

Two TCF7L2 Variants Associated with Type 2 Diabetes in the Han Nationality Residents of China

Nadeem Anjum, Arbab Jehangir and Yu Liu

ABSTRACT

Objective: To determine the risk of type II diabetes (T2D) with two transcription factor 7-like 2 gene (TCF7L2) variants in the Han nationality population of Guangdong, China.

Study Design: Case control study.

Place and Duration of Study: International School, Clinical Laboratory of Over-sea Chinese Hospital, Department of Biochemistry, Medical School, Jinan University, China, from July 2014 to June 2016.

Methodology: Two single nucleotide polymorphisms (SNPs) of gene TCF7L2 were genotyped by PCR amplification and sequencing of the specific DNA fragments, rs7903146 and rs12255372, by PCR amplification and sequencing of the specific DNA fragments in 339 T2D patients and 191 control subjects. Odds ratio was determined.

Results: The minor allele of the two variants was significantly associated with T2D; the risk to develop T2D for rs7903146 (IVS3C >T) was found to be greater than that for rs12255372 (IVS4G >T), with allelic odds ratio (OR) of 1.39 and 1.27 respectively. Furthermore, T2D risk associated with homozygosity of the minor alleles was found to be greater than that for heterozygosity.

Conclusion: The two variants of the gene TCF7L2 are important genetic risk factors for the T2D development in the Han ethnic group in China.

Key Words: Diabetes mellitus, Transcription factor 7-like 2 gene (TCF7L2) variants, Han nationality, China.

INTRODUCTION

Currently, the global prevalence of diabetes is estimated to be 8.5% in the adult population,¹ of which around 90% are patients with type 2 diabetes (T2D). In China, the prevalence of T2D was found to be 9.1% of the inland residents in 2014, especially in the urban areas.² Nevertheless, this disease has been forming a huge economic burden for the country. T2D is predicted to increase to epidemic proportions in the coming decades, primarily due to lifestyle changes, particularly obesity.^{3,4} Except the strong environmental elements, genetic components associated with T2D risk have been confirmed by twin studies, family studies and studies of T2D prevalence in different ethnic groups.⁵⁻⁷ Compared to Indonesia, which was estimated to have a prevalence of 5.8%, the rate of diabetes in Malaysia (10.9%) was believed to be about 2-fold higher.⁸ This significance in diabetic prevalence between the South Asian countries cannot be explained by cultural and lifestyle factors alone. Therefore, ethnic difference in prevalence of

diabetes could be related to the difference in genes, especially different allele frequencies of predisposing genes.

Grant and colleagues reported that a gene located in the chromosome 10q25.2 was highly associated with T2D risk, which was later identified to be transcription factor 7-like 2 (TCF7L2, Gene ID: 6934), or TCF4.⁹ TCF7L2 is a high mobility group (HMG) box-containing transcription factor and a key member of the Wnt signalling pathway which plays important roles in blood glucose homeostasis. Grant *et al.* found that a variant within exon 3 of TCF7L2 named DG10S478 was significantly associated with T2D in an Icelandic case control cohort ($p < 2.1 \times 10^{-9}$). This variant was replicated in Danish and American cohorts. In addition, heterozygous and homozygous carriers of this variant showed high risk of T2D (1.45 and 2.41, respectively). Subsequently, two single nucleotide polymorphisms (SNPs) within intron 3 of TCF7L2, rs7903146 and rs12255372, were confirmed to be strongly associated with T2D risk. In human pancreatic islets, insulin gene enhancer binding protein 1 (ISL1) is a direct target of TCF7L2, and both regulates the production and processing of proinsulin through the TCF7L2-ISL1 signal transduction network.¹⁰ Therefore, the molecular mechanisms by which TCF7L2 influences glucose metabolism is a complicated process, and the effects of SNPs of TCF7L2 on the development of T2D remains unclear, suggesting a need of more studies on the association between TCF7L2 SNPs and T2D risk.

In the present study, the objective determined the association of the two SNPs of TCF7L2, rs7903146 and

Department of Biochemistry, International School, Clinical Laboratory of Over-sea Chinese Hospital, Medical School, Jinan University, 510632, China.

Correspondence: Prof. Yu Liu, Department of Biochemistry, International School, Clinical Laboratory of Over-sea Chinese Hospital, Medical School, Jinan University, 510632, China.

E-mail: youliu668@gmail.com

Received: June 21, 2017; Accepted: February 19, 2018.

rs12255372, with the risk of T2D by analysis of the specific DNA sequences among the Han nationality residents in Guangdong, China. We hope to study the different effects of the two SNPs on susceptibility of the local residents to T2D.

METHODOLOGY

It was a case control study, conducted at International School, Clinical Laboratory of Over-sea Chinese Hospital, Department of Biochemistry, Medical School, Jinan University, China, from July 2014 to June 2016.

Two hundred and fifty-nine patients with T2D and 106 healthy people were selected for the study. Those with serious other medical disorders (e.g. cancer) or those under specific medical treatment were excluded. The venous blood samples were drawn in tubes with EDTA as anticoagulant and stored at -4°C before DNA extraction. Tris, EDTA, SDS, proteinase K, phenol, chloroform, isopropanol, ethanol, agarose and EB were Sigma's products. Trizole, PCR reagents and enzymes were purchased from Invitrogen; whereas, other chemicals were products of Shanghai Chemicals Inc.

Blood samples were added with Red Cell Lysis Buffer (RCLB) and mixed completely by inversion, span for 10 minutes at 1,200g, discarding the supernatant and re-suspending the cell pellet in white cell lysis buffer (WCLB). One hundred and fifty μl of saturated NaCl was added to 400 μl of white cell lysate, vortex; and centrifuge at 12,000 RPM for 5 minutes. The supernatant was transferred to a 1.5 ml tube (550 μl) adding 1000 μl ethanol, mixing, spinning at 12,000 RPM for 5 minutes. The precipitate was saved. The sample was washed with 1 ml 70% ethanol, air dried and re-suspended in 100 μl of dilute TE (1:4 w/ H_2O). The samples were stored at -70°C for PCR use.

To determine the two SNPs in DNA samples, four pairs of primers were designed. One was for rs7903146 (C) the sense primer was GAGCTAAGCACTTTTTAGATAC and antisense was ATAACCTCTCACACTGCTGATCAGT, product size was 350bp. Second was for rs7903146 (T) the sense primer was GAGCTAAGCACTTTTTAGATAT and antisense was AGACAGAATGAGACCCTGTCTCTGA, product size was 488bp. Third was for rs12255372 (G) the sense primer was CAGGAATATCCAGGCAAGAATG and antisense was GACACTCTGGCCACATCCAAGTGAG, product size 443bp. Fourth was for rs12255372 (T) the sense primer was CAGGAATATCCAGGCAAGAATT and antisense GACTTTCAGTATAGAGGTCTGTGAT, product size 581bp. PCR reaction was carried out at 94°C for 5 minutes, and then 30 cycles at 94°C for 30 seconds, 58°C for 40 seconds, 72°C for 40 seconds, and finally at 72°C for 8 minutes. After PCR analysis, the TCF7L2 variants were determined by comparison among the band positions in agarose gel, and later confirmed by DNA sequencing.

The study was approved by the Ethical Committee of the Medical School, Jinan University. The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki.

Statistical analysis was performed using SPSS version 20 (SPSS Inc, Chicago IL). Baseline characteristics were transformed to a normal distribution as appropriate and differences were tested using analysis of covariance. Allele frequencies were obtained using the percentage of each allele in the group. Alleles and genotypes were tested for association with type 2 diabetes using logistic regression. The comparison for the T2D cohorts with control subjects is presented as odds ratios (OR) with their corresponding 95% confidence interval (CI). Statistical significance was taken as $p < 0.05$.

RESULTS

The clinical data of the subjects recruited are listed in Table I. Age of diagnosis, and the data of clinical tests, including blood cholesterol and HbA1c were available for those with diabetes. Blood pressures were not studied for either the diabetic group and the control group. No significant differences in the pattern of gender and age were found between the two groups.

As shown in Figure 1, the positions and sizes of the four PCR fragments were in good accordance with the primer designation. The major alleles rs7903146 (C) and rs12255372 (G) only showed a single band in lanes 1

Table I: Clinical characteristics of subjects studied.

	Diabetic subjects	Control subjects
Number	339	191
Gender M/F n (%)	176/163 (51.91/48.08)	104/87 (54.45/45.54)
Age at study (years)	48.4 \pm 110.2	47.7 \pm 10.6
Age at diagnosis (years)	46.5 \pm 10.8	47.7 \pm 10.6
HDL cholesterol (mmol/l)	1.3 \pm 0.7	1.41 \pm 0.8
HbA1c (%)	8.4 \pm 2.0	N/A*

*No records for the healthy group.

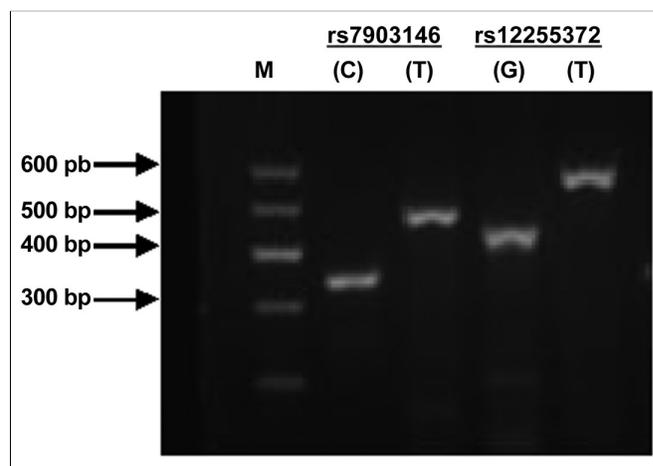
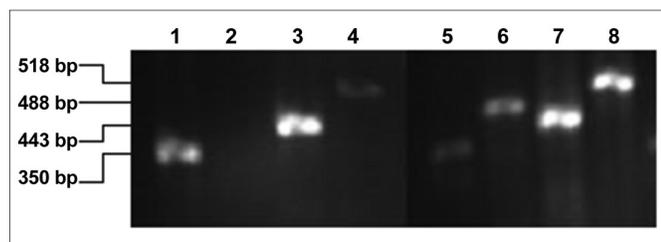


Figure 1: Identification of PCR fragments from the four variants; (from left to right): Marker, rs7903146 (C) and (T) from two control subjects, respectively; rs12255372 (G) and (T) from two T2D patients, respectively.

Table II: Association of TCF7L2 variants with type 2 diabetes.

rs7903146 (IVS3C>T)	C	0.64	0.72	C/C	160 (47.2)	110 (57.6)	1.00	
				C/T	117 (34.5)	56 (29.3)	1.43 (1.18 - 1.65)	<0.01
	T	0.36	0.28	T/T	62 (18.3)	25 (13.1)	1.70 (1.28-2.27)	<0.01
				Allelic OR ^c (95% CI):		1.39 (1.22 - 1.61)		<0.01
rs12255372 (IVS4G>T)	G	0.66	0.71	G/G	158 (46.6)	105 (54.9)	1.00	
				G/T	131 (38.6)	63 (33.0)	1.38 (0.96 - 2.04)	<0.01
	T	0.34	0.29	T/T	50 (14.8)	23 (12.1)	1.44 (1.21 - 1.93)	<0.01
				Allelic OR ^c (95% CI):		1.27 (1.06 - 1.53)		

aF = allele frequency; bOR: odds ratio for minor allele hetero- or homo-zygous genotype; cOR = allelic odds ratio (95% CI).

**Figure 2:** Electrophoresis patterns of TCF7L2 variants.

Lane 1 & 2: rs7903146 (C/C) and 3 & 4: rs12255372 (G/G) from two control subjects, respectively; Lane 5 & 6: rs7903146 (C/T) and 7 & 8: rs12255372 (G/T) from two T2D patient subjects, respectively.

(350bp) and 3 (443bp), respectively, while lane 2 (488bp) and 4 (581bp) were bands of the minor alleles 7903146 (T) and rs12255372 (T), respectively. In Figure 2, lanes 1, 2 and 3, 4 from control subjects showed homozygote genotypes rs7903146 (C/C) and rs12255372 (G/G), respectively. However, a band was found in each of lanes 5, 6 and 7, 8 from T2D samples, showing heterozygote genotypes rs7903146 (C/T) and rs12255372 (G/T), respectively. The PCR results provided with clear bands for judgments of the alleles and genotypes, and most of the interested variants were later confirmed by sequencing.

Of the 530 samples (339 (63.9%) of type 2 diabetic patients and 191 (36%) healthy people), 263 (50%) were found to be rs12255372 [G/G] and 270 (51%) be rs7903146[C/C]. However, both rs12255372 [G/T, T/T] and rs7903146 [C/T, T/T] genotypes were found higher in diabetic patients than the control subjects (Table II). The genotypic odds ratios (OR) for homozygotes rs7903146 (T/T) and rs12255372 (T/T) were found to be 1.70 (95% CI 1.01-2.88) and 1.44 (95% CI 0.83-2.51), respectively, and the allelic ORs for rs7903146 (T) and rs12255372 (T) were 1.52 (95% CI, 1.06-2.17, $p < 0.05$) and 1.40 (95% CI, 0.98-2.00, $p > 0.05$), respectively.

DISCUSSION

TCF7L2, or TCF4, is widely expressed in the body, especially in the pancreas, where it plays important roles in regulation of insulin and glucagon production and secretion. TCF7L2 is a key member in the Wnt signalling pathway. After the receptor is bound by Wnt glycoprotein (ligand), Wnt signal is transduced through the pathway. Free β -cat is accumulated and forms β -cat/TCF complex, which recruit nuclear coactivators such as

cAMP response element binding protein (CBP), increasing the expression of Wnt target genes. In maturation of insulin from proinsulin prohormone convertase 1 (PCSK1) and convertase 2 (PCSK2) cleave proinsulin molecule to yield mature insulin and C-peptide. It was found that both PCSK1 and PCSK2 mRNA expressions were significantly reduced when TCF7L2 is silenced in human pancreatic islets, suggesting that TCF7L2 is involved in proinsulin maturation.¹⁰ Shu and colleagues reported that islet TCF7L2 protein production was reduced in type 2 diabetes, suggesting a possible promoting role of the transcription factor in islet insulin production.¹¹ Paradoxically, subjects with rs7903146T allele were found to have higher expression of TCF7L2 in the pancreatic islets,^{12,13} indicating different molecular mechanisms for the effects of TCF7L2 on the islet insulin regulation.

In the Wnt pathway, TCF7L2 and ISL1 form an important regulatory circuit which plays critical roles in proinsulin expression and processing *via* regulation of MafA, Pdx1, Nkx6.1, Pcsk1 and Pcsk2.¹⁰ In addition, TCF7L2 is also involved in pancreas development,¹⁴ and is required for cytoprotection of β cells and maintaining the secretory function of mature β cells.^{15,16} Accordingly, the people carrying one or more of SNPs of TCF7L2 might have higher risk of developing T2D than others. It was reported that the effects of TCF7L2 variant genotypes are essentially co-dominant.⁹ In this regard, T2D patients with different TCF7L2 variant genotypes may exhibit a variety of phenotypes and clinical symptoms.

Strong association between T2D and the hypervariable marker in TCF7L2 has been demonstrated by many other studies.¹⁷⁻¹⁹ However, it is not clear how the polymorphisms within TCF7L2 intron regions increase the risk of T2D development. Yi *et al.* reported that lithium, which functions similarly as Wnt ligands, showed stimulatory effects on proglucagon expression in intestinal endocrine L cells, but not in pancreatic α -cells, suggesting tissue-specificity of the Wnt signalling pathway, and the effects of Wnt ligands might vary with types of tissues.²⁰ It was reported that carriers of the TT genotypes of SNP rs7903146 exhibited 5-fold increase in TCF7L2 mRNA expression in the islets. Since expression of TCF7L2 was positively correlated with insulin gene expression, the variant rs7903146 could be

related to insulin resistance.¹² The TT genotype of rs7903146 was also significantly associated with higher fasting plasma insulin level and the homeostasis model assessment of insulin resistance in case of new-onset diabetes,²¹ suggesting that the Wnt signalling pathway plays critical roles in regulation of TCF7L2 expression itself. Therefore, an abnormal feedback mechanism due to rs7903146 might explain the molecular mechanism, thereby, this variant confers T2D risk.²²

CONCLUSION

The present results provide a strong evidence for association between T2D risk and SNP rs7903146 and rs12255372 in TCF7L2. The odds ratio for SNP rs7903146 TT genotype is 1.70 (95% CI 1.28-2.27) in the Han population of Guangdong, China.

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