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ARTICLE

Replication study for the association of *TCF7L2* with susceptibility to type 2 diabetes in a Japanese population

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Abstract
Aims/hypothesis The transcription factor 7-like 2 gene (*TCF7L2*) has been shown to be strongly associated with an increased risk of type 2 diabetes in white populations. To further investigate the involvement of *TCF7L2* in conferring susceptibility to type 2 diabetes, we examined the association of *TCF7L2* polymorphisms with type 2 diabetes in a Japanese population.
Subjects and methods We analysed four SNPs (rs12255372, rs7903146, rs7901695 and rs11196205) and one tetranucleotide repeat polymorphism (DG10S478) in 1,630 Japanese subjects with type 2 diabetes and 1,064 control subjects.
Results All investigated polymorphisms were significantly associated with type 2 diabetes, and rs12255372 showed the strongest association (T vs G, χ^2=9.20, p=0.0024, odds ratio=1.70, 95% CI=1.20–2.41), although the frequency of the risk allele in our population was much lower than that in white populations. The microsatellite polymorphism showed an almost complete linkage disequilibrium to rs1255372 when the alleles with longer repeats (+8, +12) were considered as minor alleles and showed an association with type 2 diabetes (χ^2=5.34, p=0.021, odds ratio=1.50, 95% CI=1.06–2.12).
Conclusions/interpretation These results indicate that *TCF7L2* might be a strong candidate for conferring susceptibility to type 2 diabetes across different ethnicities.

Keywords Association study · Gene polymorphism · Microsatellite marker · TCF7L2 · Transcription factor 7-like 2 · Type 2 diabetes

Abbreviations

GLP-1	glucagon-like peptide 1
LD	linkage disequilibrium
OR	odds ratio
SNP	single nucleotide polymorphism

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Introduction

Type 2 diabetes affects more than 200 million individuals worldwide, and its prevalence continues to increase in many countries, including Japan. Although the precise mechanisms underlying the development and progression of type 2 diabetes have not been elucidated, a combination of multiple genetic and/or environmental factors is considered to contribute to the pathogenesis of the disease [1]. To date, several genes have been postulated as candidates for conferring

susceptibility to type 2 diabetes [2–8]; however, studies have produced conflicting results, probably due to a combination of small effect sizes being sought in sample sizes too small, using levels of statistical confidence that were not strict enough, or due to differences between the study populations in terms of environmental circumstances or ethnicity [1, 9].

The transcription factor 7-like 2 gene (*TCF7L2* [MIM 602228]) on chromosome 10q25 [10, 11], part of the Wnt signalling pathway [12], has been shown to be strongly associated with an increased risk of type 2 diabetes in Icelandic, Danish and US populations [13]. Five single nucleotide polymorphisms (SNPs) and one tetranucleotide repeat polymorphism (DG10S478) within *TCF7L2* have shown strong evidence of an association with type 2 diabetes in these three cohorts, and two of the SNPs (rs12255372 and rs7903146) showed strong linkage disequilibrium (LD) with composite at-risk alleles of the microsatellite marker (DG10S478). The associations of the SNPs rs12255372 and rs7903146 with decreased insulin secretion were also reported in US subjects with impaired glucose tolerance [14]. Although replication studies [15–21] have confirmed the role of *TCF7L2* in conferring susceptibility to type 2 diabetes in white populations, the pathophysiological mechanisms affected by the variations within *TCF7L2* and the effect of the gene in other ethnic populations have yet to be fully established.

The aim of the present study was to determine whether the previously investigated variations within *TCF7L2* are associated with susceptibility to type 2 diabetes in Japanese subjects.

Subjects and methods

Subjects and DNA preparation DNA samples were obtained from peripheral blood samples from 1,630 type 2 diabetes patients recruited from the outpatient clinic of Shiga University of Medical Science, Kawasaki Medical School (978 men, 652 women; age 61.5±11.6 years; duration of diabetes 11.5±13.9 years; HbA_{1c} 7.4±1.6%; fasting plasma glucose 9.1±3.5 mmol/l; BMI 23.7±3.9 kg/m^2 [all values are expressed as means±SD]). Diabetes was diagnosed according to the WHO criteria [22]. Type 2 diabetes was clinically defined as a disease with gradual adult onset. Subjects who tested positive for anti-GAD antibodies and those with mitochondrial disease (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episode [MELAS]) or MODY were excluded. We also examined 1,064 control subjects who were enrolled from an annual health check conducted either at Juntendo University or Keio University School of Medicine (Tokyo, Japan; 638 men, 426 women; age 45.5±9.5 years; HbA_{1c} 4.7±0.4%; fasting plasma glucose 5.1±0.5 mmol/l; and BMI 22.9±3.0 kg/m^2).

Written informed consent was obtained from all participants, and DNA extraction was performed using the standard phenol–chloroform procedure. The protocol was approved by the ethics committee of The Institute of Physical and Chemical Research.

Genotyping A microsatellite marker (DG10S478) with a tetranucleotide repeat polymorphism was analysed with regard to fragment sizes for each allele using the ABI Prism 3700 Automatic DNA Sequencer and GeneScan and Genotyper software (Applied Biosystems, Foster City, CA, USA). The SNPs within *TCF7L2* (rs12255372, rs7903146, rs7901695 and rs11196205) were analysed using the TaqMan assay (Applied Biosystems). The success rate of this assay was >95%, and there was almost 100% agreement between the results of genotyping and the results of direct sequencing.

Statistical analysis Statistical analyses for the association study and calculation of linkage disequilibrium (LD) coefficients (r^2) were performed as described previously [23]. The differences between the case and control groups in terms of genotype distribution or allele frequency were analysed using Pearson's χ^2 test.

Results

Association study The clinical characteristics of the subjects are summarised in Table 1. We calculated the power of our sample size to identify associations between the SNPs and type 2 diabetes using a dominant model for the minor allele. In this case–control setting, the prevalence of type 2 diabetes was assumed to be 10%. Our study had >90% power (β) at the p=0.05 significance level of detecting a

Table 1 Clinical characteristics of the subjects

	Type 2 diabetic subjects (n=1,630)	Control subjects (n=1,064)	p value
Sex (male:female)	978:652	638:426	0.9845[a]
Age (years)[b]	61.5±11.6	45.5±9.5	<0.0001
BMI (kg/m^2)[b]	23.7±3.9	22.9±3.0	<0.0001
FPG (mmol/l)[b]	9.1±3.5	5.1±0.5	<0.0001
HbA_{1c} (%)[b]	7.4±1.6	4.7±0.4	<0.0001
Duration of diabetes (years)[b]	11.5±13.9	–	–

[a] χ^2 test
[b] Values are expressed as means ± SD

Table 2 Genotype distribution and allele frequencies of SNPs and DG10S478 in *TCF7L2*

SNP	Genotype (*n* [%])			Allele (%)		MAF in white populations[a]
rs12255372	GG	GT	TT	G	T	
Case	1,515 (92.9)	112 (6.9)	3 (0.2)	96.4	3.6	36.6
Control	998 (95.7)	45 (4.3)	0 (0.0)	97.8	2.2	28.5
rs7903146	CC	CT	TT	C	T	
Case	1,450 (89.6)	165 (10.2)	4 (0.2)	94.7	5.3	39.7
Control	980 (91.8)	85 (8.0)	2 (0.2)	95.8	4.2	30.1
rs7901695	TT	CT	CC	T	C	
Case	1,440 (88.9)	173 (10.7)	4 (0.2)	94.4	5.6	38.4
Control	966 (91.5)	88 (8.3)	2 (0.2)	95.6	4.4	29.3
rs11196205	GG	CG	CC	G	C	
Case	1,384 (86.8)	198 (12.4)	13 (0.8)	93.0	7.0	53.2
Control	952 (89.7)	107 (10.1)	2 (0.2)	94.8	5.2	46.4
DG10S478[b]	AA	AB	BB	A	B	
Case	1497 (93.1)	111 (6.9)	0 (0)	96.5	3.5	36.0
Control	962 (95.3)	47 (4.7)	0 (0)	97.7	2.3	26.3

[a] Combined data from [13–21]
[b] A: short allele (allele −8, 0, 4), B: long allele (allele 8, 12)

genotypic relative risk (γ) of 1.5 for the SNPs with a minor allele frequency of 0.03 in our sample.

We genotyped four SNPs (rs12255372, rs7903146, rs7901695 and rs11196205) and the microsatellite (DG10S478) within *TCF7L2* reported to be associated with type 2 diabetes [13], and found that the minor allele frequencies of these polymorphisms were much lower in our Japanese population than in the white populations previously studied (Table 2). All four SNPs and the microsatellite polymorphism were significantly associated with type 2 diabetes, and rs12255372 was more strongly associated than the other SNPs (T vs G, χ^2=9.20, p=0.0024, odds ratio [OR]=1.7, 95% CI=1.20–2.41; Table 3). The genotype distributions of all SNPs were in Hardy–Weinberg equilibrium (p=0.48 for rs12255372, p=0.91 for rs7903146, p=0.99 for rs7901695, p=0.58 for rs11196205)

With regard to the microsatellite polymorphism (DG10S478), we identified five alleles in our study sample and, as observed in other populations, allele 0 was the most frequent (ESM Tables 1 and 2). We also found that DG10S478 was similar to rs12255372 in its association with type 2 diabetes (Table 3). Subsequent analysis for LD among these polymorphisms was performed; the microsatellite polymorphism showed complete LD to rs12255372 when the alleles with longer repeats (+8, +12) were considered as minor alleles (Table 4).

Table 3 Associations of SNPs in *TCF7L2* with type 2 diabetes

SNP		χ^2	*p* value	OR	95% CI
rs12255372	Genotype	9.51	0.0086	–	–
(G>T)	G vs T	9.20	0.0024	1.70	1.20–2.41
	GG vs GT+TT	8.49	0.0036	1.68	1.18–2.40
	GG+GT vs TT	1.92	0.1656	–	–
rs7903146	Genotype	3.90	0.1426	–	–
(C>T)	C vs T	3.81	0.0510	1.30	1.00–1.68
	CC vs CT+TT	3.89	0.0485	1.31	1.00–1.72
	CC+CT vs TT	0.10	0.7487	1.32	0.24–7.21
rs7901695	Genotype	4.17	0.1241	–	–
(T>C)	T vs C	4.06	0.0439	1.30	1.01–1.68
	TT vs CT+CC	4.17	0.0411	1.32	1.01–1.72
	TT+CT vs CC	0.10	0.7568	1.31	0.24–7.15
rs11196205	Genotype	8.07	0.0177	–	–
(G>C)	G vs C	6.91	0.0085	1.37	1.08–1.73
	GG vs CG+CC	5.25	0.0219	1.33	1.04–1.70
	GG+CG vs CC	4.45	0.0348	4.35	0.98–19.32
DG10S478	A vs B	5.51	0.019	1.52	1.07–2.15
	AA vs AB	5.34	0.021	1.50	1.06–2.12

A short allele (allele −8, 0, 4), *B* long allele (allele 8, 12)

Table 4 Linkage disequilibrium coefficient (r^2) among the four SNPs and the DG10S478 polymorphisms

	rs12255372	rs7903146	rs7901695	rs11196205
DG10S478 (allele X)[a]	0.61	0.69	0.66	0.27
DG10S478 (allele 8 and allele 12)	0.99	0.53	0.52	0.48
rs12255372		0.53	0.52	0.48
rs7903146			0.96	0.27
rs7901695				0.28

[a] Allele X denotes all alleles except for allele 0

Logistic regression analysis revealed that carriers of the risk allele of rs12255372 were associated with susceptibility to the disease even after adjusting for age and BMI (OR=2.06, 95% CI 1.20–3.52, p=0.0083).

Discussion

In the present study the four SNPs and the microsatellite polymorphism analysed were found to be significantly associated with type 2 diabetes.

Growing evidence suggests that *TCF7L2* is a strong susceptibility gene to type 2 diabetes in white populations [13–21]. To date, all studies in white populations have found the SNPs within the gene to be significantly associated with type 2 diabetes, and the estimated population-attributable risk was shown to be approximately 20% [13, 15, 16]. Although the contribution of *TCF7L2* in conferring susceptibility to type 2 diabetes has been widely investigated in white populations, the effects of *TCF7L2* should also be evaluated in different ethnic groups, because it is well known that there are significant differences in the frequencies of certain genetic variations among different ethnic groups [6, 9].

Our results indicated that the frequencies of the minor allele of rs12255372, the SNP that showed the strongest association with the disease, were substantially lower in our Japanese population vs white populations (type 2 diabetic patients: 3.6 vs 36.6%, $p=1\times10^{-299}$; control subjects: 2.2 vs 28.5%, $p=4\times10^{-149}$) [13, 16, 18, 19], as were the frequencies of the other SNPs. In addition, the LD pattern for this locus in the Japanese population also appeared to be different from that in white individuals; the longer allele of the microsatellite polymorphism showed complete LD to rs12255372 in our sample, whereas the X allele (alleles other than allele 0) showed complete LD to the SNP in white populations [13]. There are therefore clear ethnic differences with regard to this locus between the populations. However, we did identify a consistent association of this gene with type 2 diabetes in our Japanese population, further validating the contribution of *TCF7L2* to conferring susceptibility to the disease. Since the frequencies of the risk allele were much lower in our population, the estimated population-attributable risk was approximately 2.4%, suggesting that the genetic contribution of these polymorphisms to type 2 diabetes is relatively weak.

Previous studies have focused on the role of *TCF7L2* in oncogenesis and cancer progression [24–27]. Functional analyses are required to reveal the role of this gene in type 2 diabetes and to determine how variants of this gene confer susceptibility to the disease. Florez et al. recently reported the association between SNPs (rs12255372 and rs7903146) and decreased insulin secretion in US subjects with impaired glucose tolerance [14]. Damcott et al. also reported an association between SNPs (rs7901695 and rs7903146) and insulin resistance in white subjects [17]. Based on the fact that there is a putative TCF-binding motif within the promoter region of the gene encoding proglucagon [28], Yi et al. [12] suggested that *TCF7L2* was capable of regulating the expression of this gene, whose protein product is enzymatically cleaved to produce glucagon-like peptide 1 (GLP-1), which is secreted from gut endocrine L cells and is involved in glucose homeostasis. We therefore examined whether the plasma total GLP-1 concentrations were correlated with the polymorphisms in our control subjects; however, we did not identify any significant correlations (H. Maegawa, H. Yamamoto, T. Tani, A. Kashiwagi [all affiliated with Shiga University of Medical Science, Otsu, Japan], and T. Hayashi and S. Maeda; unpublished observations). Since it was reported that adipose tissue *TCF7L2* expression is decreased in subjects with type 2 diabetes [19], *TCF7L2* might play some roles in the adipocytes, such as the regulation of adipogenesis by altering transcriptional regulation of the genes encoding CCAAT/enhancer-binding protein-α (*CEBPA*) and peroxisome proliferator-activated receptor-γ (*PPARG*). However, the precise mechanism by which *TCF7L2* and its variants influence susceptibility to type 2 diabetes remains to be elucidated.

In summary, we have identified significant associations of *TCF7L2* variants with type 2 diabetes in a Japanese population, and have shown that differences exist in the allele frequencies and the pattern of LD between different ethnic populations.

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Duality of interest None of the authors has a conflict of interest to declare.

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