RESEARCH NOTE

Transcription factor 7-like 2 (*TCF7L2*) variations associated with earlier age-onset of type 2 diabetes in Thai patients

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Introduction

Type 2 diabetes (T2D) is a complex disorder caused by the interaction between genetic predisposition and environmental factors (Freeman and Cox 2006). Recent progress in genetic and genomic research of T2D has shown that genes involved in pancreatic beta-cell development and function are involved in pathogenesis of T2D. Identification of these genes will provide a better understanding of pathogenesis, which may lead to the improvement of diagnosis, treatment, and prevention of this increasingly prevalent and costly condition.

Genomewide linkage analysis has revealed that a region on chromosome 10q contained a T2D susceptibility gene, which was later ascribed to possess intronic variations of the transcription factor 7-like 2 (*TCF7L2*). The variation of *TCF7L2* was associated with a two-fold increase of T2D risk in the Icelandic population (Grant *et al*. 2006). This association has been replicated in cohorts of European, Asian and African descent. The precise mechanism by which variations of *TCF7L2* predispose to T2D is not clear. It has been suggested that *TCF7L2* encodes a transcription factor that is expressed in foetal pancreas and involved in Wnt signalling pathway through the regulation of glucagon-like peptide (GLP-1), which has a primary role in glucose homeostasis (Cauchi *et al*. 2006). Since there is no previous genetic study describing the *TCF7L2* variation on T2D risk in Thais, we therefore investigated the association of five variants at rs7896340, rs7901695, rs7903146, rs12255372, and rs11196205 of *TCF7L2* with T2D by high resolution melting analysis. The associations between SNP genotype, allele or haplotype frequencies and T2D or its clinical characteristics were evaluated. To our knowledge, this gene has not been previously tested in the Thai population and this study is the first to report the association between *TCF7L2* variations and T2D in Thai patients.

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Keywords. type 2 diabetes; *TCF7L2* gene; Thai.

Materials and methods

Subjects

The studied subjects included 407 Thai individuals, including 205 T2D patients were recruited from the Diabetic Clinic, Siriraj Hospital, Bangkok, Thailand. The patients were above 40 years and were unrelated. Diabetes was defined according to the ADA criteria. Normoglycemic subjects ($n = 202$) were recruited among those who underwent annual health checkup at the same hospital. The inclusion criteria were fasting plasma glucose < 5.6 mmol/L and no family history of diabetes. The study protocol was approved by the Institutional Ethics Committee and informed consent was obtained from all subjects before recruitment into the project.

TCF7L2 SNP genotyping

Genomic DNA was extracted from peripheral blood leucocyte by the standard phenol–chloroform method. Five variants of *TCF7L2* were genotyped by melting curve analysis using simple probes specific to each variant in a single run on a real-time PCR LightCycler 480 machine (Roche, Mannheim, Germany) in 96-well plate format. The details of PCR primers and reaction conditions are provided in table 1 and figure 1 of electronic supplementary material at http://www.ias.ac/jgenet/.

Table 1. Clinical and biochemical characteristics of type 2 diabetic patients (T2D) and normoglycemic control subjects.

	Control	T2D	*P* value
N	202	205	
Men (%)	28.29	28.71	0.9254
Age (years)	53.34 ± 9.06	53.75 ± 11.00	0.6776
BMI (kg/m^2)	23.85 ± 3.20	28.02 ± 5.53	2.49×10^{-18}
Weight (kg)	59.08 ± 9.83	69.76 ± 15.33	1.69×10^{-15}
Waist (cm)	82.24 ± 9.29	88.27 ± 12.14	3.77×10^{-8}
Hip (cm)	95.53 ± 7.01	99.21 ± 10.99	7.21×10^{-5}
Waist/hip ratio	0.86 ± 0.06	0.89 ± 0.08	1.14×10^{-5}
Systolic BP (mm Hg)	116.00 ± 14.86	133.09 ± 17.91	9.19×10^{-23}
Diastolic BP (mm Hg)	71.27 ± 9.90	81.43 ± 10.40	1.7×10^{-21}
Fasting plasma glucose (mmol/L)	87.88 ± 6.38	206.75 ± 98.93	5.42×10^{-41}
Total cholesterol (mmol/L)	205.74 ± 36.27	228.08 ± 49.08	3.03×10^{-7}
Triglyceride (mmol/L)	110.15 ± 59.69	205.13 ± 17.57	8.14×10^{-9}
LDL (mmol/L)	124.83 ± 33.26	144.15 ± 43.73	8.37×10^{-7}
HDL (mmol/L)	62.02 ± 17.31	50.28 ± 14.44	6.7×10^{-13}

Data are means ± SD.

Data analysis

Genotype distributions were tested at each polymorphic locus for deviation from Hardy–Weinberg equilibrium (HWE) before marker-trait association study. SNPs were tested for departure from HWE using an exact test of HWE proportions for the combined group of cases and controls, and then for cases only, and controls only. Association between genotypes and T2D was tested by logistic regression. We performed logistic regression analysis with and without adjustment for age and sex as well as body mass index (BMI). For the analysis of genotype–phenotype association, logistic regression including sex, age, age at diagnosis, BMI, weight, waist, hip, waist/hip ratio, systolic–diastolic blood pressure, fasting plasma glucose (FPG), total cholesterol, triglyceride, LDL-cholesterol, and HDL-cholesterol, as covariates were evaluated.

Tests for association of each SNP or haplotype with T2D were performed by using the Haploview software (Barrett *et al*. 2005) and Haplo.score in R software (http://www.biostat.wustl.edu/genetics/geneticssoft/manuals/haploscore/haplo.score.html). This provides a global test of association and haplotype-specific test. SPSS software v17.0 (SPSS, Chicago, USA) was used for general statistical analysis.

Results

Clinical characteristics of subjects and genotype calling

A total of 407 unrelated subjects including 202 T2D patients and 205 control subjects were recruited for analysis of *TCF7L2* variations. Clinical characteristics and biochemical profiles of the participating subjects are provided in table 1.

For each SNP, a single labelled probe was used to target each polymorphic locus. We performed genotyping of five SNPs located within the *TCF7L2* gene on 407 DNA samples. In this experiment, three groups of genotypes (common homozygote, heterozygote, and rare homozygote) could directly be discriminated. Genotyping quality was tested by including three control samples with known genotypes in each 96-well assay. The average agreement rate of genotype calling of duplicate samples was > 99%.

Association study

Genotype distribution at each polymorphic locus of *TCF7L2* was tested and found to be in HWE. Genotype and allele frequencies of five variants were compared between the two groups. Two SNPs (rs7896340 and rs11196205) were significantly associated with T2D with *P* values of 0.0088 and 0.0121, respectively, after adjusting for age, sex, BMI (table 1). For both SNPs, the association was consistent with a dominant model of inheritance with about twice the odds ratio (table 2).

The minor allele G at SNP rs7896340 of *TCF7L2* significantly increases T2D risk with odds ratio 2.34, (95% CI 1.22–4.47, $P = 0.009$). The correlations of *TCF7L2* genotypes with other covariates including sex, age, age at diagnosis, BMI, weight, waist, hip, waist/hip ratio, systolic–diastolic blood pressure, FPG, total cholesterol, triglyceride, LDL-cholesterol, and HDL-cholesterol were evaluated. In the group of T2D patients, the G allele was associated with earlier age-onset of disease (AA = 50.32 ± 10.78 year, AG + GG = 45.51 ± 10.52 year, $P = 0.0127$) (table 3). A similar trend was also observed for SNP, rs11196205. However, in the group of normoglycemic subjects, *TCF7L2* genotypes did not show any association with clinical characteristics (table 2).

The two SNPs (rs7896340 and rs11196205) defined four haplotypes (table 4). The haplotype that consisted of two minor alleles (GG) was more frequent in the group of T2D

Table 2. Genotypic distribution of five SNPs of *TCF7L2* by glycemic status.

	Controls (*n*)	T2D (*n*)	OR (95% CI)*	*P* value*
rs7896340				
AA	182	163	1	0.008
AG	22	36	1.89 (1.03–3.23)	
GG	1	3	3.35 (0.35–32.52)	
AG+GG	23	39	2.34 (1.22–4.47)	0.009
rs11196205				
CC	182	163	1	0.012
CG	22	36	1.83 (1.03–3.23)	
GG	1	3	3.35 (0.35–32.52)	
CG+GG	23	39	2.23 (1.18–4.26)	0.013
rs7901695				
CC	180	173	1	0.54
CT	24	26	1.13 (0.62–2.04)	
TT	1	3	3.12 (0.32–30.30)	
CT+TT	25	29	1.23 (0.62–2.45)	0.54
rs7903146				
CC	183	172	1	0.106
CT	21	25	1.26 (0.68–2.33)	
TT	1	4	4.23 (0.47–38.19)	
CT+TT	22	29	1.59 (0.78–3.22)	0.198
rs12255372				
GG	191	179	1	0.088
GT	13	20	1.64 (0.79–3.40)	
TT	1	3	3.20 (0.33–31.06)	
GT+TT	14	23	1.96 (0.86–4.44)	0.109

*Adjusted for age, sex and BMI.

subjects ($P = 0.018$). Conversely, the three haplotypes carrying at least one of the minor alleles or none at SNP rs7895340 or rs11196205 (i.e. AC, AG, or GC) were more frequent in the control subjects than in T2D subjects. Moreover, haplotype GG was also associated with earlier age-onset of the disease with *P* value of 0.002.

Table 3. Mean trait values stratified by *TCF7L2* SNP rs7896340 genotype.

	Controls				Cases			
	AA	AG	GG	*P* value*	AA	AG	GG	*P* value*
N	182	22	1		163	36	3	
Age	53.21	54.45	51.00	0.6491	54.77	49.19	50.33	0.0153
Age at diagnosis	–	–	–	–	50.32	44.72	45.00	0.0127
BMI (kg/m^2)	23.76	24.79	19.65	0.4278	28.20	26.77	32.95	0.6560
Weight	58.83	61.75	46.00	0.4904	69.83	68.42	81.67	0.8100
Waist	81.92	85.55	69.00	0.3032	88.28	87.75	94.00	0.8363
Hip	95.28	98.09	85.00	0.2979	99.29	98.00	109.67	0.7816
Waist/hip ratio	0.86	0.87	0.81	0.6218	0.89	0.89	0.85	0.8716
Systolic BP (mm Hg)	116.07	116.36	96.00	0.6559	134.33	127.50	133.33	0.0748
Diastolic BP (mm Hg)	71.32	71.27	62.00	0.6981	81.58	80.56	83.33	0.7612
FPG (mmol/L)	88.05	86.36	89.00	0.3131	208.13	204.86	154.00	0.5366
Total cholesterol (mmol/L)	206.26	201.05	214.00	0.6209	229.19	224.39	212.00	0.4572
Triglyceride (mmol/L)	112.23	93.55	98.00	0.1759	209.18	193.42	125.33	0.5103
LDL (mmol/L)	125.20	121.55	130.00	0.7009	144.16	144.28	142.00	0.9762
HDL (mmol/L)	61.75	63.73	73.00	0.4765	49.89	52.47	45.00	0.6033

*ANOVA was used to compare mean levels of continuous characteristics across genotypes.

Table 4. Haplotype frequencies in T2D patients and control subjects.

rs7895340/ rs11196205 Haplotype	Control ($n = 205$)	Case ($n = 202$)	OR (95% CI)	P value
AC	0.939	0.896	1	–
GG	0.0561	0.104	1.89 (1.12–3.19)	0.018
AG	0.0024	0	0	1
GC	0.0024	0	0	1

Discussion

T2D is an inheritable metabolic disorder of polygenic nature (Ridderstrale and Groop 2009). Though the theoretical analyses emphasized the power of genetic association study in common multifactorial diseases, the identification of genes that increase risk of T2D has not been very successful. The genes implicated in T2D confer only modest effects on the disease risk and in many cases have yielded inconsistent results in replication efforts. Only few associations, notably the Pro12Ala polymorphism in the peroxisome proliferator activated receptor (*PPARG*) gene (Buzzetti *et al*. 2004), the Glu23Lys polymorphism in the *KCNJ11* gene (Gloyn *et al*. 2003), and the genetic variants of calpain-10 genes (Weedon *et al*. 2003) have been convincingly replicated. Recently, researchers have identified a linkage signal on chromosome 10q that exhibited strong association of a common microsatellite (DG10S478) of *TCF7L2* with T2D in Icelandic population and this was later replicated in the populations from the US and Denmark (Grant *et al*. 2006). DG10S478 is located within well-defined linkage disequilibrium (LD) block of 92.1 kb that encompassed exon 4 and parts of two large flanking introns of *TCF7L2*. Five SNPs (rs12255372, rs7903146, rs7901695, rs11196205 and rs7895340) of *TCF7L2* existing within the LD block showed similar robust associations with T2D (Grant *et al*. 2006). Further studies in other Europeans, African Americans, Mexican Americans, and Asian Indians confirmed the strong association with an estimated population attributable risk of 17–28% (Cauchi *et al*. 2006; Humphries *et al*. 2006; Chandak *et al*. 2007; Weedon 2007). Several genomewide association studies independently confirmed the strong association of SNP rs7903146 of *TCF7L2* with T2D (Scott *et al*. 2007). These data convincingly demonstrated that the genetic variants within *TCF7L2* gene, especially T allele of SNP rs7903146, are associated with the risk of developing T2D in several ethnic groups. In contrast, the frequency of SNP rs7903146 T allele is relatively low (∼2%) in Chinese and Japanese populations (Chang *et al*. 2007; Hayashi *et al*. 2007; Sandhu *et al*. 2007), raising the question of whether these variants are major contributors of T2D in the Asian population. Further, genetic association study of T2D in Thai population has been lacking. Therefore, we conducted this study to determine whether variations of *TCF7L2* are associated with T2D in Thai patients or not.

We demonstrated that SNPs rs7895340 and rs11196205 of *TCF7L2* showed a significant association with T2D in Thai patients similar to SNP rs7903146 in the Europeans. Moreover, our results emphasized the role of genetic heterogeneities in the risk of T2D since different variations of *TCF7L2* conferred T2D risk in different populations. These findings provided novel evidence supporting the role of the *TCF7L2* in pathogenesis of T2D in Thais. Nonetheless, the underlying mechanism by which genetic variations within *TCF7L2* introns confer susceptibility of T2D remains to be elucidated.

Interestingly, we showed significant association of SNPs rs7895340 and rs11196205 of *TCF7L2* with age-onset of diabetes. The patients who carried minor allele of these two SNPs were diagnosed to have diabetes at earlier age. Variants of *TCF7L2* had been reported to influence the age-onset of T2D in Mexican Americans and Caucasians (Lehman *et al*. 2007; Silbernagel *et al*. 2011). Still, it has not been reported in Asians.

We did not observe any association of SNP rs7903146 of *TCF7L2* and T2D in our population possibly due to its relatively low-allele frequency in Thai population. This finding was in concerted with Chinese and Japanese populations. However, a larger sample size may be required to detect the impact of this variant on T2D risk in Thais.

We have shown that variations of *TCF7L2* associate with the risk and age onset of T2D in Thais. Further study would be necessary to elucidate the mechanism by which these intronic *TCF7L2* variants involve in pathogenesis of T2D.

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