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Association of *TLR2* and *TLR4* non-missense single nucleotide polymorphisms with type 2 diabetes risk in a southern Chinese population: a case-control study

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ABSTRACT. Toll-like receptors (TLRs), the triggers of the innate and adaptive immune responses, are involved in the pathogenesis of type 2 diabetes mellitus (T2DM). Several studies have investigated the effects of genetic polymorphisms in *TLR4* and *TLR2*, but they have yielded limited results. We investigated whether non-missense genetic

polymorphisms in the regulatory regions of *TLR4* and *TLR2* were related to T2DM in a southern Chinese population. Single nucleotide polymorphisms (SNPs) in *TLR4* (rs1927911, rs11536889, rs1927907, rs1927906, rs1927914, rs7873784, and rs2149356) and *TLR2* (rs1898830, rs3804099, rs4696480, and rs3804100) were genotyped in 552 T2DM and 552 unrelated age- and gender-matched controls by SNaPShot Multiplex assay. Genotypes GG (OR = 0.09, 95%CI = 0.01-0.83, P = 0.03) and CG (OR = 0.08, 95%CI = 0.01-0.74, P = 0.03) of the 3′-untranslated region (UTR) SNP rs7873784 in *TLR4*, and genotype AG (OR = 0.67, 95%CI = 0.46-0.97, P = 0.04) and allele G (OR = 0.88, 95%CI = 0.79-0.97, P = 0.01) of the intron SNP rs1898830 in *TLR2* were identified as protective against the development of T2DM in southern Chinese people. In contrast, a meta-analysis of rs1927911 and rs1927914 showed no association. Haplotypes AGTT (OR = 0.34, 95%CI = 0.15-0.77, P = 0.01) and AATT (OR = 1.20, 95%CI = 1.01-1.44, P = 0.05) in *TLR2* were significantly associated with susceptibility to T2DM. Our results suggest that the effects of non-missense polymorphisms located in the regulatory regions of *TLR4* and *TLR2* should not be neglected in T2DM association analysis.

Key words: Toll-like receptor 4; Toll-like receptor 2; Type 2 diabetes; Non-missense single nucleotide polymorphisms; Chinese

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency (Kumar et al., 2005). As of 2010, it was estimated that there were 285 million patients globally with T2DM, making up about 90% of diabetes cases. In China, diabetes has become a major public health problem. The age-standardized prevalence of total diabetes and pre-diabetes are 9.7 and 15.5%, respectively, accounting for 92.4 million adults with diabetes and 148.2 million adults with pre-diabetes (Yang et al., 2010).

Chronic low-grade inflammation and activation of the innate immune system have been implicated in the pathogenesis and prediction of T2DM and associated complications (Pickup, 2004). Toll-like receptors (TLRs) are among the most important components of the innate immunity pathway. TLRs that start a signaling pathway through the nuclear factor kappa b (NF-κB) could have an impact on the development or progression of diabetes (Wong and Wen, 2008).

Interestingly, the expression levels of TLR2, TLR4, MyD88, phosphorylated IRAK-1, Trif, TICAM-1, IRF-3, and NF-κB p65 are increased in obese subjects (Creely et al., 2007) and T2DM subjects (Dasu et al., 2010). The probable cause is that the increasing levels of various biochemical factors, including endotoxins (lipopolysaccharides), fatty acids, etc., which appear in obese people, are thought to increase the activation of TLR2 and TLR4 (Miller et al., 2005; Park et al., 2006). In addition, obesity is always accompanied by high blood glucose, which induces the expression of TLR2 in human monocytes (Dasu et al., 2008). Activated TLR2 and TLR4 mediate the inflammatory response through MyD88 signaling, which in-

creases the release of various pro-inflammatory factors (IL-6, TNF-α, IL-1β, etc.), resulting in the agglomeration of inflammatory cells (Yang et al., 2008). Ultimately, a microenvironment of inflammation in local tissues exacerbates β-cell dysfunction (Yin et al., 2014), insulin resistance, and progression to T2DM (Hotamisligil 2006; Donath and Shoelson, 2011). This has been proven in a mouse model in which inhibition of TLR2 expression dramatically improved insulin sensitivity (Caricilli et al., 2008; Kuo et al., 2011).

Moreover, evidence suggests that genetic variations in the *TLR4* gene greatly influence immune responses towards pathogenic challenges and disease outcomes (Noreen et al., 2012), although most of these studies have been restricted to two missense polymorphisms, Asp299Gly and Thr399Ile, in the *TLR4* gene. The alleles 299Gly and 399Ile are associated with a lower prevalence of T2DM (Bagarolli et al., 2010). However, in the Augsburg Region there was no association between Asp299Gly and T2DM (Illig et al., 2003). The Asp299Gly polymorphism of the *TLR4* gene is associated with early-onset diabetic retinopathy in T2DM patients, and the G allele of Asp299Gly is an independent risk factor for early-onset diabetic retinopathy (Buraczynska et al., 2009). Recently, it has been reported that *TLR4* +3725G/C and +11367G/C polymorphisms may be novel protective factors against T2DM in the Chinese population (Jiang et al., 2013). However, only a few studies have focused on the effect of *TLR2* polymorphisms in the progression of T2DM. Maldonado-Bernal et al. (2011) found that the *TLR2* polymorphism (R753Q) failed to play a major role in the progress of T2DM in a Mexican population. Additionally, Liu et al. (2012) found no statistically significant difference between *TLR2* polymorphisms (Arg677Trp and Arg753Gln) and the risk of T2DM in Chinese subjects. The single nucleotide polymorphisms (SNPs) located in regulatory regions, such as the 5'-untranslated region (UTR), where the promoter or transcription factor binding sites are located, and the 3'-UTR, which may influence the expression of mRNAs, should be regarded as important SNPs owing to their potential influence on disease susceptibility. Overall, evidence that demonstrates the effects of *TLR2* and *TLR4* gene polymorphisms on T2DM is still limited.

Therefore, the aim of the present study was to determine whether *TLR4* and *TLR2* are associated with T2DM in a southern Chinese population. We evaluated the association between seven SNPs (rs1927911, rs11536889, rs1927907, rs1927906, rs1927914, rs7873784, and rs2149356) situated in the 3'-UTR, introns, 5'-UTR, and transcriptional regions of the *TLR4* gene, and four SNPs (rs1898830, rs3804099, rs4696480, and rs3804100) situated in the intron and coding-synon in *TLR2*, and T2DM in a Chinese population.

MATERIAL AND METHODS

Study subjects and laboratory analysis methods

This case-control study included 552 Chinese patients diagnosed with T2DM according to the 2003 American Diabetes Association criteria (Genuth et al., 2003). They were sequentially recruited from The First Affiliated Hospital of Jinan University between September 2011 and January 2013 in Guangzhou, China. The healthy controls (N = 552) were volunteers with normal fasting glucose and no family history of diabetes recruited during the same period and matched with the T2DM patients by gender and age (± 5 years). All individuals involved in this study were unrelated Chinese adults. Written informed consent was obtained from all

subjects. The Jinan University Ethics Committee approved the study protocol. Weight and height were measured to calculate body mass index (BMI). All patients studied were evaluated by medical doctors. A venous blood specimen (5 mL) was collected from each participant using a vacuum tube containing ethylenediaminetetraacetic acid anticoagulation and stored at -85°C until required. The genomic DNA from the peripheral blood of each subject was obtained using a QIAamp DNA kit (Qiagen, Hilden, Germany). The quality and quantity of DNA were checked using a NanoDrop spectrophotometer (Thermo Fisher, Wilmington, DE, USA). DNA samples were amplified using an ABI GeneAmp® 9700 Dual 384 machine, and mass spectrometry was carried out by matrix-assisted laser desorption/ionization time-of-flight.

SNP selection

The SNPs were selected from The International HapMap website (http://hapmap.ncbi.nlm.nih.gov) using genotype data from the Han Chinese in Beijing, China, and the National Center for Biotechnology Information SNP database, providing they met the following criteria: a) R-square cut-off > 0.8; b) minor allele frequency of SNP > 0.05; and c) Hardy-Weinberg equilibrium (HWE) P value cut-off > 0.001. We then combined the data from the National Center for Biotechnology Information website with the results of a literature review of the public database. This strategy identified seven SNPs in the *TLR4* gene (rs1927911, rs11536889, rs1927907, rs1927906, rs1927914, rs7873784, and rs2149356) and four SNPs in *TLR2* (rs1898830, rs3804099, rs4696480, and rs3804100). Detailed information about these SNPs (alleles, gene position, and minor allele frequency) is summarized in Table 1.

Table 1. General information for toll-like receptor 4 gene (*TLR4*) and *TLR2* polymorphisms.

Gene	dbSNP ID	Chrom. Pos	SNP location	Alleles	MAF (%)	HWE P value*	Call rate (%)
TLR4	rs1927914	120464725	5'-near gene	C/T	36.8	0.892	99.9
	rs1927911	120470054	Intron	C/T	36.8	0.921	100
	rs1927907	120472764	Intron	A/G	22.2	0.970	99.0
	rs2149356	120474199	Intron	A/C	36.8	0.905	99.3
	rs11536889	120478131	3'-UTR	C/G	25.0	0.209	99.3
	rs7873784	120478936	3'-UTR	C/G	10.2	0.626	99.4
	rs1927906	120480115	3'-near gene	A/G	5.9	0.689	99.9
TLR2	rs1898830	154608453	Intron	A/G	47.0	0.521	93.3
	rs3804099	154624656	Coding-synon	T/C	27.0	0.149	98.2
	rs4696480	154607126	Intron	A/T	44.0	0.270	98.7
	rs3804100	154625409	Coding-synon	C/T	24.0	0.397	99.8

dbSNP = database single nucleotide polymorphism; MAF = minor allele frequency, HWE = Hardy-Weinberg equilibrium; UTR = untranslated region. *HWE P value cut-off: 0.001.

Genotyping assay

The polymerase chain reaction primers are shown in Table 2. Genomic DNA (5-10 ng) was amplified in a final volume of 5 μL as follows: initial denaturation at 94°C for 4 min followed by 40 cycles at 94°C for 20 s, annealing at 56°C for 30 s, and extension at 72°C for 1 min. Shrimp alkaline phosphatase (SAP) enzyme solution (2 μL) (including 0.17 μL SAP buffer, 0.3 μL SAP enzyme, and 1.53 μL water) was added and the mixture was incubated at 37°C for 40 min and at 85°C for 5 min, then stored at 4°C. After adding 2 μL High Plex iLEX

Gold (Invitrogen, USA) reaction mixture (including 0.94 μL primer mix, 0.2 μL Gold buffer, 0.2 μL termination mix, 0.041 μL enzyme, and 0.619 μL water) to each sample, we conducted a thermocycle as follows: initial denaturation at 94°C for 30 s followed by 40 cycles at 94°C for 5 s, annealing at 52°C for 5 s, extension at 80°C for 5 s, then final extension at 72°C for 3 min. Finally, we acquired the spectra.

Statistical analysis

Tests were assessed using the Haploview (v.4.2) program and SAS software (v.9.3) to analyze deviation from HWE and to compare allelic frequencies between cases and controls (Barrett et al., 2005). The HWE for SNP genotypes among controls was tested using a goodness-of-fit χ^2 test. After adjustment for potential covariates, the odds ratios ORs and 95%CIs were calculated to assess the associations between the 11 SNPs and T2DM risk, using conditional logistic regression. The potential covariates included age, gender, and BMI. Continuous variables are reported as means ± SD and categorical variables as numbers of cases. The statistical significance limit was set at P = 0.05. The PLINK software was used to establish a log-additive model and calculate ORs, 95%CIs, and P values. Meta-analysis was performed using the Stata software (v.12.0) to pool the studies by Singh et al. (2013; 2014), Peng et al. (2014), and our own. The statistical power of the present study was evaluated using the Quanto software. The prevalence of T2DM was estimated at 5%.

Table 2. Polymerase chain reaction primers and amplicon sizes.

Gene	dbSNP ID	Primers	Amplicon size (bp)
TLR4	rs1927914	F: ACGTTGGATGGTGCTTGGAGGATATTACAG	
		R: ACGTTGGATGGTAGCAAGTGCAATGTAAG	125
	rs1927911	F: ACGTTGGATGTCAAGATGTCCAGACCTTCC	
		R: ACGTTGGATGCATCACTTTGCTCAAGGGTC	109
	rs1927907	F: ACGTTGGATGGGGTATCCAGTGGATTGAAG	
		R: ACGTTGGATGCTATTAAGGTAGACCACCTC	125
	rs2149356	F: ACGTTGGATGTTGTTAGTTGGTAGCCAAG	
		R: ACGTTGGATGGAGTATCTGTGACACTTATG	104
	rs11536889	F: ACGTTGGATGGAACCCCATTAATTCCAGAC	
		R: ACGTTGGATGTTTCCTGTTGGGCAATGCTC	109
	rs7873784	F: ACGTTGGATGATGAGAGGTACCCTCTTAAC	
		R: ACGTTGGATGGCTCTAAAGATCAGCTGTAT	108
	rs1927906	F: ACGTTGGATGTGCTTGTCCACCTCACCTG	
		R: ACGTTGGATGCTCCTTCCTATCAGTTCCCT	85
TLR2	rs1898830	F: ACGTTGGATGGATCCCCTATTTTCTAGCAC	
		R: ACGTTGGATGTGAATGAGCAAGCAAATACC	141
	rs3804099	F: ACGTTGGATGTATGCTGCTTCATATGAAGG	
		R: ACGTTGGATGGATCTACAGAGCTATGAGCC	99
	rs4696480	F: ACGTTGGATGCTCACCATGTGATGCTTTCC	
		R: ACGTTGGATGGGGAAGTCCAAGATTGAAGG	102
	rs3804100	F: ACGTTGGATGTTCCAGTGTCTTGGGAATGC	
		R: ACGTTGGATGTGCCTGAAACTTGTCAGTGG	118

dbSNP = database single nucleotide polymorphism; TLR = toll-like receptor; F = forward; R = reverse.

RESULTS

The general characteristics of the 552 T2DM cases and the 552 controls are summa-

rized in Table 3. There were no significant differences in age or gender between the T2DM patients and the healthy control group (P > 0.05), but there was a significant difference in BMI between T2DM patients and the control group (P < 0.01). Only 84 patients (23.9%) did not have any complication, and at least 76.1% (268/352) of patients had complications.

Table 3. General characteristics of the study population.

Characteristics	Case (N = 552)	Control (N = 552)	P value*
Age, years (mean ± SD)	62.12 ± 13.26	62.36 ± 12.99	0.754
Gender			
Male	269	267	0.904
Female	283	285	
BMI (kg/m^2)	24.38 ± 3.38	21.94 ± 2.73	0.000*

BMI = body mass index. *The Mann-Whitney test was used to analyze differences. **P < 0.05.

Association of *TLR4* polymorphisms with T2DM

The SNPs genotyped were in HWE (P > 0.05). The genotype distributions of the eleven polymorphisms are shown in Table 4. In the *TLR4* gene, compared with the CC genotype of 3'-UTR SNP rs7873784, subjects with GG (OR = 0.09, 95%CI = 0.01-0.83; P = 0.03) and GC (OR = 0.08, 95%CI = 0.01-0.74; P = 0.03) had a lower risk of having T2DM after adjusting for the conventional risk factors such as age, gender, and BMI. In the *TLR2* gene, the subjects with the heterozygote AG (OR = 0.67, 95%CI = 0.46-0.97, P = 0.04) and the allele G (OR = 0.88, 95%CI = 0.79-0.97, P = 0.01) in SNP rs1898830 had a lower risk of having T2DM. In the log-additive model, there was a significant difference in SNP rs1898830 (OR = 0.78, 95%CI = 0.67-0.95; P = 0.01). The genotypes of rs1927914, rs1927911, rs1927907, rs2149356, rs11536889, rs1927906, rs3804099, rs4696480, and rs3804100 displayed no association with T2DM.

Meta-analysis for rs1927911 and rs1927914

The rs1927911 and rs1927914 SNPs, reported by Singh et al. (2013, 2014) and Peng et al. (2014), were subjected to meta-analysis to evaluate the pooled effects. The results indicated that there was high heterogeneity in rs1927911 (I^2 = 85.0%) and rs1927914 (I^2 = 61.0%), and the overall effect of rs1927911 (OR = 1.01, 95%CI = 0.81-1.28, P > 0.05) and rs1927914 (OR = 1.19, 95%CI = 0.94-1.51, P > 0.05) failed to show a significant difference (Figure 1).

Haplotype analysis of *TLR4* and *TLR2* with T2DM

We identified six types of haplotypes in *TLR4* (rs1927914, rs1927911, rs1927907, rs2149356, rs11536889, rs7873784, rs1927906) and five types of haplotypes in *TLR2* (rs4696480, rs1898830, rs3804099, rs3804100) with frequencies above 2%. Compared with the haplotype TGTT, the haplotypes AGTT (OR = 0.34, 95%CI = 0.15-0.77, P = 0.01) and AATT (OR = 1.20, 95%CI = 1.01-1.44, P = 0.05) in *TLR2* were significantly associated with T2DM (Table 5).

Table 4. Association of the toll-like receptor 4 (*TLR4*) and *TLR2* genes with risk of type 2 diabetes mellitus (T2DM).

Gene	Genotype	Case (N = 552)	Control (N = 552)	Adjusted OR (95%CI)[a]	Adjusted P value[a]
TLR4	rs1927914				
	CC	68	78	1.00 (Ref)	
	TT	216	228	1.24 (0.75-2.06)	0.410
	CT	266	244	1.36 (0.83-2.25)	0.220
	T/C	698/402	700/400	0.99 (0.83-1.18)	0.930
	Log-additive	-	-	1.01 (0.85-1.21)	0.900
	rs1927911				
	TT	68	76	1.00 (Ref)	
	CC	216	227	1.15 (0.69-1.92)	0.590
	TC	266	246	1.24 (0.75-2.05)	0.400
	C/T	698/402	700/398	0.98 (0.83-1.17)	0.880
	Log-additive	-	-	1.01 (0.85-1.20)	0.900
	rs1927907				
	GG	331	336	1.00 (Ref)	
	AA	28	26	1.89 (0.71-5.04)	0.200
	GA	185	186	0.99 (0.62-1.59)	0.970
	A/G	241/847	238/858	1.03 (0.84-1.26)	0.810
	Log-additive	-	-	1.02 (0.83-1.25)	0.840
	rs2149356				
	AA	68	80	1.00 (Ref)	
	CC	216	228	1.20 (0.72-2.00)	0.480
	AC	263	241	1.34 (0.81-2.22)	0.260
	C/A	695/399	697/401	1.00 (0.84-1.19)	0.980
	Log-additive	-	-	1.01 (0.85-1.20)	0.900
	rs11536889				
	GG	306	290	1.00 (Ref)	
	CC	35	28	1.14 (0.58-2.27)	0.700
	GC	204	232	0.87 (0.64-1.19)	0.390
	C/G	274/816	288/812	0.95 (0.78-1.15)	0.580
	Log-additive	-	-	0.93 (0.77-1.14)	0.500
	rs7873784				
	CC	8	2	1.00 (Ref)	
	GG	441	444	0.09 (0.01-0.83)	0.030*
	CG	96	100	0.08 (0.01-0.74)	0.030*
	G/C	978/112	988/104	0.92 (0.69-1.22)	0.560
	Log-additive	-	-	1.10 (0.82-1.46)	0.530
	rs1927906				
	AA	495	497	1.00 (Ref)	
	GG	2	1	2.02 (0.12-34.79)	0.630
	AG	53	55	0.87 (0.51-1.49)	0.620
	G/A	57/1043	57/1049	1.01 (0.69-1.47)	0.980
	Log-additive	-	-	1.00 (0.69-1.46)	0.990
TLR2	rs1898830				
	AA	195	151	1.00 (Ref)	
	AG	241	249	0.67 (0.46-0.97)	0.038*
	GG	96	115	0.70 (0.45-1.10)	0.129
	G/A	433/631	479/551	0.88 (0.79-0.97)	0.007*
	Log-additive	-	-	0.78 (0.67-0.95)	0.009*
	rs3804099				
	TT	289	279	1.00 (Ref)	
	TC	205	229	0.82 (0.60-1.14)	0.247
	CC	43	34	1.17 (0.63-2.16)	0.602
	C/T	291/783	297/787	0.99 (0.86-1.14)	0.874
	Log-additive	-	-	0.98 (0.81-1.19)	0.873
	rs4696480				
	TT	97	93	1.00 (Ref)	
	TA	258	294	0.81 (0.53-1.23)	0.328
	AA	190	158	1.10 (0.70-1.73)	0.675
	A/T	638/452	610/480	1.06 (0.97-1.13)	0.225
	Log-additive	-	-	0.90 (0.75-1.07)	0.220
	rs3804100				
	TT	316	315	1.00 (Ref)	
	TC	193	208	0.88 (0.64-1.20)	0.427
	CC	35	28	1.10 (0.56-2.18)	0.765
	C/T	263/825	264/838	1.00 (0.87-1.17)	0.906
	Log-additive	-	-	1.01 (0.83-1.23)	0.910

[a]Adjusted for age, gender, and body mass index (BMI). *P < 0.05.

Table 5.

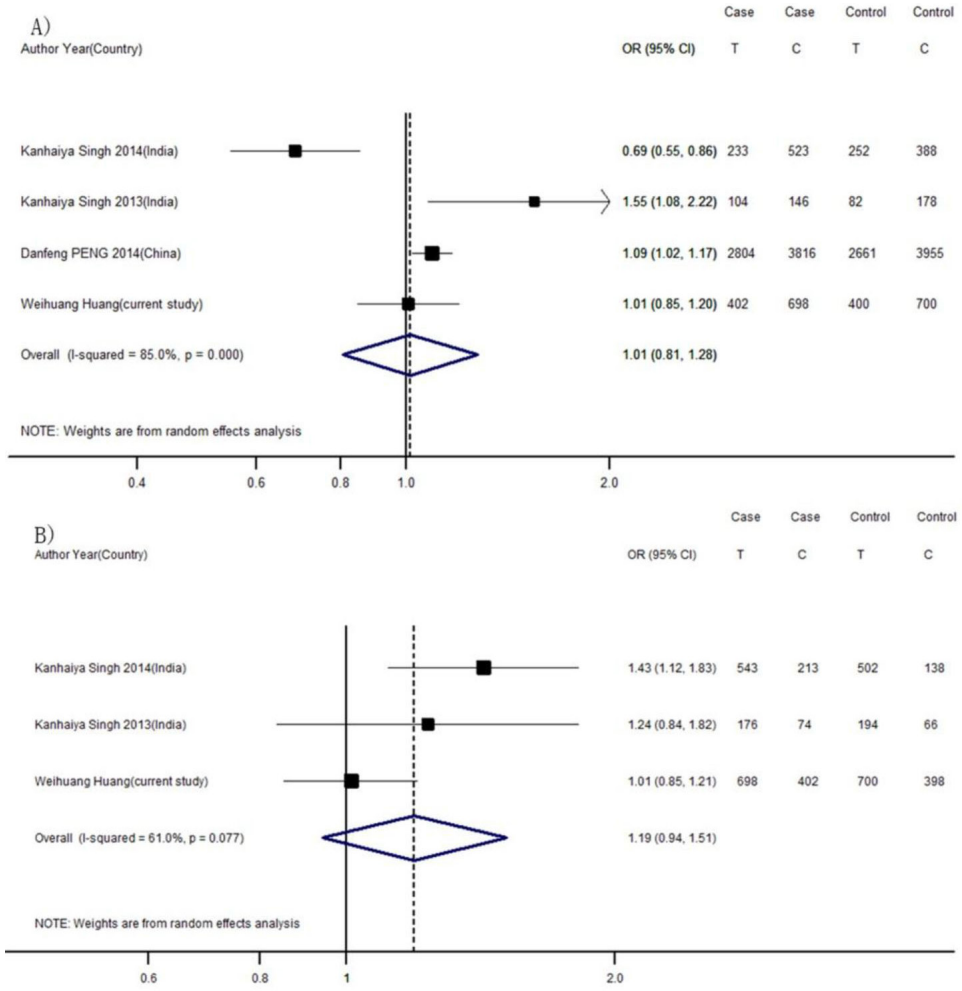

Figure 1. Forest plot of toll-like receptor 4 gene (*TLR4*) polymorphisms and type 2 diabetes mellitus (T2DM) risk. **A.** Pooled OR of rs1927911 for T versus C; **B.** Pooled OR of rs1927914 for C versus T.

Table 5. Association of haplotypes with type 2 diabetes mellitus (T2DM).

Gene	Haplotype	Case	Control	OR	P value
TLR4	TCGCGGA	38.2	37.0	1.05 (0.89-1.25)	0.569
	TCGCCGA	24.9	26.0	0.95 (0.78-1.15)	0.572
	CTAAGGA	17.2	16.6	1.05 (0.84-1.31)	0.705
	CTGAGCA	10.1	9.1	1.12 (0.84-1.48)	0.417
	CTAAGGG	5.0	5.0	0.99 (0.68-1.46)	0.998
	CTGAGGA	4.0	5.0	0.79 (0.53-1.18)	0.244
TLR2	TGTT	39.3	42.7	0.87 (0.74-1.04)	0.117
	AATT	30.7	27.0	1.20 (1.01-1.44)	0.050*
	AACC	22.4	22.1	1.01 (0.83-1.24)	0.900
	AACT	3.9	3.3	1.20 (0.77-1.89)	0.418
	AGTT	0.7	2.1	0.34 (0.15-0.77)	0.007*

*$P < 0.05$.

DISCUSSION

We presented evidence for associations between non-missense polymorphisms located in the regulatory regions of *TLR4* and *TLR2* genes and the risk of T2DM in a southern Chinese population. We identified SNP rs7873784 in *TLR4* and SNP rs1898830 in *TLR2* as being significantly associated with T2DM risk in the Chinese population.

The 3'-UTR SNP rs7873784 appeared to be the significant locus associated with T2DM in the Chinese population. The homogeneous genotype CC of rs7873784 was a risk factor for T2DM. The allele C frequency of rs7873784 was 9.9% in our study, which is less than the 14.6% found in the CEU population (Utah residents with ancestry from northern and Western Europe) and the 27.8% found in the YRI population (Yoruba in Ibadan, Nigeria) based on the data from the HapMap project. To our knowledge, the effect of rs7873784 variation on T2DM has not been described in a case-control study before, although one case-cohort study on rs7873784 found no direct association with the incidence of T2DM (Kolz et al., 2008). Chen et al. (2005) reported that the CC and CG genotypes in rs7873784 were associated with a decrease in prostate cancer risk in a US population. Bertinetto et al. (2012) reported that the G allele and the GG genotype were associated with IgA nephropathy with proteinuria for rs7873784 in *TLR4* in a north Italian population. Transcriptional regulation is also changed when the allele in rs7873784 is changed from C to G, and transcription factor STATx cannot bind to the *TLR4* gene, as predicted by the transcription factor search tool. Combined with above reports, the current study implies that 3'-UTR rs7873784 might play a role in the development of T2DM, and seems to be a good candidate for a functional SNP.

We also suggest that rs1898830 (AG) and rs1898830 (G) are a "protective" genotype and allele, respectively. Until now, there has been no research on the relationship between *TLR2* rs1898830 and susceptibility to T2DM. However, the *TLR2* polymorphism rs1898830 has been associated with maternal atopy, bronchiolitis obliterans, and pulmonary tuberculosis in previous studies (Chen et al., 2010; Kastelijn et al., 2010; Liu et al., 2011). rs1898830, which is located in the intron region of *TLR2*, can cause the decline of lung function in cystic fibrosis patients (Haerynck et al., 2013). Moreover, Taniguchi et al. (2013) have reported that the genotype AG in rs1898830 is associated with congenital cytomegalovirus infection. Liu et al. (2011) found that the GG genotype of rs1898830 in combination with maternal atopy leads to a decrease in regulatory T cell markers, indirectly pointing to potentially insufficient immunosuppression. In addition, Chen et al. (2011) suggest that rs1898830 is associated with TLR2-mediated cellular activation, which is caused by the effect of rs13150331 on transcriptional activities of the *TLR2* gene promoter. Variation of the gene promoter may affect mRNA transcription efficiency, stability, translation, etc. The mechanism by which TLR2 takes effect may be partly based on the above evidence, although it requires further clarification. We supposed that after being stimulated by high glucose and free fatty acids (Ehses et al., 2010), *TLR2* with genotype rs1898830 (AA) was more likely to induce the expression of IKK-β and NF-κB pathway target genes, and also facilitated secretion of pro-inflammatory cytokines and chemokines, including TNF, IL-6, IL-1β, CCL2, and CCL3 (Cai et al., 2005; Donath and Shoelson, 2011). These factors can induce the aggregation of inflammatory cells, promoting inflammation in islet, adipose, and liver tissues, and resulting in insulin resistance and ultimately the development of T2DM (Donath and Shoelson, 2011). However, there exists another possibility that the intron region of DNA could regulate the expression of genes through circular RNA (Zhang et al., 2013). Thus, variations at the intron could contribute to

the function of DNA. However, more investigation is required to elucidate the underlying mechanism of this effect.

For the other nine loci, none of the genotypes showed any significant association with T2DM in the southern Chinese population. Unfortunately, the SNPs rs1927911, rs1927907, rs11536889, rs1927906, rs3804099, and rs3804100 with negative results lacked the genetic power (lower than 80%) to detect the true "causal" associations. Further, we performed meta-analyses on rs1927911 and rs1927914 but did not find any associations. The sample size was not large enough to detect associations and there was high heterogeneity among the studies included. Therefore, more studies are required.

With regards to haplotype analysis, compared with the control subjects, the frequency of the AATT haplotype was higher in T2DM subjects, suggesting that haplotype AATT was associated with increasing T2DM susceptibility. In contrast, the AGTT haplotype was associated with lower risk of disease susceptibility. As we expected, the AGTT and AATT haplotypes differed only in the rs1898830 (A/G) allele, which indicates that rs1898830 (A/G) influences the effects of the haplotypes on the risk of T2DM. However, estimation of the haplotype was based on statistical calculation without biological evidence. Thus, further analysis of the underlying mechanisms of *TLR2* haplotype contribution to T2DM susceptibility is needed.

In conclusion, we have provided evidence that polymorphisms in *TLR4* and *TLR2* are associated with T2DM. The genotypes GG and CG of 3'-UTR SNP rs7873784, and the genotype AG and allele G of intron SNP rs1898830 protected against the development of T2DM in southern Chinese people. In addition, haplotypes AATT and AGTT were significantly associated with susceptibility to T2DM. Although the variant at 3'-UTR in *TLR4* decreases the stability of the *TLR4* mRNA according to other studies, the exact functional effect of rs7873784 on the expression of *TLR4* still requires further investigation.

Conflicts of interest

The authors declare no conflict of interest.

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