7L2 genotyping

GeneBank database of NCBI was adopted to find the complete sequence of *TCF7L2* in human chromosome 10 and the sequences of 2 single nucleotide polymorphisms (SNPs). Then, complying with general primer design principles, we designed the polymerase chain reaction (PCR) primers using Primer Premier 5.0 software. The detailed sequences were listed in Table 1.

Table 1

Primer sequences of *TCF7L2* rs7903146 and rs290487.

SNP	Primer sequence	Primer length, bp
rs7903146		
Forward	5′-CAAATTCATGGGCTTTCT-3′	151
Reverse	5′-CCTTCCCTGTAACTGTGG-3′	
rs290487		
Forward	5′-GCTGCCATATTGTTTACT-3′	112
Reverse	5′-ATGATTTGTACTGGGTTG-3′	

SNP = single nucleotide polymorphisms.

PCR amplification reaction system was a total volume of 25 μL, including 2.5 μL 10× PCR loading buffer, 2.0 μL dNTP mix, 1.0 μL forward primer, 1.0 μL reverse primer, 2.0 μL *Taq* DNA polymerase, 2.0 μL genomic DNA template, 2.0 μL $MgCl_2$ solution, and 12.5 μL sterilization ddH_2O. The PCR amplification conditions were 94°C predenaturation for 5 minutes; followed by 36 cycles of 94°C degeneration for 30 seconds, 51°C annealing for 30 seconds, 72°C extension for 30 seconds, and finally 72°C extension for 10 minutes. The PCR products were checked in 1% agarose gel electrophoresis (AGE).

The enzyme digestion reaction system was 20 μL, including 2.0 μL 10× buffer R, 1.0 μL restriction enzyme (*Acc*II for rs7903146, *Mlu*I for rs290487), 10 μL PCR products, and 7.0 μL double-distilled water. And then the PCR products were digested for 12 hours in water bath of 37°C. Enzyme digestion products were separated by 4% AGE.

2.4. Statistical analysis

PASW Statistics 18.0 software was used to analyze the data and all data were expressed by $\bar{x} \pm s$ or %. Genotype and allele frequencies of *TCF7L2* rs7903146 and rs290487 polymorphisms were calculated through direct counting. Linkage disequilibrium (LD) and its correlation coefficient (D′ value) were calculated by haploview software. If existed LD, haplotypes would be analyzed. The chi-squared test was used to check whether the genotype distributions of the study population matched Hardy–Weinberg equilibrium (HWE). The comparison of genotypes, alleles, and haplotypes between 2 groups were also tested by chi-squared test. The effect of *TCF7L2* polymorphisms on DN was evaluated with odds ratio (OR) and 95% confidence interval (CI). $P < .05$ was considered as the statistically significant difference.

3. Results

3.1. General characteristics of study objects

As shown in Table 2, the genotypes distribution of *TCF7L2* gene rs290487 polymorphism was conformed to HWE, while rs7903146 was not. But because the study population was all T2DM patients, the SNP would unnecessarily be in HWE. Moreover, the patients were all Chinese Han population and they had no blood relationship with each other. In addition, there was no significant different between the 2 groups by gender and age ($P > .05$ for both).

3.2. The genotype distributions of TCF7L2 polymorphisms in case and control groups

The results of genotype and allele distributions in *TCF7L2* rs7903146 and rs290487 SNPs between 2 groups were displayed in Table 2. The CC, CT, and TT genotype frequencies in rs7903146 were 60.00%, 28.89%, and 11.11% in the case group and 71.88%, 25.00%, and 3.12% in the control group. The C and T allele frequencies were 74.44% and 25.56% in case group and 84.38% and 15.62% in control group. The results showed that the TT genotype had a significant higher frequency in case group than that of control group ($P < .05$). Moreover, T allele frequencies in DN patients were also higher than that in the patients without DN ($P = .02$). All results demonstrated that *TCF7L2* rs7903146 was correlated with DN susceptibility, and T allele might significantly increase the occurrence risk of DN compared with C allele (OR = 1.85, 95% CI = 1.11–3.10).

Table 2

Frequency comparisons of genotypes and alleles in *TCF7L2* gene polymorphisms.

Genotype/allele	Case, n = 90 (%)	Control, n = 96 (%)	χ^2	*P*	OR (95% CI)	PHWE
rs7903146						.02
CC	54 (60.00)	69 (71.88)	—	—	1.00	
CT	26 (28.89)	24 (25.00)	0.94	.33	1.38 (0.72–2.68)	
TT	10 (11.11)	3 (3.12)	5.15	.02	4.26 (1.12–16.24)	
C	134 (74.44)	162 (84.38)	—	—	1.00	
T	46 (25.56)	30 (15.62)	5.64	.02	1.85 (1.11–3.10)	
rs290487						.82
CC	12 (13.33)	19 (19.79)	—	—	1.00	
TC	41 (45.56)	47 (48.96)	0.58	.45	1.38 (0.60–3.18)	
TT	37 (41.11)	30 (31.25)	2.31	.13	1.95 (0.82–4.65)	
C	65 (36.11)	85 (44.27)	—	—	1.00	
T	115 (63.89)	107 (55.73)	2.57	.11	1.41 (0.93–2.13)	

95% CI = 95% confidence interval, OR = odds ratio, PHWE = *P* value for Hardy–Weinberg equilibrium.

For rs290478, the frequencies of CC, TC, and TT genotypes were, respectively, 13.33%, 45.56%, 41.11% and 19.79%, 48.96%, 31.25% in case and control groups. And C and T allele frequencies were 36.11% and 63.89% in case group and 44.27% and 55.73% in control group, respectively. There was no significant difference between the 2 groups in genotypes and alleles distributions ($P > .05$), which indicated that this polymorphism had no correlation with the susceptibility of DN.

3.3. Haplotype analysis of TCF7L2 rs7903146 and rs290487 polymorphisms

The LD and haplotype analyses between rs7903146 and rs290487 of *TCF7L2* were performed by haploview software ($D' = 1.0$). Three haplotypes were constructed including C–C, C–T, and T–T. The frequencies were, respectively, 27.78%, 55.00%, and 17.22% in the case group and 37.50%, 53.65%, and 8.85% in control group (Table 3). The data showed that T–T haloptype had obvious difference in 2 groups ($P = .01$), which suggested that it might be associated with the risk of DN (OR = 2.63, 95% CI = 1.31–5.25).

4. Discussion

DM is a group of metabolic diseases characterized by hyperglycemia. Hyperglycemia is caused by a deficiency in insulin secretion or by a biological action, or both. High blood glucose in DM has existed for a long time, resulting in chronic damage and dysfunction of various organizations, in particular to eye, kidney, heart, blood vessels, and nerve. Diabetes can accompany with a variety of complications,[19] including some serious long-term complications, such as cardiovascular disease,[20] retinopathy,[21] neuropathy,[22] and microvascular disease,[23] as well as chronic renal failure, also known as DN, which is the main cause of adults hemodialysis in developing countries.[4] DN, as a typical microvascular complications, has been an important factor affecting the prognosis of patients, resulting in a major death and disability in patients with diabetes. At present, the specific pathogenesis of DN is not completely understood. DN is caused by the comprehensive action of multiple factors disease, involving genetic factors, high blood glucose, polyols and myo-inositol metabolism, changes of glomerular hemodynamics, abnormal blood coagulation, protein nonenzymatic glycation, oxidative stress, lipid metabolism, and vascular growth factor expression in abnormal factors. Clinical studies have shown that there is a certain difference in the incidence of DN among individuals.[24] In recent years, various studies on DN from various aspects of the pathophysiology of the kidney have been performed to explore the genetic factors that cause the disease, and some genetic susceptibility genes related to DN have also been found, such as angiotensin-converting enzyme (*ACE*) gene,[25] aldose reductase (*AR*) gene,[26] glucose transporter gene,[27] and interleukin-10 (*IL-10*).[28] Therefore, to explore the relationship between the pathogenesis of DN and genetic susceptibility and further effectively retard the progression of DN have become a solution desiderated problem in clinic.

TCF7L2 is a transcription factor containing DNA-binding domain. Its encoding gene is widely expressed in the human body, which is mainly expressed in human pancreatic beta cells and adipose tissue.[29] As β-catenin receptors, it indirectly mediates Wnt-signaling pathway, participates in the regulation of cell proliferation and differentiation, and maintains the stability of plasma glucose.[30] TCF7L2 initial research focused on tumor development and embryonic development. The current study suggests that *TCF7L2* may lead to increased apoptosis of islet cells by the gene overexpression in pancreatic beta cells due to the gene mutation, the mutation can also result in the expression of glucagon like peptide-1 by affect incretin axis Wnt-signaling transduction pathway. Besides, *TCF7L2* may involve in insulin synthesis, processing, secretion or affect insulin sensitivity, and

Table 3

Analyses of linkage disequilibrium and haplotypes in *TCF7L2* rs7903146 and rs290487 polymorphisms.

Haplotype SNP1–SNP2	Case, 2n = 180 (%)	Control, 2n = 192 (%)	χ^2	*P*	OR (95% CI)
C–C	50 (27.78)	72 (37.50)	—	—	1.00
C–T	99 (55.00)	103 (53.65)	1.97	.16	1.38 (0.88–2.18)
T–T	31 (17.22)	17 (8.85)	7.69	.01	2.63 (1.31–5.25)

95% CI = 95% confidence interval, OR = odds ratio, SNP1 = rs290487, SNP2 = rs7903146.

other ways, which finally take part in the development of diabetes.

TCF7L2 is a transcription factor in Wnt-signaling pathway, with high level in most human tissues, containing the heart, lung, brain, liver, kidney, placenta, adipose tissues, and pancreatic β-cells.[31] And previous study has provided a novel function of Wnt/β-catenin for the protection of kidney damage and in delay of the progression of DN.[32] In addition, there are a number of studies on the association of the *TCF7L2* gene variants with DN.[33,34]

In present study, the genotype and allele distributions of *TCF7L2* rs7903146 polymorphism were correlated with the susceptibility to DN in Chinese Han population. The data indicated that TT genotype and T allele frequencies of *TCF7L2* gene were significantly higher in DN patients than the controls, which indicated that T allele may be a candidate susceptible factor for DN. The previous study has also showed that the T allele of the rs7903146 *TCF7L2* variant is associated with DN in patients with early onset of diabetes in Caucasians of Polish origin, which has also been confirmed in another Caucasians population.[15] Besides, a recent study from South India has also reported the association of the rs7903146 polymorphism of *TCF7L2* gene with T2DM and DN.[31] These results were all consistent with the results of our study. In view of rs290487, it was the first time to study its effect on DN, but we did not find any significant difference between the case and control groups based on either genotypes or alleles. However, our study population is restricted to the Chinese Han population and whether the association of *TCF7L2* rs290487 polymorphisms with DN in the other population was similar to our result needs to be further studied in other larger sample size or different populations. In addition, the result was checked by the correlation between the haplotypes formed by *TCF7L2* rs7903146 and rs290487 polymorphisms and DN, T–T haplotype was discovered to obviously increase the risk suffering from DN.

In summary, *TCF7L2* rs7903146 polymorphism is significantly correlated with DN risk in Chinese Han population, while rs290487 is not. Meanwhile, there are several limitations in our study. First, the sample size was not large enough to obtain the exact results. Second, interactions between genetic and environmental factors were ignored in this study. Therefore, in order to early diagnose and early treat for DN, further researches to verify this conclusion should be conducted with well design and enough large sample size or various populations.

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