Association of transcription factor 7-like 2 (TCF7L2) gene polymorphism with type 2 diabetes mellitus in Chinese Korean ethnicity population

Kui-Chen Zhou, MMa,*, Hong-Wei Liu, MMb, Chen Wang, MMc, Yan-Jun Fu, MMa, Feng Jin, MBd

Abstract
Presently, data on the type 2 diabetes mellitus (T2DM) in Chinese Korean ethnicity are very scarce. This study aimed to explore the relationship between the transcription factor 7-like 2 (TCF7L2) and T2DM in Chinese Korean ethnicity population. This case-control study involved 43 T2DM Chinese Korean ethnicity patients (T2DM group) and 43 healthy Chinese Korean ethnicity normoglycemic subjects as controls (Control group). All included participants aged from 40 to 75 years old. Clinical and biological data were collected to determine the phenotypic traits. The restriction fragment length polymorphism-polymerase chain reaction was used to analyze the TCF7L2 by genotyping for rs7903146 (C/T). Spectrophotometer with Chronolab kits was used to conduct the biochemical analyses. TCF7L2 was associated with T2DM in the Chinese Korean ethnicity population (P < .01 for alleles, and P < .05 for genotypes). Significant differences were found in 2 groups regarding the T allele (37.2% T2DM patients vs 15.1% healthy subjects, P < .01), and G allele (62.8% T2DM patients vs 84.9% healthy subjects, P < .01). The risk genotypes were GG (83.7% T2DM patients, vs 44.2% healthy control, P < .01), GT (4.7% T2DM patients, vs 20.9% healthy control, P = .04), and TT (11.6% T2DM patients, vs 34.9% healthy control, P = .01). The results of this study demonstrated that TCF7L2 is associated with T2DM in the Chinese Korean ethnicity population, which is an important risk factor for T2DM in this population.

Abbreviations: BMI = body mass index, DM = diabetes mellitus, HDL = high-density lipoprotein, LDL = low-density lipoprotein, OR = ratio risk, RFLP-PCR = restriction fragment length polymorphism-polymerase chain reaction, SPSS = statistical package for the social sciences, T2DM = type 2 diabetes mellitus, TCF7L2 = transcription factor 7-like 2.

Keywords: Chinese Korean ethnicity population, transcription factor 7-like 2, type 2 diabetes mellitus

1. Introduction
Type 2 diabetes mellitus (T2DM) is one of the most types of diabetes. It is a metabolic disease and is characterized by chronic hyperglycemia and disturbances in carbohydrates.[1] It has been confirmed that T2DM results from the insulin secretion impaired, insulin resistance in peripheral tissues and hepatic glucose output increase.[1] Previous studies also confirmed that it can cause significant morbidity, disability, and early mortality, and is also associated with a huge economic burden for both family and the society.[2,3] It has reported that about 381.8 million adults suffered from diabetes mellitus (DM) in 2013 around the world.

This number will expand by 55% in 2035, which means 591.9 million adults will be affected with DM.[2]

T2DM interacts between genetic and environmental factors. It associated with several susceptibility genes, identified by the high throughput genome-wide association studies.[4–9] One of the most important factors is the transcription factor 7-like 2 (TCF7L2), which involves in insulin secretion.[10–13] Previous studies have also confirmed that TCF7L2 association is highly reproducible in various ethnic populations with T2DM.[10–21]

The association between the TCF7L2 and T2DM has not been studied in Chinese Korean ethnicity population in China till date. The aim of this study was to investigate the association between the TCF7L2 rs11196203 (G/T) gene polymorphism and T2DM in Chinese Korean ethnicity population.

2. Methods
2.1. Ethics statement
This study was approved by the ethics committee of First Affiliated Hospital of Jiamusi University and was conducted in accordance with the Helsinki Declaration. All the participants were required to provide the written informed consent.

2.2. Study population
This case-control study included 43 T2DM Chinese Korean ethnicity patients (T2DM group) and 43 healthy Chinese Korean ethnicity normoglycemic participants as controls (Control group). All included participants aged from 40 to 75 years old. In addition, all T2DM patients were diagnosed according to
performed by using Statistical Package for the Social Sciences (SPSS). The data of allele and genotype frequencies were analyzed and drop-out rate, therefore, the required sample size of this study was estimated at 36 participants with a ratio risk (OR) of 10 and an expected allele frequency of 0.10 in the general population by using the Power and Sample Size calculation software. The minimum size of each group was determined to be the risk allele (37.2% T2DM patients vs 15.1% healthy subjects, P < .01), while the G allele was determined to be the protective allele (62.8% T2DM patients vs 84.9% healthy subjects, P < .01) (Table 2). The frequencies of the genotypes were as follows with GG genotype (83.7% T2DM group, vs 44.2% control group, P < .01), GT genotype (4.7% T2DM group, vs 20.9% control group, P = .04), and TT genotype (11.6% T2DM group, vs 34.9% control group, P = .01) (Table 2).

4. Discussion
It is reported that a variety of population who suffered from the T2DM. However, the epidemiological data of T2DM are limited, especially on its genetic determinants. This study aimed to explore the association between the TCF7L2

2.3. Biochemical assays
Fasting plasma glucose, serum cholesterol and triglycerides, high-density lipoprotein (HDL)-cholesterol, and low-density lipoprotein (LDL)-cholesterol were measured. Chronolab kits (Tiangen Biotech Engineering Co, Ltd, Beijing, China) were used to test the biochemical and molecular assays.

2.4. DNA extraction and genotyping
The Chelex method was used to extract DNA from blood samples on filter paper, and then stored at −20°C. The TCF7L2 rs11196205 (G/T) polymorphism was genotyped using the following primers: Forward 5’-GAA AGT AGT CCA CAT TTA TAA CTT CG-3’, Reverse 5’-TTT GCC CAA TAA TAT GCC ATG AAA -3’ (Great Wall Biotechnology Engineering Co, Ltd, Shanghai, China). The final reaction volume of 25 μL was used for polymerase chain reaction (PCR) perform. It consisted of 0.1 μL genomic DNA, 1.5 μL each primer, 2.5 μL MgCl₂ of PCR buffer, 2 μL deoxynucleotide triphosphate, 5 μL Hot Start Taq DNA polymerase (QIAGEN) and 16.9 μL nuclease-free water. The PCR was conducted on a BIOMETRA T3 Thermal Cycler under the following conditions: 95°C for 2 minutes, followed by 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and a final extension of 72°C for 5 minutes. The amplicons were digested with Helicobacter pylori CH4 III (HpyCH4III) restriction enzyme at 65°C for 3 hours. The electrophoresis on a 2% agarose gel was used to separate the products, which were visualized under a UV transilluminator.

2.5. Sample size calculation
The sample size was calculated based on the disease variant with a ratio risk (OR) of 10 and an expected allele frequency of 0.10 in the general population by using the Power and Sample Size Calculation software. The minimum size of each group was estimated at 36 participants with a = 0.5, β = 0.8. Assuming a 20% drop-out rate, therefore, the required sample size of this study was estimated to be 86 participants, with 43 assigned to each group.

2.6. Statistical analysis
The data of allele and genotype frequencies were analyzed and performed by using Statistical Package for the Social Sciences (SPSS) software (v.17.0, SPSS, Chicago, IL). The Mann–Whitney U test was used to analyze the quantitative data. The Chi-square test or Fisher exact test was used to analyze the qualitative variables. The statistical significance level was set at P < .05.

3. Results
The characteristics are summarized in Table 1. No significant differences of age, sex, BMI, systolic blood pressure, diastolic blood pressure, and triglycerides were detected between 2 groups. However, there were significant differences of waist-to-hip ratio (P < .01), fasting plasma glucose (P < .01), total cholesterol (P < .01), HDL cholesterol (P < .01), and LDL cholesterol (P < .01) between 2 groups.

All findings were showed in Table 2. The results of this study demonstrated that TCF7L2 rs7903146 (C/T) was associated with T2DM (P < .01 for alleles, and P < .05 for genotypes). The minor allele was T and the major allele was G. The T allele was determined to be the risk allele (37.2% T2DM patients vs 15.1% healthy subjects, P < .01), while the G allele was determined to be the protective allele (62.8% T2DM patients vs 84.9% healthy subjects, P < .01) (Table 2). The frequencies of the genotypes were as follows with GG genotype (83.7% T2DM group, vs 44.2% control group, P < .01), GT genotype (4.7% T2DM group, vs 20.9% control group, P = .04), and TT genotype (11.6% T2DM group, vs 34.9% control group, P = .01) (Table 2).

Table 1
Characteristics of the study population between 2 groups.

| Characteristics | T2DM group (n=43) | Control group (n=43) | P value |
|-----------------|-------------------|----------------------|---------|
| Mean age, yr    | 56.9 (10.7)       | 58.2 (11.1)          | .58     |
| Sex             |                   |                      |         |
| Male            | 20 (46.5)         | 18 (41.9)            | .66     |
| Female          | 23 (53.5)         | 25 (58.1)            | .66     |
| BMI, kg/m²      | 27.9 (3.5)        | 28.1 (3.7)           | .80     |
| WHR             | 0.9 (0.1)         | 1.0 (0.1)            | <.001   |
| SBP, mm Hg      | 129.4 (15.1)      | 132.8 (16.3)         | .32     |
| DBP, mm Hg      | 73.2 (6.8)        | 80.1 (7.3)           | .55     |
| FPG, g/L        | 154.4 (14.2)      | 198.6 (18.5)         | <.01    |
| TC, mg/dL       | 45.8 (5.5)        | 50.2 (5.6)           | <.01    |
| LDL-C, mg/dL    | 83.3 (6.7)        | 114.0 (18.9)         | <.01    |
| HDL-C, mg/dL    | 138.7 (16.6)      | 144.3 (17.4)         | .13     |

Data are present as mean ± standard deviation or number (%).

BM=body mass index, DBP=diastolic blood pressure, FPG=fasting plasma glucose, HDL=high density lipoprotein cholesterol, LDL=low density lipoprotein cholesterol, SBP=systolic blood pressure, TC=total cholesterol, TG=triglycerides, WHR=waist-to-hip ratio.

Table 2
Association between the TCF7L2 and T2DM.

| TCF7L2 | T2DM group (n=43) | Control group (n=43) | OR (95% CI) | P value |
|--------|-------------------|----------------------|-------------|---------|
| rs11196205 (G/T) | 54 (62.8) | 73 (84.9) | 0.30 (0.14, 0.63) | <.01   |
| G      | 32 (37.2)         | 13 (15.1)            | 3.33 (1.60, 6.94) | <.01   |
| T      | 86 (100.0)        | 86 (100.0)           | –          | –       |
| Genotypes |                   |                      |             |         |
| GG     | 36 (83.7)         | 19 (44.2)            | 6.50 (2.37, 17.82) | <.01   |
| GT     | 2 (4.7)           | 9 (20.9)             | 0.18 (0.04, 0.91) | .04    |
| TT     | 5 (11.6)          | 15 (34.9)            | 0.25 (0.08, 0.76) | .01    |
| Total (%) | 43 (100.0) | 43 (100.0)           | –          | –       |

Data are present as number (%).

OR=odd ratio, T2DM=type 2 diabetes mellitus, TCF7L2=transcription factor 7-like 2.
rs11196205 (G/T) polymorphism and T2DM in Chinese Korean ethnicity population in China. It has been found that the frequency of the minor T allele was 30%, which was comparable to the 30.15% in the Czech population,\(^\text{[26]}\) 34.45% in the Iranian population,\(^\text{[27]}\) and 36.15% in the Arab population.\(^\text{[28]}\) The variation of the T allele frequency among the different population could account for the genetic diversity among those different ethnic groups.\(^\text{[29]}\) However, the T allele was found with a significant association of OR 3.92 (95% CI 2.04–7.67, \(P<0.01\)). The result of the present study is consistent with the previous studies in diverse ethnic populations\(^\text{[14,15]}\) with a strong association between TCF7L2 and the risk of T2DM.

The results of this study showed an association between the rs11196205 (G/T) polymorphism of the TCF7L2 gene and T2DM in Chinese Korean ethnicity population in China. Presently, although the pathophysiology of T2DM remains unclear, such substantial evidence still suggests that the TCF7L2 gene can help to predict the development of T2DM in several ethnic populations. Thus, it is also can be considered as a very important tool to identify the Chinese Korean ethnicity population at risk. In addition, the confirmed association between TCF7L2 and T2DM in an independent population can also provide evidence for the further study of TCF7L2 and its related molecules and pathways as potential therapeutic targets for T2DM.

Although the association between the TCF7L2 and T2DM in Chinese Korean ethnicity population was confirmed, this study still suffered from several limitations. First, the sample size was pretty small, which may lead to the deviation from Hardy–Weinberg equilibrium in this study. Thus, a larger sample size studies are needed to verify this results in the future. Second, healthy participants in the control groups should also be carefully selected in order to avoid confounding by population stratification. Third, lacking of randomization, allocation, and blinding may result in high risk of selection.

5. Conclusion

The rs11196205 (G/T) polymorphism of the TCF7L2 gene is associated with T2DM in Chinese Korean ethnicity population. Thus, it can help clinical genetic test to predict the occurrence of T2DM in Chinese Korean ethnicity population.

Author contributions

Conceptualization: Kui-Chen Zhou, Hong-Wei Liu, Chen Wang, Yan-Jun Fu, Feng Jin.

Data curation: Kui-Chen Zhou, Hong-Wei Liu, Chen Wang, Yan-Jun Fu, Feng Jin.

Formal analysis: Kui-Chen Zhou.

Investigation: Hong-Wei Liu, Yan-Jun Fu.

Methodology: Kui-Chen Zhou, Chen Wang.

Project administration: Yan-Jun Fu.

Resources: Kui-Chen Zhou, Chen Wang, Feng Jin.

Software: Kui-Chen Zhou, Chen Wang.

Supervision: Hong-Wei Liu, Chen Wang.

Validation: Hong-Wei Liu, Yan-Jun Fu, Feng Jin.

Writing – original draft: Kui-Chen Zhou, Hong-Wei Liu, Chen Wang, Yan-Jun Fu, Feng Jin.

Writing – review and editing: Kui-Chen Zhou, Hong-Wei Liu, Chen Wang, Yan-Jun Fu, Feng Jin.

References

[1] Guillausseau PJ, Meas T, Virally M, et al. Abnormalities in insulin secretion in type 2 diabetes mellitus. Diabetes Metab 2008;34(Suppl 2): S43–8.
[2] Cannon A, Handelsman Y, Heile M, et al. Burden of illness in type 2 diabetes mellitus. J Manag Care Spec Pharm 2018;24(9 Suppl):S5–13.
[3] Seshasai SRK, Kaptoge S, Thompson A, et al. Diabetes mellitus, fasting glucose, and risk of cause-specific death. N Engl J Med 2011;364:829–41.
[4] Sladék R, Rocheleau G, Rung J, et al. A genomewide association study identifies novel risk loci for type 2 diabetes. Nature 2007;445:881–5.
[5] Saxena R, Gianniny L, Burtt NP, et al. Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in non-diabetic individuals. Diabetes 2006;55:2890–5.
[6] Prabhanjan M, Suresh RV, Murthy MN, et al. Type 2 diabetes mellitus disease risk genes identified by genome wide copy number variation scan in normal populations. Diabetes Res Clin Pract 2016;113:160–70.
[7] Kwon YJ, Lee YJ, Park BJ, et al. Total serum bilirubin and 8-year incident type 2 diabetes mellitus: the Korean Genome and Epidemiology Study. Diabetes Metab 2018;44:346–53.
[8] Park SK, Ryoo JH, Oh CM, et al. The risk of type 2 diabetes mellitus according to 2-h plasma glucose level: the Korean Genome and Epidemiology Study (KoGES). Diabetes Res Clin Pract 2017;134:130–7.
[9] Go MJ, Hwang JY, Park TJ, et al. Genome-wide association study identifies two novel loci with sex-specific effects for type 2 diabetes mellitus and glycemic traits in a korean population. Diabetes Metab J 2014;38:375–87.
[10] Hsiao TJ, Lin E. A common rs7903146 variant of the transcription factor 7-like 2 gene is associated with type 2 diabetes mellitus and fasting glucose in a Taiwanese population. Diabetes Metab 2017;43: 83–5.
[11] Lin PC, Lin WT, Yeh YH, et al. transcription factor 7-like 2 (TCF7L2) rs7903146 polymorphism as a risk factor for gestational diabetes mellitus: a meta-analysis. PLoS One 2016;11:e0153044.
[12] Pourahmadi M, Erfanian S, Moradzadeh M, et al. Non-Association between rs7903146 and rs12255372 polymorphisms in transcription factor 7-like 2 gene and type 2 diabetes mellitus in Jahrom city, Iran. Diabetes Metab J 2015;39:512–7.
[13] Ding Y, Hu Z, Yuan S, et al. Association between transcription factor 7-like 2 rs7903146 polymorphism and diabetic retinopathy in type 2 diabetes mellitus: a meta-analysis. Diab Vasc Dis Res 2015;12:436–44.
[14] Al-Safar H, Hassoun A, Almazrouei S, et al. Association of the genetic polymorphisms in transcription factor 7-like 2 and peroxisome proliferator-activated receptors- γ with type 2 diabetes mellitus and is interaction with obesity status in Emirati population. J Diabetes Res 2015;2015:129695.
[15] Pagán A, Sabater-Molina M, Olza J, et al. A gene variant in the transcription factor 7-like 2 (TCF7L2) is associated with an increased risk of gestational diabetes mellitus. Eur J Obstet Gynecol Reprod Biol 2014;180:77–82.
[16] Kang S, Nie Z, Zhang D. Association of the rs7903146 polymorphism in transcription factor 7-like 2 (TCF7L2) gene with gestational diabetes mellitus: a meta-analysis. Gynecol Endocrinol 2013;29:873–7.
[17] Klein H, Haslinger P, Bancher-Todesca D, et al. Transcription factor 7-like 2 gene polymorphisms and gestational diabetes mellitus. J Matern Fetal Neonatal Med 2012;25:1783–6.
[18] Kurzawski M, Dziewanowski K, Kędzierzewska K, et al. Association of transcription factor 7-like 2 (TCF7L2) gene polymorphism with posttransplant diabetes mellitus in kidney transplant patients medicated with tacrolimus. Pharmacol Rep 2011;63:826–33.
[19] Kang ES, Kim MS, Kim YS, et al. A variant of the transcription factor 7-like 2 (TCF7L2) gene and the risk of posttransplantation diabetes mellitus in renal allograft recipients. Diabetes Care 2008;31:63–8.
[20] Shaat N, Lennmark A, Karlsson E, et al. A variant in the transcription factor 7-like 2 (TCF7L2) gene is associated with an increased risk of gestational diabetes mellitus. Diabetologia 2007;50:972–9.
[21] Watanabe RM, Allayee H, Xian AH, et al. Transcription factor 7-like 2 (TCF7L2) is associated with gestational diabetes mellitus and interacts with adiposity to alter insulin secretion in Mexican Americans. Diabetes 2007;56:1481–5.
[22] WHO-IDF. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF Consultation.
Available at: http://www.idf.org/webdata/docs/WHO_IDF_definition_diagnosis_of_diabetes.pdf. [Accessed on January 30, 2017].

[23] Friedewald WT, Levy RI, Fredrickson DS. Estimation of low density lipoprotein cholesterol without the use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.

[24] Gauderman WJ, Morrison JM. Quanto: A Computer Program for Power and Sample Size Calculations for Genetic-Epidemiology Studies; 2006. Available at: http://biostats.usc.edu/software. [Accessed on April 1, 2017].

[25] Včeláč J, Vejražková D, Vaňková M, et al. T2D Risk haplotypes of the TCF7L2 gene in the Czech population sample; the association with free fatty acids composition. Physiol Res 2012;61:229–40.

[26] Alami FM, Ahmadi M, Bazrafshan H, et al. Association of the TCF7L2 rs12255372(G/T) variant with type 2 diabetes mellitus in an Iranian population. Genet Mol Biol 2012;35:413–7.

[27] Ahmadi O, Al-Rubeaan K, Mohamed G, et al. Weak or no association of TCF7L2 variants with Type 2 diabetes risk in an Arab population. BMC Med Genet 2008;9:e72.