Gene Expression, Vol. 16, pp. 177–185
Printed in the USA.

1052-2166/15 $90.00 + .00
DOI: http://dx.doi.org/10.3727/105221615X14399878166195
E-ISSN 1555-3884
www.cognizantcommunication.com

Association of Genetic Variants of *SIRT1* With Type 2 Diabetes Mellitus

Junfeng Han,*[1] Meilin Wei,*[1] Qianqian Wang,* Xu Li,* Chaoyu Zhu,* Yueqin Mao,* Li Wei,* Yongning Sun,† and Weiping Jia*

*Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Key Clinical Center for Metabolic Disease, Shanghai, China
†Department of Traditional Chinese Medicine, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

SIRT1 has been demonstrated in nutrient-sensing and insulin-signaling pathways in in vivo and in vitro experiments, but there is minimal information concerning the association between gene polymorphisms of *SIRT1* and type 2 diabetes mellitus (T2DM) in a Chinese Han population. Using case-control design, we recruited 310 unrelated T2DM patients from inpatients at Shanghai Jiao Tong University Affiliated Sixth People's Hospital, while 301 healthy controls were volunteers from the community for regular medical checkup. All participants were genotyped within the *SIRT1* region. The following five SNPs rs10509291, rs12778366, rs10997870, rs10823112, and rs4746720 cover 100% of common genetic variations (minor allele frequency ≥ 0.05) within the *SIRT1* gene ($r^2 \geq 0.8$). The genotypes of *SIRT1* gene polymorphisms were analyzed by the Snapshot assay and DNA sequencing. The resulting data show that there was significant genetic differentiation in rs10823112 [$p = 0.003$; OR (95% CI) = 1.515 (1.152–1.994) for genotype], rs4746720 [$p = 0.024$; OR (95% CI) = 1.37 (1.037–1.674) for genotype], and rs10509291 [$p = 0.002$; OR (95% CI) = 1.551 (1.179–2.04) for genotype] between T2DM and control subjects. However, the result of rs4746720 was no longer significant after correction for multiple testing (p after Bonferroni correction = 0.12); the results of rs10509291and rs10823112 were still significantly different between the two groups (p after Bonferroni correction = 0.01 and 0.015, respectively). Linear regression analyses adjusting for age, gender, and body mass index (BMI) showed that HbA1c and HOMA-IR in subjects with rs10509291 AA genotype were higher than those with TT genotype in T2DM group ($p = 0.045$, $p = 0.035$, respectively). Together, our data show that genetic variation of the *SIRT1* gene is related to insulin resistance and increase risk of T2DM in Chinese Han population. The risk allele A at *SIRT1* rs10509291 was closely associated with T2DM, and subjects who were homozygous of the A allele were more likely to develop T2DM.

Key words: Type 2 diabetes; Genetic variants; *SIRT1*; Chinese Han population

INTRODUCTION

Diabetes mellitus (DM) is one of the most common public health problems with a high incidence, numerous complications, high disability rate, low awareness rate, and heavy economic burden. According to the Diabetes Atlas 2014 from the International Diabetes Federation, there are 387 million people living with diabetes worldwide, and every 7 s one person dies from diabetes (1). Type 2 diabetes mellitus (T2DM) constitutes more than 90% of the cases of diabetes, and the prevalence has been dramatically increasing in developing countries, especially in China. The prevalence of diabetes in China, in 2007, was estimated to be 9.7%, with a further 15.5% of the population at high risk of diabetes with impaired glucose tolerance (2). T2DM is characterized by insulin resistance (IR) and insufficient compensatory insulin secretion (3). In addition to the environmental factors, genetic factors have been demonstrated to play an important role in the pathogenesis of T2DM. Recently, along with the powerful genome-wide association study, the candidate gene approach can also guide a better understanding of the pathophysiology of complex diseases. Up to now, more than 60 loci have been confirmed to confer susceptibility to T2DM (4). However, all these variants could only explain approximately 10–15% of the genetic mechanism of the disease. This has been attributed to the prevalent sedentary lifestyle and high-calorie diet that affect human physiology

[1]These authors provided equal contribution to this work.
Address correspondence to Li Wei, Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, Shanghai Key Clinical Center for Metabolic Disease, 600 Yishan Road, Shanghai 200233, China. Tel: 18930173636; Fax: +8621-64519943; E-mail: weili63@hotmail.com *or* Yongning Sun, Department of Traditional Chinese Medicine, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, 600 Yishan Road, Shanghai 200233, China. Tel: 18930177579; Fax: +8621-64519943; E-mail: Ynsun2002@126.com

and the level of expression of the genes involved mainly in fuel metabolism. However, most of the GWAS were performed in Caucasian population; therefore, it is imperative to explore the specific genetic architecture of T2DM in Chinese population.

The human sirtuin *SIRT1*, a NAD^+ histone deacetylase, is regulated by stress and nutritional status, regulating many transcription factors that modulate endocrine signaling and cellular metabolism in different physiological contexts (5). *SIRT1* has been involved in many cellular processes and implicated in human diseases, such as obesity, type 2 diabetes, cancer, and neurodegenerative diseases. Increasing dosage of *SIRT1* in pancreatic β-cells improves glucose tolerance and enhances insulin secretion in response to glucose in β-cell-specific *SIRT1*-overexpressing transgenic mice (6). The role of *SIRT1* has been demonstrated in nutrient-sensing and insulin-signaling pathways, as well as in the regulation of stress responses that determine cell survival, apoptosis, and proliferation (7). During fasting or calorie restriction, *SIRT1* expression is differentially regulated (8,9) in tissues. It is upregulated in liver to increase glucose output through suppressing gluconeogenesis and in white adipose tissue to facilitate free fatty acid release. In contrast, *SIRT1* is downregulated in pancreatic β-cells leading to reduced insulin secretion (10). Thus, *SIRT1*, which is considered as a convincing candidate gene that may contribute substantially to glucose metabolism, should be investigated on the relationship between *SIRT1* locus and

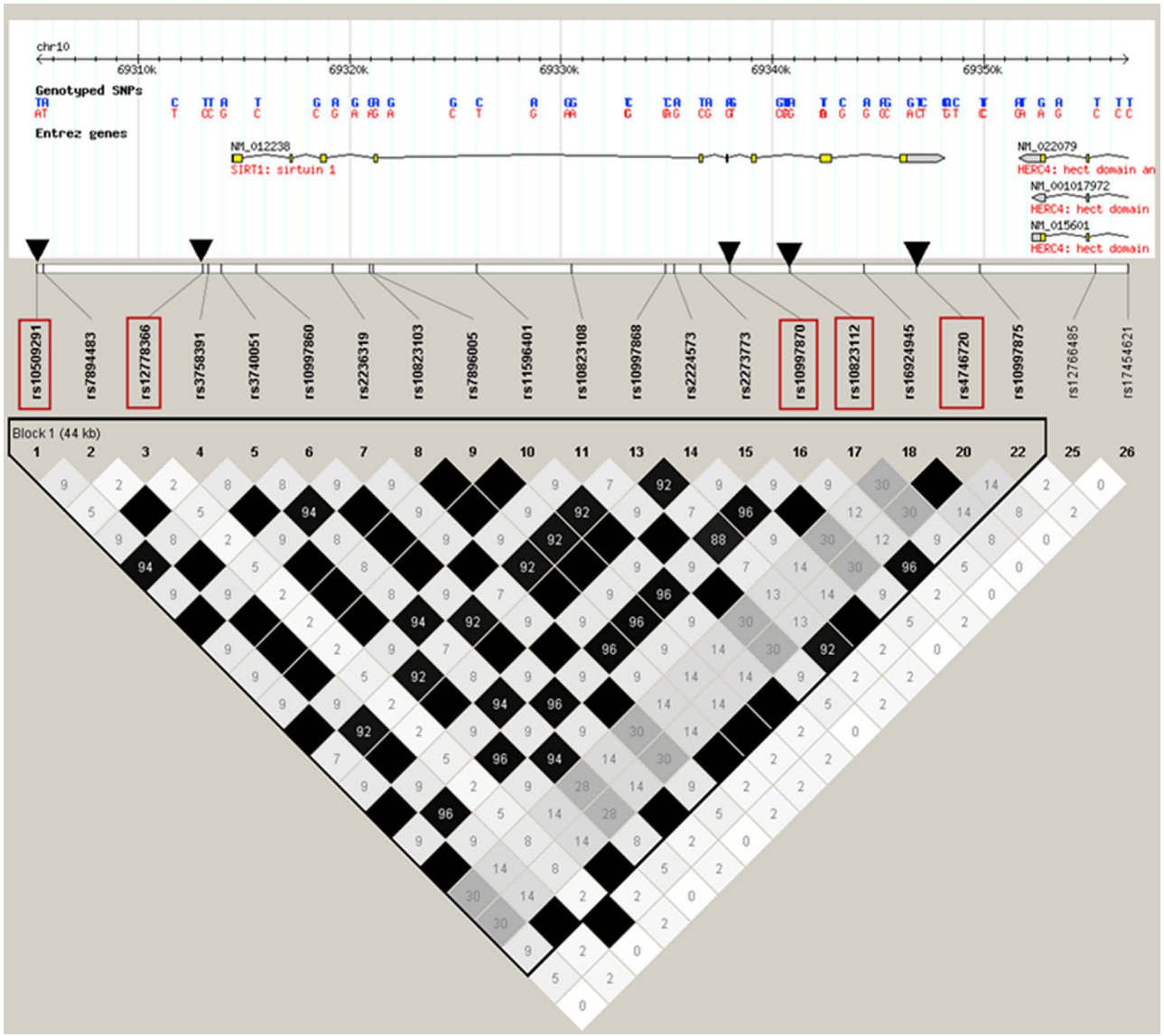

Figure 1. Genomic region of human chromosome 10 harboring the *SIRT1* gene and LD data of informative SNPs within this region (HapMap data). Shades of gray show the strength of the pairwise linkage disequilibrium based on r^2, and numbers indicate the value of r^2 expressed as a percentage.

Table 1. Primer Sequences

		Sequence (5′–3′)
PCR primers		
Rs10509291	F	TTCCAACTACGCTATCAATCT
	R	CAGATAGAAGCCAAGGGTGT
Rs12778366FF		TAAGGCTTCTAGGACTGGAG
	R	CTAAGGTCCTATCTACATCC
Rs10997870FF		ATGGTACAGAAAGATTCAGG
	R	GGTATTGAAAGGTTCTCGTG
Rs10823112FF		CCCATATCTCGTGGCTTTAG
	R	GCAAATTGTCTTGTATTCTAC
Rs4746720FF		GTACTCAAAATCTGTTACGC
	R	AGTTAGCTGCCACAGTTTTG
SNP primers		
Rs12778366		TTTTTTTTTTTTTTTTATTTCATCTGGTCACCACT
Rs10997870		TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGTGTATTTTACCAAAATTCC
Rs10823112		TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAACAGAATTTCACAGCTAA
Rs4746720		TTTTTTTTTTTTTTTTTTTTTTTTTAAATCTGTTACGCTAAACTT

F, forward primer; R, reverse primer.

susceptibility for T2DM. Nevertheless, up to now, there are no reports focusing on *SIRT1*. In view of this, the aim of the current study is to investigate possible correlations between genetic variation in the *SIRT1* gene and related clinical traits of T2DM in a Chinese cohort.

MATERIALS AND METHODS

Subjects

A total of 611 participants of Han ancestry residing in Shanghai were recruited. Among them, 310 patients with T2DM were newly diagnosed and not receiving any treatments. This sample will guarantee detection of gene and loci for common alleles influencing T2DM with an OR of ≥1.3 reaching at least 80% power. The diagnosis of T2DM was made according to the 1999 WHO criteria (11). Exclusion criteria included 1) patients with a history of ketoacidosis; 2) patients requiring continuous insulin treatment; 3) patients having exocrine pancreatic disease; or 4) patients with exceptionally early age of disease onset (<30 years). The control group was recruited from subjects who came for routine physical examination. In the present study, criteria for including the control participants were 1) over 40 years old; 2) normal oral glucose tolerance test (OGTT) assessed by a standard 75-g oral glucose with fasting plasma glucose levels of 5.6 mmol/L and 2-h plasma glucose of 7.8 mmol/L; and 3) without known family

Table 2. Clinical Characteristics of T2DM Patients and Control Subjects

	Controls ($n=301$)	T2DM Patients ($n=310$)	p
Age (years)	52.4±6.8	53.6±9.1	0.06
Sex (M/F)	160/141	148/162	0.19
BMI (kg/m^2)	22.8±4.1	25.5±5.2	**<0.001**
WHR	0.86±0.12	0.92±0.15	**<0.001**
Hypertension [n (%)]	66 (21.9%)	108 (34.7%)	**<0.001**
SBP (mmHg)	124.1±13.3	131.5±15.9	**<0.001**
DBP (mmHg)	79.1±8.1	86.5±11.4	**<0.001**
Fasting glucose (mmol/L)	5.3±0.4	8.7±2.9	**<0.001**
HbA1c (%)	5.6±0.34	8.1±2.1	**<0.001**
Triglycerides (mmol/L)	1.5±0.4	2.3±1.5	**<0.001**
Total cholesterol (mmol/L)	4.8±0.8	5.1±2.4	0.04
HDL-C (mmol/L)	1.2±0.3	1.0±0.3	**<0.001**
LDL-C (mmol/L)	3.0±0.7	3.1±0.9	0.12
Uric acid (μmol/L)	310±92	324±98	0.06
Fasting insulin (μU/ml)	6.7 (4.57–8.68)	10.42 (6.7–1.98)	**<0.001**
HOMA-IR	1.56 (1.05–2.1)	3.7 (2.2–6.3)	**<0.001**
HOMA-β	75.6 (52.3–101.1)	47.2 (27.6–79.6)	**<0.001**

HOMA-IR and HOMA-β were subjected to natural logarithm transformation prior to statistical analysis. p values <0.05 are shown in bold.

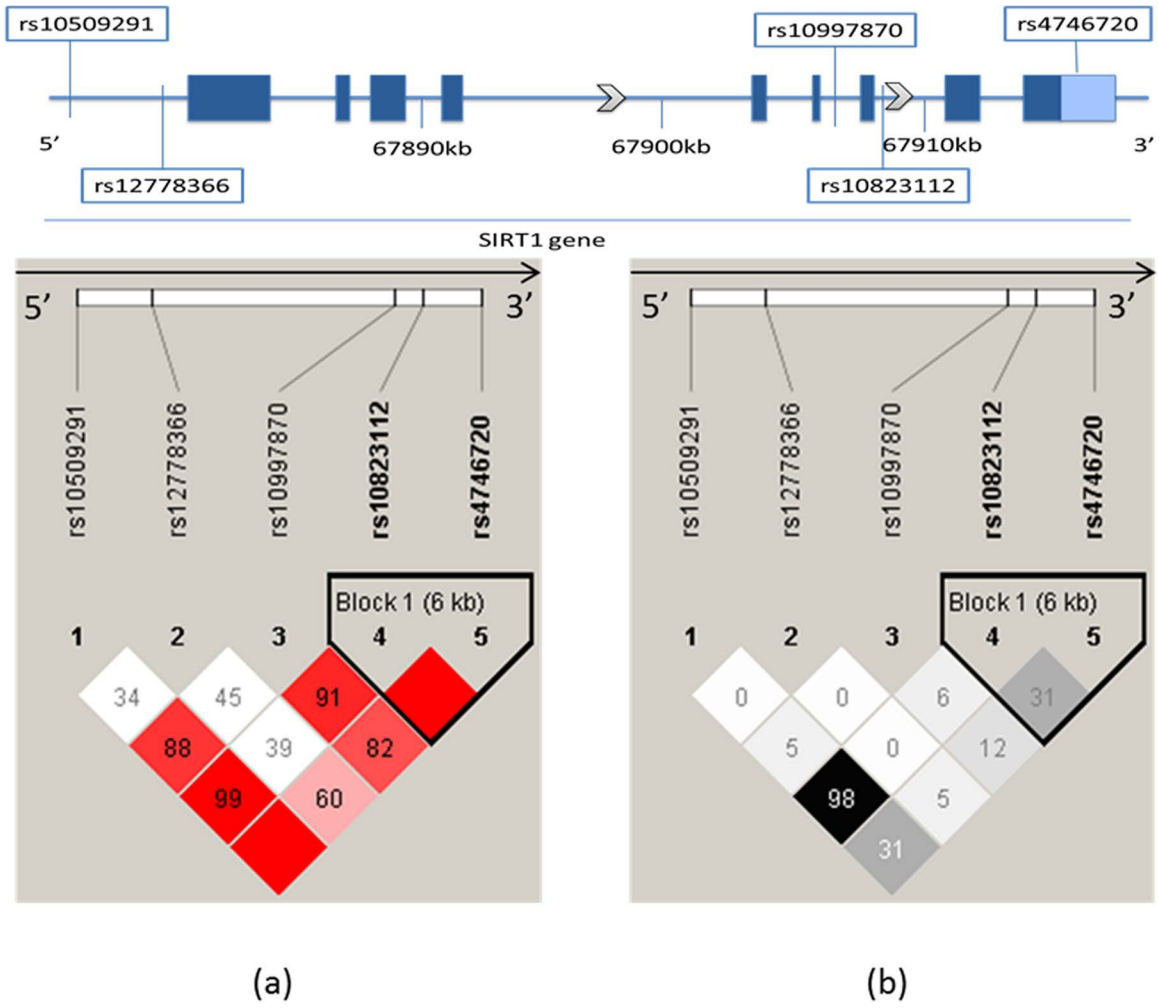

Figure 2. Schematic of *SIRT1* gene structure and Linkage disequilibrium maps for SNPs genotyped in *SIRT1* region. *SIRT1* gene is composed of nine exons and spans 33.7 kb on chromosome 10q21.3. The exons are represented as dark blue boxes. The light blue represents the region of 3′UTR. (a) Shades of red demonstrate the strength of the pairwise linkage disequilibrium based on *D*′, and numbers represent the value of *D*′ expressed as a percentage. (b) Shades of gray show the strength of the pairwise linkage disequilibrium based on r^2, and numbers indicate the value of r^2 expressed as a percentage.

history of diabetes as per questionnaire. Anthropometric data were collected at the time of recruitment. A written informed consent for this study was obtained from all the participants and was approved by the institution review board of Shanghai Jiao Tong University Affiliated Sixth People's Hospital.

Clinical Measurements

All participants underwent a demographic, anthropometric, and biochemical investigation as described previously (12). Briefly, anthropometric parameters such as height, weight, blood pressure, and waist and hip circumference were measured. Body mass index (BMI) and the waist-to-hip ratio (WHR) were calculated using the following formula: BMI = weight (kg)/height $(m)^2$; WHR = abdominal circumference (cm)/hip circumference (cm). For the controls, OGTTs were assessed by standard 75 g glucose in the morning after an overnight fast, and blood samples were obtained at 0 and 2 h during OGTTs. Plasma glucose, serum insulin, and lipid profile were measured. Homeostasis model assessment (HOMA), which was calculated by fasting plasma glucose and insulin, was used for estimating insulin resistance index and β-cell function (13). We used the following formulae: HOMA-IR = FINS (fasting insulin, μIU/ml) × FPG (mmol/L)/22.5; HOMA-β = 20 × FINS (μIU/ml)/FPG (mmol/L) − 3.5.

Single Nucleotide Polymorphism (SNP) Selection and Genotyping

Genomic DNA was isolated from a 2-ml blood sample using a DNA extraction kit. The *SIRT1* gene is encoded in nine exons interrupted by eight introns and spans 33.7 kb

Table 3. Genotype and Allelic Frequencies of *SIRT1* SNP Polymorphism in T2DM Patients and Control Subjects

SNP Sites	Controls	T2DM	*p**	OR (95% CI)	*p*†	*p*‡
Rs10509291						**0.01**
Genotype						
TT [n (%)]	182 (60.5%)	153 (49.3%)	**0.013**	1		
AT [n (%)]	102 (33.9%)	127 (41%)		1.56 (1.09–2.22)	**0.016**	
AA [n (%)]	17 (5.6%)	30 (9.7%)		2.3 (1.17–4.56)	**0.016**	
Allele						
T [n (%)]	466 (77.4%)	433 (69.8%)	**0.003**	1.551 (1.179–2.04)	**0.002**	
A [n (%)]	136 (22.6%)	187 (30.2%)				
Rs12778366						0.1141
Genotype						
TT [n (%)]	232 (77.1%)	217 (70%)	0.077	1		
CT [n (%)]	65 (21.6%)	83 (26.8%)		1.31 (0.88–1.95)	0.183	
CC [n (%)]	4 (1.3%)	10 (3.2%)		3.23 (0.873–11.953)	0.079	
Allele						
T [n (%)]	529 (87.9%)	517 (83.4%)	**0.028**	1.433 (1.05–2.024)	**0.041**	
C [n (%)]	73 (12.1%)	103 (16.6%)				
Rs10997870						0.9988
Genotype						
GG [n (%)]	206 (68.4%)	218 (70.3%)	0.639	1		
TG [n (%)]	87 (28.9%)	81 (26.1%)		0.958 (0.655–1.402)	0.827	
TT [n (%)]	8 (2.7%)	11 (3.5%)		1.287 (0.493–3.357)	0.606	
Allele						
G [n (%)]	499 (82.9%)	517 (83.4%)	0.819	1.021 (0.744–1.402)	0.896	
T [n (%)]	103 (17.1%)	103 (16.6%)				
Rs10823112						**0.015**
Genotype						
AA [n (%)]	181 (60.1%)	153 (49.4%)	**0.017**	1		
GA [n (%)]	104 (34.6%)	129 (41.6%)		1.546 (1.08–2.213)	**0.017**	
GG [n (%)]	16 (5.3%)	28 (9%)		2.218 (1.099–4.474)	**0.026**	
Allele						
A [n (%)]	466 (77.4%)	435 (70.2%)	**0.004**	1.515 (1.152–1.994)	**0.003**	
G [n(%)]	136 (22.6%)	185 (29.8%)				
Rs4746720						0.12
Genotype						
CC [n(%)]	79 (26.2%)	54 (17.4%)	**0.029**	1		
CT [n(%)]	140 (46.5%)	165 (53.2%)		1.82 (1.175–2.82)	**0.007**	
TT [n(%)]	82 (27.2%)	91 (29.4%)		1.81 (1.112–2.947)	**0.017**	
Allele						
C [n (%)]	298 (49.5%)	273 (44%)	0.055	1.37 (1.037–1.674)	**0.024**	
T [n (%)]	304 (50.5%)	347 (56%)				

p values <0.05 are shown in bold.
*Chi-square test.
†Logistic regression analysis after adjustment with age, sex, and BMI.
‡*p* values after Bonferroni correction.

on chromosome 10q21.3. In the present study, five tagging SNPs were selected according to the HapMap Phase III (release 27) Han Chinese database (http://www.hapmap.org/) using the threshold of $r^2 \geq 0.8$, which stretched 10 kb in the upstream to 10 kb in the downstream of the *SIRT1* gene region (Fig. 1). The five tagging SNPs could tag 100% SNPs (five SNPs out of 21 SNPs in the HapMap Chinese Han sample) with a minor allele frequency (MAF) of >0.05. Rs10509291 and rs12778366 are located in the putative promoter region of the *SIRT1* gene; rs10997870 is located in intron 6, and rs10823112 is located in intron 7 of the *SIRT1* gene, while rs4746720 lies in the 3′ untranslated region (3′UTR). Among the SNPs, four of them (rs12778366, rs10997870, rs10823112, rs4746720) were genotyped using the primer extension of multiplex products with detection by the Snapshot assay according to the standard Applied Biosystems Snapshot Multiplex Kit protocol (14). Rs10509291 was genotyped by direct DNA sequencing methods. The designed primers are listed in Table 1. Randomly selected samples were validated by

direct sequencing using ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA) to exclude any genotyping error from Snapshot assay and for quality control. Results of the Snapshot assay and DNA sequencing analysis were 100% concordant.

Statistical Analysis

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL, USA). The Hardy–Weinberg equilibrium test was performed by chi-square test in the cases and controls separately for each variant before association analysis. SNPs that failed this test ($p < 0.01$ in the controls) should be excluded. Pairwise linkage disequilibrium including $|D'|$ and r^2 was estimated using Haploview (version 4.2) (15). Continuous data were shown as mean ± standard deviation (±SD) if normally distributed, or as medians (interquartile range) if nonnormally distributed. Differences in clinical characteristics among the groups were analyzed using two-tailed Student's *t*-tests, Mann–Whitney *U*-test, or ANOVA where appropriate. Nonnormally distributed variables were subjected to natural logarithm transformation to obtain a normal distribution prior to statistical analysis. Differences in allele and genotype frequencies between T2DM and normal controls were analyzed using Pearson's chi-square test. Logistic regression analysis was performed to calculate genotype and allele-specific odds ratio (OR) with 95% confidential intervals (CIs) after adjustment with age, sex, and BMI as covariates. In all hypothesis tests, two-tailed values of $p < 0.05$ were considered statistically significant. Multiple testing correction was performed by Bonferroni correction, which was made for *p* value for the results of any SNP by multiplying the number of SNPs.

RESULTS

Clinical Characteristics of Subjects

Anthropometric and biochemical characteristics of all participants are summarized in Table 2.

There was no significant difference in age and sex between the two groups. However, T2DM patients had significantly higher BMI, WHR, systolic blood pressure (SBP), diastolic blood pressure (DBP), HbA1c, triglycerides, total cholesterol, fasting glucose, and fasting insulin levels compared with control subjects. In contrast, high-density lipoprotein cholesterol (HDL-C) and HOMA-β were lower in the T2DM group compared with controls. As expected, T2DM patients have insulin resistance compared to controls, as evaluated by HOMA-IR.

Association of SIRT1 *Gene Polymorphisms With T2DM*

All five SNPs were in Hardy–Weinberg equilibrium for control groups ($p = 0.59$, 0.82, 0.74, 0.83, 0.23, respectively). Pairwise linkage disequilibrium indicated that these five SNPs were in modest linkage disequilibrium and formed one haplotype block (rs10823112 and rs4746720) in this region (Fig. 2). There were significant differences in allele frequency and genotype distribution between the control and T2DM groups at rs10509291, rs12778366, and rs10823112 for allele by using chi-square test (Table 3). Logistic regression analysis, adjusting for age, gender, and BMI, resulted in a significant risk association at rs10509291 [$p = 0.002$; OR (95% CI) = 1.551 (1.179–2.04) for genotype], rs10823112 [$p = 0.003$; OR (95% CI) = 1.515 (1.152–1.994) for genotype], and rs4746720 ($p = 0.024$; OR (95% CI) = 1.37 (1.037–1.674) for genotype] and between two groups in an additive model. However, the result of rs4746720 was no longer significant after correction for multiple testing (*p* after Bonferroni correction = 0.12). For the haplotype analysis, by comparing the frequencies between the patients and control subjects, we found that haplotype GT in a block comprised by rs10823112–rs4746720 indicated a significant association with type 2 diabetes ($p = 0.004$). After adjusting for multiple testing, the statistical significance was still significant (*p* after Bonferroni correction = 0.02, Table 4).

Association Analyses of the rs10509291 Genotype With Clinical Characteristics

In addition, we further found that rs10509291 and rs10823112 were in almost completely linkage disequilibrium ($D' = 0.99$, $r^2 = 0.98$) in this study. So we analyzed the effect of the SNP rs10509291 on clinical characteristics under an additive model. As shown in Table 5, linear regression analyses adjusting for age, gender, and BMI showed that HbA1c and HOMA-IR were higher in subjects with rs10509291 AA genotype than those with TT genotype in the T2DM group ($p = 0.045$, $p = 0.035$, respectively).

DISCUSSION

In the current study, we genotyped five tagging SNPs in the *SIRT1* region on type 2 diabetes in a Chinese

Table 4. Associations of One Haplotype in *SIRT1* Region With Type 2 Diabetes

	Haplotype Frequencies			
Haplotype	Controls	T2DM	*p**	*p*†
Block (rs10823112–rs4746720)				
AC	0.495	0.44	0.055	0.12
AT	0.279	0.261	0.484	0.93
GT	0.226	0.299	**0.004**	**0.02**

p values <0.05 are shown in bold.
*Chi-square test.
†*p* values after Bonferroni correction.

Table 5. Association Analyses of the rs10509291 Genotype With Clinical Characteristics

	Controls					T2DM				
	TT ($n=182$)	TA ($n=102$)	AA ($n=17$)	p	p*	TT ($n=153$)	TA ($n=127$)	AA ($n=30$)	p	p*
Age (years)	52.33±6.65	52.38±7.29	53.41±6.26	0.660	/	54.8±11.5	52.8±11.4	51.1±7.27	0.104	/
BMI (kg/m^2)	23.61±4.37	23.66±3.87	23.19±1.64	0.393	/	22.29±8.40	23.3±7.71	23.44±8.76	0.852	/
Fasting glucose (mmol/L)	5.33±0.46	5.21±0.38	5.3±0.36	0.205	0.195	8.83±2.89	8.57±3.06	9.06±2.69	0.929	0.866
HbA1c (%)	5.43±0.37	5.53±0.27	5.58±0.3	0.071	0.057	7.91±1.93	8.21±1.78	8.62±1.95	**0.04**	**0.045**
Triglycerides (mmol/L)	1.55±1.37	1.49±0.94	1.26±0.56	0.366	0.346	2.46±2.47	2.19±2.52	2.18±1.58	0.397	0.254
TC (mmol/L)	4.89±0.1	4.7±0.85	4.97±0.89	0.348	0.344	5.14±2.79	5.21±2.16	4.76±0.81	0.675	0.641
HDL-C (mmol/L)	1.21±0.29	1.24±0.33	1.32±0.33	0.199	0.179	1.08±0.2	1.11±0.37	1.04±0.28	0.897	0.776
LDL-C (mmol/L)	3.06±0.73	2.93±0.68	3.06±0.68	0.370	0.386	3.08±1.01	3.21±0.91	3.01±0.86	0.733	0.967
Fasting insulin (μU/mL)	6.90 (4.72–8.62)	6.36 (4.22–8.93)	5.94 (4.48–8.95)	0.169	0.215	10.54 (5.94–17.4)	10.24 (6.72–14.74)	12.86 (7.74–19.06)	0.511	0.578
HOMA-IR	1.61 (1.11–2.06)	1.51 (0.95–2.18)	1.43 (1.09–1.86)	0.140	0.174	3.03 (1.95–5.06)	3.45 (2.19–5.35)	4.94 (2.59–6.77)	**0.037**	**0.035**
HOMA-β	76.91 (51.53–102.73)	76.35 (57.89–105.25)	72.89 (45.34–83.13)	0.317	0.408	42.18 (27.49–76.86)	49.39 (27.57–104.80)	60.94 (28.56–77.38)	0.494	0.468

Data are shown as mean±SD or median (interquartile range). TC, total cholesterol; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA-IR, homeostasis assessment model of insulin resistance; HOMA-β, homeostasis assessment model of β-cell function. p values <0.05 are shown in bold.
*Adjusted for age, gender, and BMI.

population. There was an association between two *SIRT1* tag SNP genotypes (rs10509291, rs10823112) and type 2 diabetes. The SNP analysis demonstrated that the A allele of rs10509291 and G allele of rs10823112 had significantly higher frequency in the T2DM group than in control subjects. However, we observed in this study that rs10509291 can completely tag rs10823112. The association of carriers of rs10509291AT with modestly higher risk of type 2 diabetes suggests a dominator semidominant role of the rs10509291A allele in affecting the phenotype. Also, the AA genotype increased the risk of T2DM with an OR of 2.3, and the OR of A allele to T allele was 1.551, indicating that the risk A allele was related with the susceptibility of T2DM in Chinese population.

SIRT1 is a nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase that plays a highly conserved role in modulating insulin signaling, the regulation of the metabolic rate, control of insulin sensitivity, fat storage and metabolism, and glucose utilization (7), providing evidence that *SIRT1* may play an important role in the pathogenesis of T2DM. For glucose homeostasis, in part by pancreatic β-cells, *SIRT1* is proposed to act as a potential master switch in the modulation of insulin secretion (11,13) and in the regulation of the activity of transcription factors and transcription coregulators (14). *SIRT1* regulates insulin secretion by repressing uncoupling protein 2 (UCP2) (15). It affects glucose metabolism in liver cells by activating the transcription coactivator PGC1a with a subsequent expression of gluconeogenic genes and a repression of glycolytic genes (8). *SIRT1* has been suggested to regulate several downstream genes (9) to maintain glucose homeostasis in the body.

Previous studies have examined the association of T2DM with *SIRT1* SNP variants in distinct ethnic populations. Two tag SNPs, rs10509291 and rs7896005, were nominally associated with type 2 diabetes in Pima Indians (10), which showed that the major (T) allele of rs10509291 was associated with increased risk for diabetes [$p = 0.01$, OR (95% CI) = 1.25 (1.05–1.48), adjusted for age, sex, birth year, and family membership]. Most of the variation in *SIRT1* falls into a single LD block (Fig. 1), which could suggest that conservation of this master regulator is essential for survival. *SIRT1* has been shown to influence energy intake and possibly contribute to obesity in European Caucasians (16,17). We did not observe an association between the *SIRT1* SNPs and BMI in Chinese Han population, which could be due to different lifestyle or environmental influences interacting with genotype. However, a case-control study in a Chinese population showed that three nucleotide variants of the *SIRT1* gene were associated with high body weight including rs10509291 (18). In this study, after adjusting for BMI, there was a strong association between *SIRT1* gene polymorphisms and type 2 diabetes, suggesting that *SIRT1* might interact with type 2 diabetes independent of BMI. This result is consistent with a study that also did not observe an association between the *SIRT1* SNPs and BMI or percent body fat in Pima Indians (19). In another cohort study, minor allele carriers of two genetic *SIRT1* variants who had been exposed to famine in utero had a 50% lower risk of developing diabetes than noncarriers but had a higher BMI, which also support *SIRT1* gene interacting with T2DM independent of BMI (20). Our study showed that HOMA-IR were higher in subjects with rs10509291 AA genotype than those with TT genotype in T2DM group. Insulin resistance is a defining pathophysiologic feature of T2DM. *SIRT1* is implicated in the regulation of glucose metabolism and insulin sensitivity. Hepatic *SIRT1* deficiency in mice impairs mTORC2/Akt signaling and results in hyperglycemia, oxidative damage, and insulin resistance (21), while overexpression of *SIRT1* in mice hepatic cells attenuates endoplasmic reticulum stress and insulin resistance in the liver (22).

In spite of the small number of participants examined in this case-control study, we were able to confirm the effect of *SIRT1* gene variants on T2DM risk among Chinese Han population. The strength of this study was that the diagnosis of all participants was based on standard 75 g OGTT in this study. Large studies with new strategies, other than the classic case-control study design, are required to consolidate our preliminary results in the future. This might have raised risk allele frequencies of *SIRT1* gene variants and made it easier to detect.

In summary, genetic variation of the *SIRT1* gene is related to the pathogenesis of T2DM in Chinese Han population. The allele A at *SIRT1* rs10509291 increased risk of T2DM. However, further functional studies are required to confirm its effect in cell lines and primary cells.

ACKNOWLEDGMENTS: This work was funded by the National Natural Science Foundation of China Project (81370956) to Li Wei, Shanghai Jiao Tong University Wuxi Research Institute Operating Grant (2011JDZX021) to Li Wei, and National Natural Science Foundation of China Project (81200564) to Junfeng Han. The authors declare no conflicts of interest.

REFERENCES

1. American DiabetesAssociation. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2010; 33(Suppl 1):S62–S69.
2. Yang W, Liu J, Shan Z, Tian H, Zhou Z, Ji Q, et al. Acarbose compared with metformin as initial therapy in patients with newly diagnosed type 2 diabetes: An open-label, non-inferiority randomised trial. Lancet Diabetes Endocrinol 2014; 2(1):46–55.
3. Terauchi Y, Takamoto I, Kubota N, Matsui J, Suzuki R, Komeda K, et al. Glucokinase and IRS-2 are required for compensatory beta cell hyperplasia in response to high-fat diet-induced insulin resistance. J Clin Invest 2007; 117(1):246–257.
4. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, et al. Twelve type 2 diabetes susceptibility

loci identified through large-scale association analysis. Nat Genet 2010; 42(7):579–589.
5. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 2004; 303(5666):2011–2015.
6. Moynihan KA, Grimm AA, Plueger MM, Bernal-Mizrachi E, Ford E, Cras-Méneur C, et al. Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice. Cell Metab 2005; 2(2):105–117.
7. Yang T, Fu M, Pestell R, Sauve AA. SIRT1 and endocrine signaling. Trends Endocrinol Metab 2006; 17(5):186–191.
8. Billings LK, Florez JC. The genetics of type 2 diabetes: What have we learned from GWAS? Ann NY Acad Sci 2010; 1212:59–77.
9. Dali-Youcef N, Lagouge M, Froelich S, Koehl C, Schoonjans K, Auwerx J. Sirtuins: The 'magnificent seven', function, metabolism and longevity. Ann Med 2007; 39(5):335–345.
10. Dong Y, Guo T, Traurig M, Mason CC, Kobes S, Perez J, et al. SIRT1 is associated with a decrease in acute insulin secretion and a sex specific increase in risk for type 2 diabetes in Pima Indians. Mol Genet Metab 2011; 104(4):661–665.
11. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998; 15(7):539–553.
12. Jia WP, Pang C, Chen L, Bao YQ, Lu JX, Lu HJ, et al. Epidemiological characteristics of diabetes mellitus and impaired glucose regulation in a Chinese adult population: The Shanghai Diabetes Studies, a cross-sectional 3-year follow-up study in Shanghai urban communities. Diabetologia 2007; 50:286–292.
13. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28(7):412–419.
14. Hurst CD, Zuiverloon TC, Hafner C, Zwarthoff EC, Knowles MA. A Snapshot assay for the rapid and simple detection of four common hotspot codon mutations in the PIK3CA gene. BMC Res Notes 2009; 2:66.
15. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21(2):263–265.
16. Weyrich P, Machicao F, Reinhardt J, Machann J, Schick F, Tschritter O, et al. SIRT1 genetic variants associate with the metabolic response of Caucasians to a controlled lifestyle intervention—The TULIP Study. BMC Med Genet 2008; 9:100.
17. Clark SJ, Falchi M, Olsson B, Jacobson P, Cauchi S, Balkau B, et al. Association of sirtuin 1 (SIRT1) gene SNPs and transcript expression levels with severe obesity. Obesity (Silver Spring) 2012; 20(1):178–185.
18. Zheng J, Chen LL, Xiao F, Hu X, Deng X, Li H. Three single nucleotide variants of the SIRT1 gene are associated with overweight in a Chinese population: A case control study. Endocr J 2012; 59(3):229–237.
19. Dong Y, Guo T, Traurig M, Mason CC, Kobes S, Perez J, et al. SIRT1 is associated with a decrease in acute insulin secretion and a sex specific increase in risk for type 2 diabetes in Pima Indians. Mol Genet Metab 2011; 104:661–665.
20. Botden IP, Zillikens MC, de Rooij SR, Langendonk JG, Danser AH, Sijbrands EJ, et al. Variants in the SIRT1 gene may affect diabetes risk in interaction with prenatal exposure to famine. Diabetes Care 2012; 35:424–426.
21. Wang RH, Kim HS, Xiao C, Xu X, Gavrilova O, Deng CX. Hepatic Sirt1 deficiency in mice impairs mTorc2/Akt signaling and results in hyperglycemia, oxidative damage, and insulin resistance. J Clin Invest 2011; 121:4477–4490.
22. Li Y, Xu S, Giles A, Nakamura K, Lee JW, Hou X, et al. Hepatic overexpression of SIRT1 in mice attenuates endoplasmic reticulum stress and insulin resistance in the liver. FASEB J 2011; 25:1664–1679.