



Research Article

Genetic Variants of *SIRT1* Gene Promoter in Type 2 Diabetes

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Type 2 diabetes (T2D) is a highly heterogeneous and polygenic disease. To date, genetic causes and underlying mechanisms for T2D remain unclear. *SIRT1*, one member of highly conserved NAD-dependent class III deacetylases, has been implicated in many human diseases. Accumulating evidence indicates that *SIRT1* is involved in insulin resistance and impaired pancreatic β -cell function, the two hallmarks of T2D. Thus, we speculated that altered *SIRT1* levels, resulting from the genetic variants within its regulatory region of *SIRT1* gene, may contribute to the T2D development. In this study, the *SIRT1* gene promoter was genetically analyzed in T2D patients ($n = 218$) and healthy controls ($n = 358$). A total of 20 genetic variants, including 7 single-nucleotide polymorphisms (SNPs), were identified. Five heterozygous genetic variants (g.4114-15InsA, g.4801G > A, g.4816G > C, g.4934G > T, and g.4963_64Ins17bp) and one SNP (g.4198A > C (rs35706870)) were identified in T2D patients, but in none of the controls. The frequencies of two SNPs (g.4540A > G (rs3740051) (OR: 1.75, 95% CI: 1.24–2.47, $P < 0.001$ in dominant genetic model) and g.4821G > T (rs35995735)) (OR: 3.58, 95% CI: 1.94–6.60, $P < 0.001$ in dominant genetic model) were significantly higher in T2D patients. Further association and haplotype analyses confirmed that these two SNPs were strongly linked, contributing to the T2D (OR: 1.442, 95% CI: 1.080–1.927, $P < 0.05$). Moreover, most of the genetic variants identified in T2D were disease-specific. Taken together, the genetic variants within *SIRT1* gene promoter might contribute to the T2D development by altering *SIRT1* levels. Underlying molecular mechanism needs to be further explored.

1. Introduction

Type 2 diabetes (T2D) is a highly heterogeneous and polygenic disease. Dysfunction of pancreatic β cells and insulin resistance in tissues are involved in the pathophysiology of T2D [1, 2]. Candidate gene association, linkage, and genome-wide association studies have identified large number of genetic loci and gene variants for T2D. A highly polygenic architecture of T2D has been established, which is dominated by common alleles with small and cumulative effects [3]. Although a small proportion of T2D cases can be explained, genetic causes and underlying molecular mechanisms of T2D remain largely unknown [4, 5]. Rare and low-frequency genetic variants that

modulate β -cell mass and function may account for the missing inheritance for T2D [6].

Sirtuins are NAD-dependent protein deacetylases and broadly regulate many cellular processes, including cell fate determination, DNA damage repair, cellular protection, calorie restriction, and energy metabolism. Sirtuins have been implicated in age-related diseases, such as cancer, diabetes, and cardiovascular and neurodegenerative diseases [7–10]. There are seven members in the mammalian sirtuin family, *SIRT1*–*7*. *SIRT1* gene is highly expressed in metabolically active tissues, including the liver, muscle, adipose tissue, heart, pancreas, and brain. *SIRT1* regulates glucose and lipid metabolism, mitochondrial biogenesis, stress responses, inflammation, autophagy, circadian rhythms, and

chromatin silencing [11]. In addition, SIRT1 is involved in the epigenetic regulation in the differentiation of the human stem cells [12, 13].

SIRT1 has been involved in insulin resistance and impaired β -cell function, which are the hallmarks of T2D [14]. In experimental animals, SIRT1 regulates insulin secretion and protects pancreatic β -cells against toxic stresses [15, 16]. In mouse pancreatic beta cells, loss of SIRT1 leads to impaired glucose sensing and insulin secretion [17]. SIRT1 ameliorates insulin resistance by repressing protein tyrosine phosphatase 1B, a major negative regulator of insulin action [18]. In a T2D rat model, SIRT1 regulates glucose homeostasis and insulin sensitivity [19]. SIRT1 improves insulin sensitivity in skeletal muscle and liver [20, 21]. In cultured 3T3-L1 adipocytes and human adipose tissues, SIRT1 functions as a suppressor of inflammation, which is strongly associated with insulin resistance [22, 23]. Therefore, SIRT1 plays an important role in the T2D development.

The human *SIRT1* gene has been mapped to chromosome 10q21.3 [24]. The expression of the *SIRT1* gene is strictly controlled at transcription level. Hypermethylated in cancer 1 (HIC1), a transcriptional repressor, directly binds the *SIRT1* gene promoter and represses its transcription [25]. P53 has been shown to upregulate *SIRT1* gene expression by binding to a P53-binding element [26]. E2F1, a cell cycle and apoptosis regulator, induces the expression of the *SIRT1* gene [27]. Proinflammatory cytokine interferon gamma IFN- γ represses *SIRT1* gene expression [28]. In human pancreatic islet cells, SIRT1 is induced by gamma-aminobutyric acid (GABA), which protects pancreatic beta cells against apoptosis [29]. *SIRT1* gene is also regulated by extracellular-signal-regulated kinase 5 in leukemic Jurkat T cells [30].

Dysregulation of gene expression has been implicated in human diseases [31]. Variations in *SIRT1* gene expression levels have been associated with obesity and T2D [32–34]. *SIRT1* gene expression in circulating peripheral blood mononuclear cells is significantly associated with abdominal visceral fat accumulation [35]. In human adipose tissue, SIRT1 mRNA expression is significantly associated with energy expenditure and insulin sensitivity [36]. Therefore, we postulated that altered *SIRT1* gene expression levels, caused by the genetic variants within its regulatory regions, may contribute to the T2D development. Identification and subsequent functional analysis of genetic variants in *SIRT1* gene associated with T2D may provide a basis for manipulating *SIRT1* gene expression with genetic approaches or pharmaceutical chemicals as potential therapies for T2D patients. In the present study, the promoter region of the *SIRT1* gene was genetically analyzed in cohorts of T2D patients and controls.

2. Materials and Methods

2.1. Study Subjects. All T2D patients ($n=218$, mean age: 52.44 years), including 123 males and 95 females, were recruited from the Division of Endocrinology, Affiliated Hospital of Jining Medical University, Jining Medical

University, Jining, Shandong, China. T2D patients were diagnosed according to the American Diabetes Association guideline (2014) with fasting plasma glucose >7.0 mmol/L, 2-hour plasma glucose level >11.1 mmol/L, and glycated hemoglobin A1c $>6.5\%$. Subjects with type 1 diabetes and other metabolic or endocrinological diseases were excluded from this study. The healthy controls ($n=358$, mean age: 52.76 years), including 206 males and 152 females, were recruited from Physical Examination Center in the same hospital. Subjects with family history of T2D were excluded. This study was approved by the Human Ethics Committee of Affiliated Hospital of Jining Medical University. Informed consent was obtained from all participants. According to the power calculations for genetic association studies, more than 200 cases were included to eliminate the bias in different genetic models in this study [37–39].

2.2. Genetic Analysis. Peripheral leukocytes were isolated and genomic DNAs were extracted with DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). The *SIRT1* gene promoter region, from -1051 bp upstream to $+57$ bp downstream to the transcription start site, was analyzed. Two overlapped DNA fragments, 551 bp and 592 bp, were amplified by PCR and directly sequenced. PCR primers were designed with the genomic sequence of human *SIRT1* gene (GenBank accession number: NG_050664.1). The 551 bp fragments (-1051 bp~ -501 bp) were generated with the PCR primers SIRT1-F1 (5'-GGAGTCACAGTGTGCCAGAA-3') and SIRT1-R1 (5'-TTTCCCCTCTCCTCACACC-3'). The 592 bp fragment (-535 bp~ $+57$ bp) was generated with the PCR primers SIRT1-F2 (5'-AGGAGCTGTCAGAACGGTGT-3') and SIRT1-R2 (5'-CCATCTTCCAACCTGCCTCTC-3'). DNA sequencing was performed with 3730 DNA Analyzer (Applied Biosystems, Foster city, CA, USA). DNA sequences were aligned and compared with wild type *SIRT1* gene promoter. For heterozygous insertion or deletion genetic variants, the *SIRT1* gene promoter regions were subcloned into T-vector and directly sequenced. All genetic variants were further confirmed with PCR-generated DNA fragments and direct sequencing.

2.3. Statistical Analysis. Distributions of genetic variants were compared between T2D patients and controls using SPSS v13.0. The frequency of single-nucleotide polymorphisms (SNPs) in T2D and control groups was tested for deviation from Hardy–Weinberg equilibrium (HWE) by Fisher's test. Pearson chi-squared test was performed to evaluate the significant differences on allele and genotype frequencies between T2D patients and controls. The statistical power was generally set as 80% for determining the sample size that may yield the acceptable probability estimates. Odds ratio (OR) values and 95% confidence intervals (CIs) were measured using unconditional logistic regression analysis. The associations in different genetic models (co-dominant, dominant, over-dominant, and recessive) were analyzed with web-based software SNPStats. Linkage disequilibrium (LD) analysis and haplotype associations were conducted with Haploview software package (version 4.2)

and SHEsis software platform. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Genetic Variants Identified in T2D Patients and Controls. A total of 20 genetic variants, including 7 SNPs, were identified in this study population. Distribution and locations of the genetic variants are summarized in Table 1 and Figure 1(a). Five heterozygous genetic variants (g.4114-15InsA, g.4801G > A, g.4816G > C, g.4934G > T, and g.4963_64Ins17bp) and one SNP (g.4198A > C (rs35706870)) were identified in 16 T2D patients, but in none of the controls. More strikingly, the heterozygous insertion genetic variant (g.4963_64Ins17bp) was found in 11 T2D patients. The chromatograms of these genetic variants are depicted in Figure 1(b). Two SNPs (g.4540A > G (rs3740051) and g.4821G > T (rs35995735)) were more significantly frequent in T2D patients compared to controls ($P < 0.01$). In contrast, five heterozygous genetic variants (g.4153G > A, g.4794G > A, g.4800G > A, g.4859A > G, and g.4932G > A) and one SNP (g.4981G > A (rs575321146)) were only found in controls. Two SNPs (g.4798A > C (rs932658) and g.4922G > C (rs2394443)) were significantly more frequent in controls compared to T2D patients, respectively ($P < 0.01$ and $P < 0.05$). In addition, two heterozygous genetic variants (g.4714G > C and g.4807C > T) and two SNPs (g.4916A > G (rs3740053) and g.4981G > T (rs575321146)) were found in both T2D patients and controls with similar frequencies ($P > 0.05$).

3.2. Association between SIRT1 Gene SNPs and T2D Risk. Seven SNPs were identified in this study, among which five SNPs were found with high frequency. Genotype distributions of the five SNPs in T2D patient and control groups (rs3740051, $P = 0.378$; rs932658, $P = 0.263$; rs35995735, $P = 0.095$; rs3740053, $P = 0.542$; rs2394443, $P = 0.263$) were in HWE ($P > 0.05$). Distributions of genotypic and allelic frequencies of each SNP are shown in Table 2. The results showed that SNPs (g.4540A > G (rs3740051) and g.4821G > T (rs35995735)) were statistically associated with T2D.

Distributions of A/A, A/G, and G/G genotypes in SNP rs3740051 were 51.8%, 43.1%, and 5.0% in the T2D patient group and 65.4%, 31.8%, and 2.8% in the control group, respectively. There were significant associations between genotype frequency and distribution with the T2D patient group in codominant, dominant, and over-dominant models ($P = 0.005$, $P < 0.001$, and $P = 0.006$). The G allele frequency of rs3740051 was higher in the T2D patient group (26.6%) than in the control group (18.7%) ($P = 0.002$).

Distributions of G/G, G/T, and T/T genotypes in SNP rs35995735 were 84.9%, 15.1%, and 0.0% in the T2D patient group and 95.3%, 4.5%, and 0.2% in the control group, respectively. There were significant associations between genotype frequency and distribution with the T2D patient group in codominant, dominant, and over-dominant models ($P < 0.001$, $P < 0.001$, and $P < 0.001$). The T allele

frequency of rs35995735 was higher in the T2D patient group (7.6%) than in the control group (2.5%) ($P < 0.001$).

In addition, distributions of A/A, A/G, and G/G genotypes in SNP rs3740053 were 52.8%, 41.7%, and 5.5% in the T2D patient group and 61.7%, 34.4%, and 3.9% in the control group, respectively. There was a significant association between genotype frequency and distribution with the T2D patient group in the dominant model ($P = 0.34$). The G allele frequency of rs3740053 was higher in the T2D patient group (26.4%) than in the control group (21.1%) ($P = 0.039$).

3.3. Associations between Haplotypes and T2D Risk. To further analyze the association between haplotypes and T2D, we characterized the linkage disequilibrium (LD) of the *SIRT1* gene promoter SNPs in T2D patients and controls. D values and R2 values were examined with Haploview (version 4.2) and SHEsis (Figure 2). Two SNPs (rs35995735 and rs3740053) had no linkage, and all other SNPs showed strong linkage. Furthermore, R2 values showed a strong linkage between SNPs rs3740051 and rs3740053 as well as between SNPs rs932658 and rs2394443. These results further confirmed that SNPs rs3740051 and rs35995735 were associated with T2D.

The haplotypes of the five SNPs (rs3740051, rs932658, rs35995735, rs3740053, and rs2394443) and their frequencies in T2D patients and controls are shown in Table 3. The haplotypes G-A-G-G-G and A-A-T-A-G were associated with T2D ($P < 0.05$ and $P < 0.001$, respectively). The haplotype A-C-G-A-C provided protection from T2D ($P < 0.001$). The most common haplotype A-A-G-A-G was not associated with T2D ($P > 0.05$).

3.4. Disease-Disease-Specificity of Genetic Variants in SIRT1 Gene Promoter. In previous studies, we have identified a number of genetic variants within *SIRT1* gene promoter in patients with acute myocardial infarction (AMI), Parkinson's disease (PD), and ventricular septal defects (VSD) [40–42]. In this study, we identified five genetic variants in T2D patients. These disease-related genetic variants are summarized in Table 4. Most genetic variants were disease-specific, including AMI (g.4198A > C, g.4324_25InsGCTG, g.4420_21InsG, and 0.4484G > C), PD (g.4614C > G, g.4794G > A, and g.4932G > A), VSD (g.4174A > G, g.4544A > T, and g.4552G > A), and T2D (g.4114-15InsA and g.4801G > A). In addition, genetic variants (g.4816G > C and g.4934G > T) were found in both AMI and T2D. Genetic variant (g.4963_64Ins17bp) was found in both VSD and T2D. Taken together, the genetic variants within *SIRT1* gene promoter had disease specificity.

4. Discussion

SIRT1 gene mutations have been reported in type 1 diabetes [43]. Genetic variations in *SIRT1* gene have been related to the risk for obesity [44–46]. In a Dutch population, *SIRT1* gene SNPs are associated with prenatal famine exposure to influence the T2D risk [47]. In Pima Indians, an upstream variant (NC_000010.10: g.69635204T > A, rs10509291) and

TABLE 1: Genetic variants within the *SIRT1* gene promoter in T2D patients and controls.

Genetic variants	Genotypes	Location ¹	T2D (n = 218)	Controls (n = 358)	P value
g.4114-15InsA	-/A	-894 bp	1	0	—
g.4153G > A	GA	-855 bp	0	1	—
g.4198A > C (rs35706870)	AC	-810 bp	1	0	—
g.4540A > G (rs3740051)	AA	-468 bp	113	234	0.005
	AG		94	114	
	GG		11	10	
g.4714G > C	CG	-294 bp	1	2	1.000
g.4794G > A	GA	-214 bp	0	5	—
g.4798A > C (rs932658)	AA	-210 bp	167	230	0.007
	AC		48	118	
	CC		3	10	
g.4800G > A	GG	-208 bp	218	347	0.016
	GA		0	10	
	AA		0	1	
g.4801G > A	GA	-207 bp	1	0	—
g.4807C > T	CT	-201 bp	1	1	—
g.4816G > C	GC	-192 bp	1	0	—
g.4821G > T (rs35995735)	GG	-187 bp	185	341	<0.001
	GT		33	16	
	TT		0	1	
g.4859A > G	AG	-149 bp	0	1	—
g.4916A > G (rs3740053)	AA	-92 bp	115	221	0.099
	AG		91	123	
	GG		12	14	
g.4922G > C (rs2394443)	GG	-86 bp	167	230	0.007
	GC		48	118	
	CC		3	10	
g.4932G > A	GA	-76 bp	0	5	—
g.4934G > T	GT	-74 bp	1	0	—
g.4963_64Ins17bp	-/17 bp	-45 bp	11	0	—
g.4981G > A (rs575321146)	GA	-19 bp	0	1	—
g.4981G > T (rs575321146)	GT	-19 bp	1	1	—

¹Locations of genetic variants upstream (-) to the transcription start site of *SIRT1* gene at 5008 of NG_050664.1. P value: comparison of genotype frequencies between T2D patients and controls.

an intron variant (NC_000010.10: g.69651125A > G, rs7896005) in *SIRT1* gene are associated with reduced insulin secretion and increased risk for T2D [48]. In this study, we analyzed the proximal promoter region of the *SIRT1* gene and found six genetic variants in 7.3% (16/218) of T2D patients. The frequencies of two SNPs (g.4540A > G (rs3740051) and g.4821G > T (rs35995735)) were significantly higher in T2D patients compared to controls. Further genotypes analysis indicated that these two SNPs had strong linkage and were significantly associated with T2D in co-dominant, dominant, and over-dominant models. Collectively, these genetic variants and SNPs may abolish, create, or modify the binding sites for transcription factors within the *SIRT1* gene promoter, which then alter *SIRT1* levels, contributing to the T2D development.

Many downstream targets of *SIRT1* have been identified, including forkhead-box transcription factors (FOXOs), peroxisome proliferator-activated receptor γ (PPAR γ), PPAR γ -coactivator 1 α (PGC-1 α), myogenic differentiation 1, p53, and autophagy-related proteins. *SIRT1* has been involved in insulin signaling by regulating insulin receptor substrate 2 and FOXO3 [49, 50]. In adipocytes, *SIRT1* increases adiponectin gene expression in maintaining energy

homeostasis [51]. In transgenic mice, adiponectin improves insulin sensitivity and acts against inflammation [52]. *SIRT1* deacetylates FOXO1 in liver, adipose tissue, and pancreatic β -cells and protects β -cells against oxidative stress [53]. *SIRT1* is involved in regulating inflammatory responses, gluconeogenesis, and levels of reactive oxygen species, which contribute to the insulin resistance [54, 55]. *SIRT1* forms a complex with FOXA2 to regulate pancreas duodenum homeobox 1 (PDX1) gene, which is essential for pancreas development and β -cell formation [56]. In addition, *SIRT1* induces autophagy by deacetylating autophagy-related (ATG) proteins, such as ATG5, ATG7, and LC3 (microtubule-associated protein 1 light chain 3 alpha) [57]. The crosstalk between *SIRT1* and autophagy has been implicated in obesity and T2D [58]. Therefore, changed *SIRT1* levels may affect pancreatic β -cell functions, insulin signaling, inflammation, autophagy, and other processes, contributing to the T2D development.

Genetic variants in *SIRT1* gene have been associated with many human diseases. T2D and coronary artery disease are closely linked. A number of genetic loci have been identified and shared in both diseases [59]. In patients with coronary artery disease, *SIRT1* gene expression levels are significantly

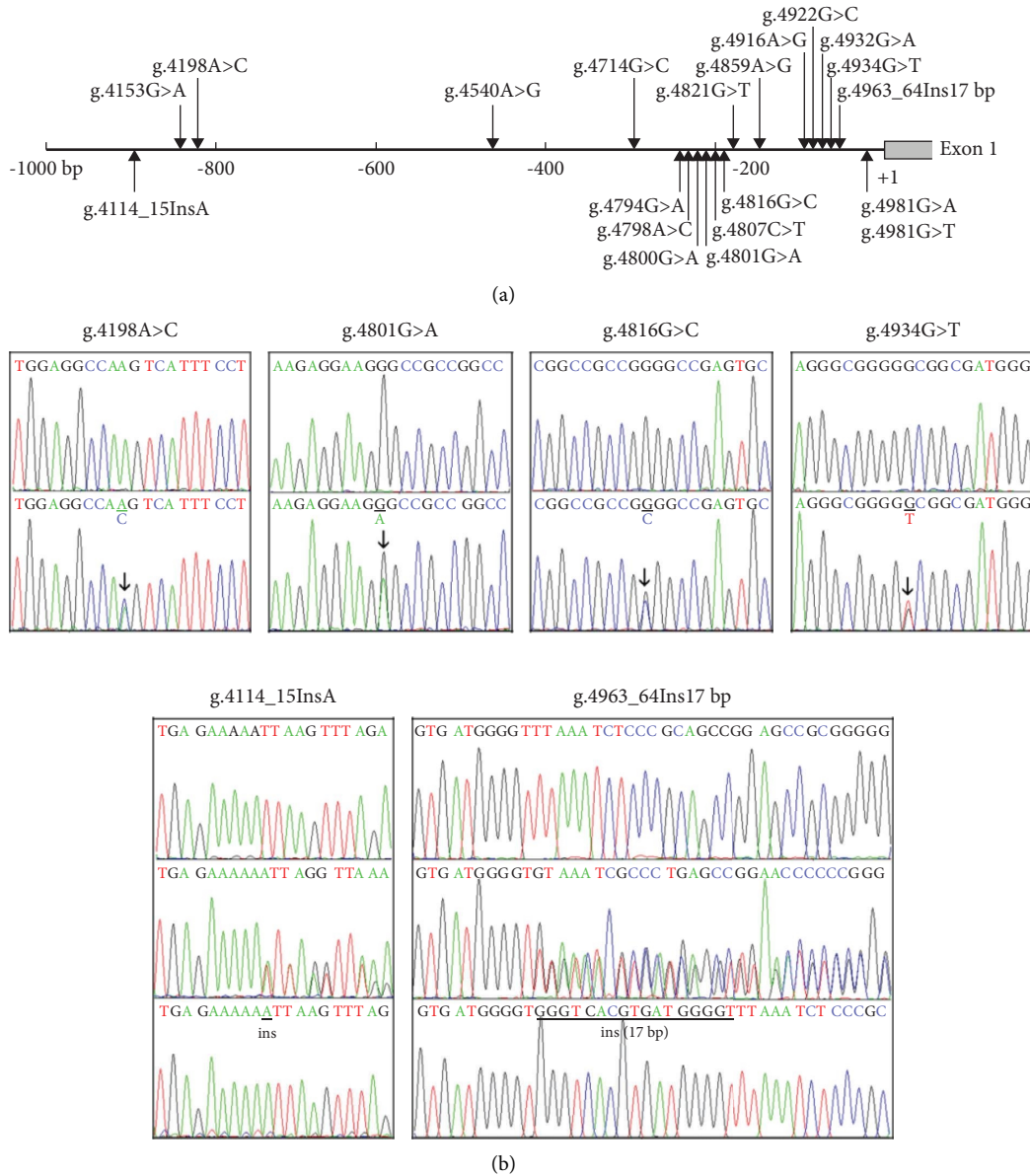


FIGURE 1: Genetic variants within the *SIRT1* gene promoter in T2D patients and controls. (a) Schematic representation of the genetic variants in the *SIRT1* gene promoter. The numbers represent the sequences of *SIRT1* gene promoter (Genbank accession number NG_050664.1). The transcription start site is at the position of 5008 of the first exon. (b) Sequencing chromatograms of the genetic variants which were only identified in T2D patients. These genetic variants were depicted in forward orientations. For genetic variants g.4198A > C (rs35706870), g.4801G > A, g.4816G > C, and g.4934G > T, top panels show the sequencing of wild type and bottom panels show the sequencing of heterozygous variants. For the insertion genetic variants, g.4114-15InsA and g.4963_64Ins17bp, top panels show the sequences of wild type, and middle panels show the sequences of heterozygous variants. To examine the exact insertion sequences, the *SIRT1* gene promoter containing insertion genetic variants are subcloned into T-vectors, and then directly sequenced. The insertion sequences are shown in bottom panels. Heterozygous genetic variants are marked with solid arrows and the sequences of insertion genetic variants are underlined.

decreased [60]. *SIRT1* gene SNPs have been associated with *SIRT1* levels in patients with cardiovascular diseases [61]. In previous studies, we have identified several genetic variants within the *SIRT1* gene promoter in AMI patients, including g.4816G > C and g.4934G > T [40]. In this study, these two genetic variants were also found in T2D patients, providing further evidence that T2D and AMI shared common molecular mechanisms.

This study has limitations. *SIRT1* gene expression was not measured directly with clinical samples due to lack of sample availability. Moreover, the effects of the SNPs (g.4540A > G (rs3740051) and g.4821G > T (rs35995735)) on *SIRT1* gene expression need further study, as have been by previous studies [62, 63]. In addition, the impact of the SNPs on the onset and progression of T2D needs to be investigated.

TABLE 2: Genotype distribution and allele frequencies of the SNPs in T2D patients and controls.

Genetic model	Genotypes	T2D (<i>n</i> = 218) (<i>n</i> (%))	Controls (<i>n</i> = 358) (<i>n</i> (%))	OR	<i>P</i> value
g.4540A > G (rs3740051)					
Codominant	AA	113 (51.8)	234 (65.4)	1.00	0.005
	AG	94 (43.1)	114 (31.8)	1.71 (1.20–2.43)	
	GG	11 (5.0)	10 (2.8)	2.28 (0.94–5.52)	
Dominant	AA	113 (51.8)	234 (65.4)	1.75 (1.24–2.47)	0.001
	AG + GG	105 (48.2)	124 (34.6)		
Recessive	AA + AG	207 (95.0)	348 (97.2)	1.85 (0.77–4.43)	0.162
	GG	11 (5.0)	10 (2.8)		
Over-dominant	AA + GG	124 (56.9)	244 (68.2)	1.62 (1.15–2.30)	0.006
	AG	94 (43.1)	114 (31.8)		
Allele	A	320 (73.4)	582 (81.3)	1.57 (1.19–2.09)	0.002
	G	116 (26.6)	134 (18.7)		
g.4798A > C (rs932658)					
Codominant	AA	167 (76.6)	230 (64.2)	1.00	0.007
	AC	48 (22.0)	118 (33.0)	0.56 (0.38–0.83)	
	CC	3 (1.4)	10 (2.8)	0.41 (0.11–1.52)	
Dominant	AA	167 (76.6)	230 (64.2)	0.55 (0.38–0.80)	0.002
	AC + CC	51 (23.4)	128 (35.8)		
Recessive	AA + AC	215 (98.6)	348 (97.2)	0.49 (0.13–1.78)	0.277
	CC	3 (1.4)	10 (2.8)		
Over-dominant	AA + CC	170 (78.0)	240 (67.0)	0.57 (0.39–0.85)	0.005
	AC	48 (22.0)	118 (33.0)		
Allele	A	382 (87.6)	578 (80.7)	0.59 (0.42–0.83)	0.002
	C	54 (12.4)	138 (19.3)		
g.4821G > T (rs35995735)					
Codominant	GG	185 (84.9)	341 (95.3)	1.00	<0.001
	GT	33 (15.1)	16 (4.5)	3.80 (2.04–7.09)	
	TT	0 (0.0)	1 (0.2)	—	
Dominant	GG	185 (84.9)	341 (95.3)	3.58 (1.94–6.60)	<0.001
	GT + TT	33 (15.1)	17 (4.7)		
Recessive	GG + GT	218 (100.0)	357 (99.8)	—	1.000
	TT	0 (0.0)	1 (0.2)		
Over-dominant	GG + TT	185 (84.9)	342 (95.5)	3.81 (2.04–7.11)	<0.001
	GT	33 (15.1)	16 (4.5)		
Allele	G	403 (92.4)	698 (97.5)	3.18 (1.77–5.71)	<0.001
	T	33 (7.6)	18 (2.5)		
g.4916A > G (rs3740053)					
Codominant	AA	115 (52.8)	221 (61.7)	1.00	0.100
	AG	91 (41.7)	123 (34.4)	1.42 (1.00–2.02)	
	GG	12 (5.5)	14 (3.9)	1.65 (0.74–3.68)	
Dominant	AA	115 (52.8)	221 (61.7)	1.45 (1.03–2.03)	0.034
	AG + GG	103 (47.2)	137 (38.3)		
Recessive	AA + AG	206 (94.5)	344 (96.1)	1.43 (0.65–3.15)	0.371
	GG	12 (5.5)	14 (3.9)		
Over-dominant	AA + GG	127 (58.3)	235 (65.6)	1.37 (0.97–1.94)	0.075
	AG	91 (41.7)	123 (34.4)		
Allele	A	321 (73.6)	565 (78.9)	1.34 (1.02–1.78)	0.039
	G	115 (26.4)	151 (21.1)		
g.4922G > C (rs2394443)					
Codominant	GG	167 (76.6)	230 (64.2)	1.00	0.007
	GC	48 (22.0)	118 (33.0)	0.56 (0.38–0.83)	
	CC	3 (1.4)	10 (2.8)	0.41 (0.11–1.52)	
Dominant	GG	167 (76.6)	230 (64.2)	0.55 (0.38–0.80)	0.002
	GC + CC	51 (23.4)	128 (35.8)		
Recessive	GG + GC	215 (98.6)	348 (97.2)	0.49 (0.13–1.78)	0.277
	CC	3 (1.4)	10 (2.8)		
Over-dominant	GG + CC	170 (78.0)	240 (67.0)	0.57 (0.39–0.85)	0.005
	GC	48 (22.0)	118 (33.0)		

TABLE 2: Continued.

Genetic model	Genotypes	T2D (n = 218) (n (%))	Controls (n = 358) (n (%))	OR	P value
Allele	G	382 (87.6)	578 (80.7)	0.59 (0.42–0.83)	0.002
	C	54 (12.4)	138 (19.3)		

OR: odds ratio. P value: comparison of the genotype frequencies between T2D patients and controls. P values less than 0.05 were shown in bold.

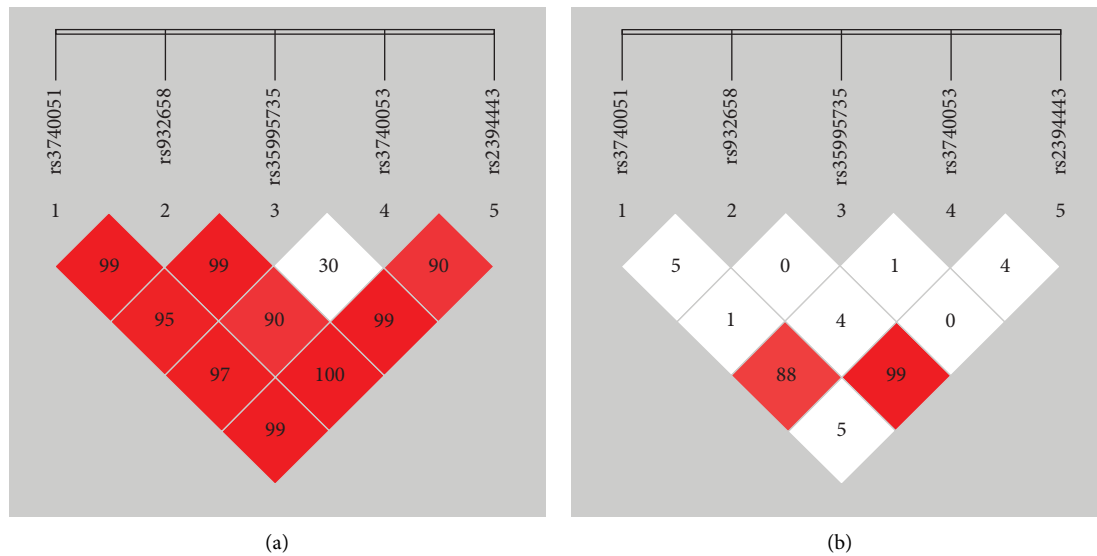


FIGURE 2: Linkage disequilibrium (LD) analysis for the five SNPs. The SNPs rs3740051, rs932658, rs35995735, rs3740053, and rs2394443 were analyzed. (a) Lewontin's standardized coefficient D' values. (b) Square of Pearson's correlation coefficient values (R2). Standard color schemes indicate different levels of LD.

TABLE 3: Haplotype analysis of the *SIRT1* gene SNPs in T2D patients and controls.

Haplotypes (SNPs 1–5)	T2D (n = 218) frequency (%)	Control (n = 358) frequency (%)	Chi2	Fisher's P	OR (95% CI)
A-A-G-A-G	240.14 (0.551)	427.06 (0.596)	2.628	>0.05	0.817 (0.639~1.043)
A-C-G-A-C	47.31 (0.109)	136.93 (0.191)	14.060	<0.01	0.511 (0.358~0.729)
G-A-G-G-G	107.29 (0.246)	131.93 (0.184)	6.186	<0.05	1.442 (1.080~1.927)
A-A-T-A-G	30.53 (0.070)	0.00 (0.000)	51.357	<0.01	—

SNPs 1–5: rs3740051, rs932658, rs35995735, rs3740053, and rs2394443. Frequency: frequencies of haplotypes. OR: odds ratio.

TABLE 4: Summary of genetic variants within the *SIRT1* gene promoters in human diseases.

Genetic variants	Genotypes	Location ¹	AMI ² (n = 327)	PD ³ (n = 97)	VSD ⁴ (n = 333)	T2D ⁵ (n = 218)
g.4114-15InsA	-/A	-894 bp	-	-	-	+
g.4174A > G	AG	-834 bp	-	-	+	-
g.4198A > C	AC	-810 bp	+	-	-	-
g.4324_25InsGCTG	-/GCTG	-684 bp	+	-	-	-
g.4420_21InsG	-/G	-588 bp	+	-	-	-
g.4484G > C	GC	-524 bp	+	-	-	-
g.4544A > T	AT	-464 bp	-	-	+	-
g.4552G > A	GA	-456 bp	-	-	+	-
g.4614C > G	CG	-394 bp	-	+	-	-
g.4794G > A	GA	-214 bp	-	+	-	-
g.4801G > A	GA	-207 bp	-	-	-	+
g.4816G > C	GC	-192 bp	+	-	-	+
g.4932G > A	GA	-76 bp	-	+	-	-
g.4934G > T	GT	-74 bp	+	-	-	+
g.4963_64Ins17bp	-/17 bp	-45 bp	-	-	+	+

¹Locations of variants upstream (-) to the transcription start site of *SIRT1* gene at 5008 of NG_050664.1; ²Cui et al. [40]; ³Zhang et al. [42]; ⁴Shan et al. [41]; ⁵this study. AMI, acute myocardial infarction; PD, Parkinson's disease; VSD, ventricular septal defects; T2D, type 2 diabetes.

In conclusion, we genetically analyzed the promoter region of *SIRT1* gene in T2D patients and controls. The genetic variants identified in T2D patients may contribute to the T2D development by changing SIRT1 levels. As natural and pharmacological compounds have been identified for regulating *SIRT1* gene expression, pharmacological targeting of *SIRT1* gene genetic variants may emerge as a novel therapy for T2D patients.

Abbreviations

AMI:	Acute myocardial infarction
ATG5:	Autophagy-related protein 5
ATG7:	Autophagy-related protein 7
FOX1:	Forkhead-box transcription factor 1
FOX3:	Forkhead-box transcription factor 3
HIC1:	Hypermethylated in cancer 1
LC3:	Microtubule-associated protein 1 light chain 3 alpha
PD:	Parkinson's disease
PGC-1 α :	PPAR γ -coactivator 1 α
PPAR γ :	Peroxisome proliferator-activated receptor γ
T2D:	Type 2 diabetes
VSD:	Ventricular septal defects.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

This study was approved by the Human Ethics Committee of Affiliated Hospital of Jining Medical University.

Consent

The participants provided written informed consent.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

SP, JZ, and BY were responsible for the conception and design of the study. SP, ZZ, and YZ conducted the experiments. SP and JZ performed the statistical analysis. SP and ZZ wrote the first draft of the manuscript. JZ and BY revised the draft of the manuscript. All authors have read and approved the submitted version.

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