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Single nucleotide polymorphisms on SHIP2 is associated with Type 2 diabetes mellitus in Chinese Han population

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Abstract. – OBJECTIVE: Type 2 diabetes mellitus (T2DM) is a chronic disease characterized by insulin resistance in the target tissue of insulin with insufficient insulin secretion in pancreatic β-cells. Src homology 2-containing 5'-inositol phosphatase 2 (SHIP2) is a lipid phosphatase that hydrolyzes PI3-kinase product PI(3,4,5)P3 to PI(3,4)P2, which contributes to the negative regulation of insulin signaling both *in vitro* and *in vivo*. Some polymorphisms of SHIP2 have been reported to be associated with the metabolic syndrome including T2DM and hypertension in British, French and Japanese T2DM population.

PATIENTS AND METHODS: In our present study, we investigated the relation between single nucleotide polymorphisms (SNPs) on SHIP2 gene and the pathogenesis of T2DM in Chinese Han population.

RESULTS AND CONCLUSIONS: Our results indicated that the genotype and allele frequency of SHIP2 (+1893CC/AA) locus in T2DM patients showed significantly different from between the healthy control population. In addition, the G allele of SHIP2 (+2945A/G) seemed to increase the susceptibility to hypertension for T2DM patients.

Key Words:
Single nucleotide polymorphisms, SHIP2, Type 2 diabetes mellitus.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic disease due to endocrine dysfunction that affects millions of people globally and is associated with multiple comorbidities and complications. T2DM is a rapidly growing health concern in both developed and developing nations[1,2]. According to the World Health Organization (WHO), in 2011, approximately 364 million people globally suffer from diabetes mellitus (DM), and it is expected that the number of DM-related deaths will double from 2005 to 2030[3]. In China, the number of patients with T2DM will double in 2030[4].

T2DM is characterized by insulin resistance in the target tissue of insulin with insufficient insulin secretion in pancreatic β-cells[5]. Phosphatidyl Inositol 3-Kinase (PI3K) dependent pathway plays a crucial role in the metabolic action of insulin[6,7]. After insulin stimulation, activated insulin receptors phosphorylate tyrosine residues of insulin receptor substrate. The phosphorylated insulin receptor substrate binds to the p85 subunit of PI3K and activates its p110 catalytic subunit[7]. The activated PI3K functions as a lipid kinase that phosphorylates the D-3 position of the phosphoinositide ring and produces PI(3,4,5)-trisphosphate [PI(3,4,5)P3]. PI(3,4,5)P3 functions as a lipid second messenger in the activation of downstream signaling of PI3K including Akt/protein kinase B and atypical protein kinase C[8-11]. This series of insulin signaling leads to glucose uptake by promoting glucose transporter 4 translocation from the cytosol to the plasma membrane[6,9,11]. The PI3K pathway also plays an important role in regulating glucose homeostasis via hepatic gene expression[6]. Inhibition of PI3K activity results in a blockade of insulin signaling including glucose uptake and glycogen synthesis. Therefore, it has been speculated that the disruptions of the regulation of PI(3,4,5)P3 metabolism are part of the pathogenesis of insulin resistance and T2DM.

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Src homology 2-containing 5'-inositol phosphatase 2 (SHIP2) is a lipid phosphatase that hydrolyzes PI3-kinase product PI(3,4,5)P3 to PI(3,4)P2, which contributes to the negative regulation of insulin signaling both *in vitro* and *in vivo*[12,13,14]. Overexpression of SHIP2 could inhibit insulin-induced activation of Akt, glucose uptake and glycogen synthesis in 3T3-L1 adipocytes and L6 myotubes via the 5 -phosphatase activity[15,16]. Loss of SHIP2 could lead to the increased sensitivity to insulin, which was characterized by severe neonatal hypoglycemia, deregulated expression of the genes involved in gluconeogenesis, and perinatal death in mice. Heterozygous knockout mice of the SHIP2 gene had increased glucose tolerance and insulin sensitivity associated with an increased recruitment of the GLUT4 glucose transporter and increased glycogen synthesis in skeletal muscles[17]. It has been reported that some polymorphisms of SHIP2 were associated with the metabolic syndrome including T2DM and hypertension in British and French T2DM population[18]. Kagawa et al[19] reported that human SHIP2 gene polymorphism was associated with T2DM in Japanese population. It is unclear whether SHIP2 phenotype is associated with T2DM in Chinese population.

In the present study, we investigated the relation between single nucleotide polymorphisms (SNPs) on SHIP2 gene and the pathogenesis of T2DM in Chinese Han population. Our study indicated that the genotype and allele frequency of SHIP2 (+1893CC/AA) locus in T2DM patients showed significantly different from between the healthy control population. In addition, the G allele of SHIP2 (+2945A/G) seemed to increase the susceptibility to hypertension for T2DM patients.

Patients and Methods

Study Participants

Blood was obtained from the following two groups and DNA was extracted for genotyping: (1) healthy donors of Chinese Han population without diabetes history and diabetes family history (n=382) and (2) Type 2 diabetes mellitus (T2DM) inpatients (cases) of Chinese Han population in the Second Affiliated Hospital, Hebei Medical University from July 2009 to June 2010 (n=376). The inclusion criteria was shown as follows: (1) complied with the World Health Organization (WHO) diagnostic criteria for diabetes in 1999: fasting plasma glucose (FPG) ≥ 7.0 mmol/L and/or 2h plasma glucose (PG 2h) ≥ 11.1 mmol/L in 75 g oral glucose tolerance test (OGTT). (2) Unrelated individuals. (3) Maturity-onset diabetes of the young (MODY) and type I diabetes were excluded. The study was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Hebei Medical University and informed consent was obtained from all recruited subjects before enrollment.

The age, gender, height, body weight Waist-hip Ratio (WHR) and hypertension history of each participant were recorded at the entry of study. The body mass index (BMI) was calculated according to the following formula: BMI=body weight (kg)/height^2 (m^2). The fasting blood glucose, glycosylated hemoglobin, serum total cholesterol (CHOL), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were routinely examined in the Department of Clinical Laboratory, the Second Affiliated Hospital of Hebei Medical University.

DNA Extraction

Venous blood (5 ml) was drawn from each subject into Vacutainer tubes containing EDTA and stored at 4°C. Genomic DNA was extracted within one week after sampling using proteinase K (Merck, Darmstadt, Germany) digestion followed by a salting out procedure according to the method of Miller et al[20].

SHIP2 SNP Genotyping

The SHIP2 genotyping was determined by PCR-restriction fragment length polymorphism (PCR-RFLP) assay. The PCR primers used for amplifying the SHIP exon 16 c1893/1894 CC/AA (SNP3) polymorphism were as follows: forward, 5'-TAAGGGCCACATGGGCTATCACCCC-3' and reverse, 5'-CCTGACACCCAGGGATACTGCAAGT-3'. PCR was performed in a 20 μl volume containing 100 ng of DNA template, 2 μl of 10 × PCR buffer, 1.5 mmol of $MgCl_2$, 1 U of *Taq* DNA polymerase (BioDev-Tech., Beijing, China), 200 μmol of dNTPs and 200 nmol of sense and antisense primer. The PCR cycling conditions were 5 min at 94°C, followed by 35 cycles of 15 s at 94°C, 40 s at 68°C and 40 s at 72°C, with a final step at 72°C for 5 min to allow for the complete extension of all PCR fragments. An 8 μl aliquot of PCR product was digested overnight at 37°C in a 10 μl reaction containing 10 U of DdeI (TakaRa Biotechnology Co. Ltd, Dalian, China) and 1 ×

reaction buffer. After 4 h digestion, the products were resolved and separated on a 2% agarose gel stained with ethidium bromide. After electrophoresis, homozygous CC alleles were represented by a DNA band with size at 118 bp, 85bp and 38bp, homozygous AA alleles were represented by DNA bands with sizes at 203bp and 38 bp, whereas heterozygous AC displayed a combination of both alleles (203bp, 118bp, 85bp and 38 bp) (Figure 1). For a negative control, each PCR reaction used distilled water instead of DNA in the reaction system.

The PCR primers used for amplifying the SHIP exon 26 c2945A/G (SNP5) polymorphism were as follows: forward, 5'-TACGTC-CTTGAAGGGGTCCCGCAC-3' and reverse, 5'-AGTGGAGGCCTTGGATGGGCCTTG-3'. PCR was performed in a 20 µl volume containing 100 ng of DNA template, 2 µl of 10 × PCR buffer, 1.5 mmol of $MgCl_2$, 1 U of *Taq* DNA polymerase, 200 µmol of dNTPs and 200 nmol of sense and antisense primer. The PCR cycling conditions were 5 min at 94°C, followed by 35 cycles of 20 s at 94 , 60 s at 63°C and 60 s at 72°C, with a final step at 72°C for 5 min to allow for the complete extension of all PCR fragments. An 8 µl aliquot of PCR product was digested overnight at 37°C in a 10 µl reaction containing 10 U of BamHI (TakaRa Biotechnology Co. Ltd, Dalian, China) and 1 × reaction buffer. After 4 h digestion, the products were resolved and separated on a 2% agarose gel stained with ethidium bromide. After electrophoresis, homozygous AA alleles were represented by a DNA band with size at 338 bp, homozygous GG alleles were represented by DNA bands with sizes at 249bp and 89 bp, whereas heterozygous AG displayed a combination of both alleles (338bp, 249bp and 89 bp) (Figure 2). For a negative control, each PCR reaction used distilled water instead of DNA in the reaction system.

Statistical Analysis

Statistical analysis was performed using SPSS 13.0 software package. Hardy-Weinberg analysis was performed to compare the observed and expected genotype frequencies using the Chi-square test. The average of the two groups was performed by using the two-sample *t* test. The association of different rates was evaluated by using Chi-square test. p value less than 0.05 was considered statistically significant.

Results

General Clinical Data

The diabetic group consists of 196 cases of males and 180 cases of females, with an average age of 56.78 ± 13.21 years. The control group consists of 233 cases of males and 149 cases of females, with an average age of 52.28 ± 12.40 years. As shown in Table I, the age, BMI and TG in the T2DM group were significantly higher than the control group ($p < 0.05$). The HDL and LDL in the T2DM group were significantly lower than the control group ($p < 0.05$). The CHOL in the T2DM group seems lower than control group, but the difference is not statistically significant ($p > 0.05$).

SNP Genotype Distribution and Allele Frequency of SHIP2 (+1893CC/AA) Locus in T2DM Patients

As shown in Table II, the distribution of SHIP2 (+1893CC/AA) locus in the T2DM group

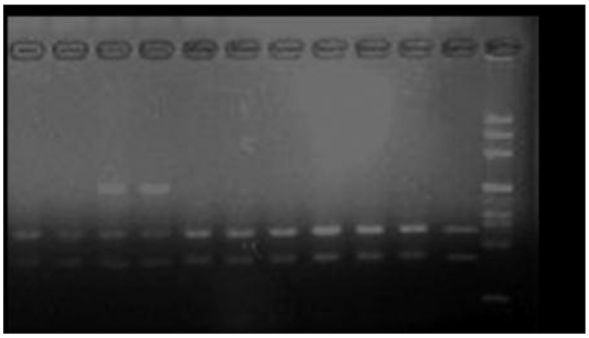

Figure 1. SHIP2 SNP3 (+1893CC/AA) genotyping by PCR-RFLP analysis. Lane 3, 4: CA heterozygous genotype; Lane 1, 2, 5, 6, 7, 8, 9, 10, 11: CC homozygous genotype; Lane 12: 500 DNA Marker.

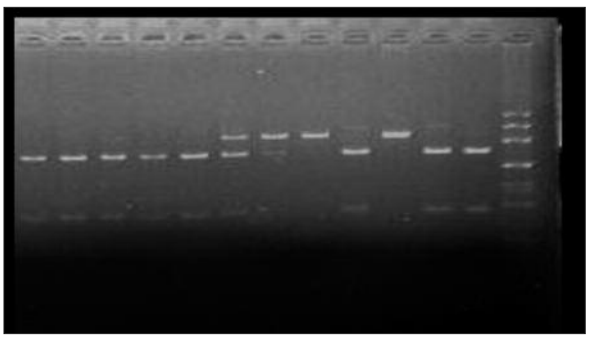

Figure 2. SHIP2 (+2945A/G) genotyping by PCR-RFLP analysis. Lane 1, 2, 3, 4, 5, 9, 11, 12: A/A homozygous genotype; Lane 6, 7: A/G heterozygous genotype; Lane 8, 10: G/G homozygous genotype; Lane 13: DNA Marker.

Table I. Comparison of clinical data between diabetes mellitus and the control groups ($x \pm s$).

Index	Diabetics	Control	*p* value
Age (years old)	56.82 ± 13.21	52 ± 12	0.035*
Gender (male/female)	196/180	133/49	0.000*
WHR	0.94 ± 0.41	0.89 ± 0.05	0.105
BMI (kg/m^2)	26.27± 3.97	25.4 ± 3.08	0.005*
CHOL (mmol/l)	5.11 ± 2.93	4.85 ± 0.84	0.109
TG (mmol/l)	2.02 ± 1.91	1.57 ± 1.00	0.000*
HDL (mmol/l)	1.44 ± 0.44	1.53 ± 0.33	0.026*
LDL (mmol/l)	2.73 ± 1.1	3.00 ± 0.70	0.001*

Note: BMI: body mass index; CHOL: total cholesterol; TG: triglycerides; HDL high-density lipoprotein; from: LDL: low density lipoprotein.

Table II. Genotype and allele frequency distribution of SHIP2 (+1893CC/AA) between T2DM group and the control group.

		Genotype frequency			Allele frequency	
Groups	n	AA	CA	CC	A	C
T2DM	376	23 (6.1%)	80 (21.3%)	273 (72.6%)	126 (16.5%)	626 (83.2%)
Controls	382	13 (3.4%)	54 (14.1%)	315 (82.5%)	80 (10.5%)	684 (89.5%)
χ^2			10.78		21.80	
p value			0.000		0.000	

was 6.1% (23 of 376) of AA type, 72.6% (273 of 376) of CC type and 21.3% (80 of 376) of CA type, respectively. The genotype distribution of SHIP2 (+1893CC/AA) locus in the control group was 3.4% (13 of 382) of AA type, 82.5% (315 of 382) of CC type and 14.1% (54 of 382) of CA type, respectively. The A allele distribution in the T2DM group and the control group was 16.8% (126 of 752) and 10.5% (80/764), respectively. The C allele distribution in the T2DM group and the control group was 83.2% (626 of 752) and 89.5% (684/764), respectively. The genotype distribution and allele frequency were in accordance with Hardy-Weinberg genetic equilibrium ($p > 0.05$). The distribution of SHIP2 (+1893CC/AA) locus genotype was significantly different between the T2DM group and the control group ($\chi^2 = 10.78$, $p = 0.00$). The allele frequency of SHIP2 (+1893CC/AA) locus was also significantly different between the T2DM group and the control group ($\chi^2 = 21.80$, $p = 0.00$).

SNP Genotype Distribution and Allele Frequency of SHIP2 (+2945A/G) Locus in T2DM Patients

As shown in Table III, the distribution of SHIP2 (+2945A/G) locus in the T2DM group was 83.5% (314 of 376) of GG type, 4.3% (16 of 376) of AA type and 12.2% (46 of 376) of AG type, respectively. The genotype distribution of SHIP2 (+2945A/G) locus in the control group was 80.6% (308 of 382) of GG type, 3.4% (13 of 382) of AA type and 16% (61 of 382) of AG type, respectively. The G allele distribution in the

Table III. Genotype and allele frequency distribution of SHIP2 (+2945A/G) between T2DM patients and controls.

		Genotype frequency			Allele frequency	
Groups	n	GG	AG	AA	G	A
T2DM	376	314 (83.5%)	46 (12.2%)	16 (4.3%)	674 (89.6%)	78 (10.4%)
controls	382	308 (80.6%)	61 (16%)	13 (3.4%)	662 (86.6%)	102 (13.4%)
χ^2			2.42		3.21	
p value			0.30		0.07	

Table IV. Association between genotype and clinical presentation of SHIP2 (+1893CC/AA) in T2DM group.

Index	Genotype			*p* values		
	CC	AA	CA	CCvsAA	CCvsCA	AAvsCA
Age (years old)	56.55 ± 14.01	56.56 ± 8.13	57.78 ± 11.55	1	0.812	0.920
WHR	0.94 ± 0.48	0.93 ± 0.06	0.92 ± 0.07	0.925	0.722	0.917
BMI (kg/m^2)	26.32 ± 4.18	26.10 ± 3.76	26.13 ± 3.26	0.792	0.696	0.975
CHOL (mmol/l)	4.97 ± 1.56	4.79 ± 1.18	5.09 ± 1.57	0.603	0.544	0.422
TG (mmol/l)	2.07 ± 2.04	1.76 ± 1.15	1.94 ± 1.59	0.452	0.579	0.695
HDL (mmol/l)	1.46 ± 0.46	1.29 ± 0.30	1.41 ± 0.42	0.080	0.388	0.253
LDL (mmol/l)	2.68 ± 1.05	2.80 ± 0.97	2.89 ± 1.27	0.610	0.136	0.739

Note: BMI: body mass index; CHOL: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; from: LDL: low density lipoprotein. *$p \leq 0.05$.

T2DM group and the control group was 89.6% (674 of 752) and 86.6% (662/764), respectively. The A allele distribution in the T2DM group and the control group was 10.4% (78 of 752) and 13.4% (102/764), respectively. The genotype distribution and allele frequency were in accordance with Hardy-Weinberg genetic equilibrium ($p > 0.05$). The distribution of SHIP2 (+2945A/G) locus genotype was not significantly different between the T2DM group and the control group ($\chi^2 = 2.42$, $p = 0.30$). The allele frequency of SHIP2 (+2945A/G) locus was also not significantly different between the T2DM group and the control group ($\chi^2 = 3.21$, $p = 0.07$).

Association Between SHIP2 (+1893CC/AA) Genotype and the Clinical Features of T2DM Patients and the Control Group

As shown in Table IV, there was no significant difference between SHIP2 (+1893CC/AA) genotype and the clinical features of T2DM patients, such as the patient's age, WHR, BMI, CHOL, TG, HDL, LDL ($p > 0.05$). In the control group, there was no significant difference between SHIP2 (+1893CC/AA) genotype and the clinical features was found ($p > 0.05$) (Table V).

Association Between SHIP2 (+2945A/G) Genotype and the Clinical Features of T2DM Patients and the Control Group

As shown in Table VI, there was no significant difference between SHIP2 (+2945A/G) genotype and the clinical features of T2DM patients, such as the patient's age, WHR, BMI, CHOL, TG, HDL, LDL ($p > 0.05$). In the control group, the BMI in AA type group was significantly decreased as compared with the GG type group (Table VII).

Association Between SHIP2 (+1893CC/AA) (+2945A/G) Genotype and the Hypertension in T2DM Patients

220 of 376 T2DM patients showed hypertension. As shown in Table VIII, the distribution of

Table V. Association between genotype and clinical presentation of SHIP2 (+1893CC/AA) in control group.

Index	Genotype			*p* values		
	CC	AA	CA	CCvsAA	CCvsCA	AAvsCA
Age (years old)	52.52 ± 12.1	50.66 ± 12.37	51.23 ± 14.44	0.72	0.625	0.920
WHR	0.88 ± 0.05	0.88 ± 0.03	0.89 ± 0.62	0.23*	0.11*	0.25*
BMI (kg/m^2)	25.44 ± 3.00	27.17 ± 3.09	24.77 ± 3.47	0.178	0.308	0.087
CHOL (mmol/l)	4.87 ± 0.86	4.82 ± 0.51	4.69 ± 0.77	0.868	0.308	0.752
TG (mmol/l)	1.60 ± 1.02	1.97 ±1.49	1.35 ± 0.74	0.374	0.258	0.178
HDL (mmol/l)	1.51 ± 0.31	1.49 ± 0.19	1.61 ± 0.40	0.997	0.524	0.651
LDL (mmol/l)	3.00 ± 0.73	3.14 ± 0.50	2.96 ± 0.58	0.645	0.786	0.582

Note: BMI: body mass index; CHOL: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; from: LDL: low density lipoprotein. *$p \leq 0.05$.

Table VI. Association between genotype and clinical presentation of SHIP2 (+ 2945A/G) in T2DM group.

Index	Genotype			*p* values		
	GG	AA	AG	GGvsAA	GGvsAG	AAvsAG
Age (years old)	57.06 ± 13.0	55.62 ± 15.44	55.58 ± 14.04	0.673	0.481	0.992
WHR	0.91 ± 0.10	0.92 ± 0.07	0.91 ± 0.07	0.997	0.999	0.856
BMI (kg/m^2)	26.32 ± 3.78	26.07 ± 4.10	25.99 ± 5.10	0.809	0.606	0.946
CHOL (mmol/l)	5.00 ± 1.607	5.01±1.37	4.82 ± 1.11	0.995	0.456	0.681
TG (mmol/l)	1.99 ± 1.82	1.46 ± 0.66	2.46 ± 2.62	0.284	0.119	0.073
HDL (mmol/l)	1.45 ± 0.44	1.59 ± 0.61	1.31 ± 0.40	0.235	0.051	0.053
LDL (mmol/l)	2.75 ± 1.14	2.87 ± 0.93	2.55 ± 0.77	0.680	0.254	0.325

Note: BMI: body mass index; CHOL: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; from: LDL: low density lipoprotein. *$p \leq 0.05$.

Table VII. Association between genotype and clinical presentation of SHIP2 (+2945A/G) in control group.

Index	Genotype			*p* values		
	AA	GG	AG	AAvsGG	AAvsAG	GGvsAG
Age (years old)	52.58 ± 12.45	49.18 ± 10.75	51.96 ± 12.96	0.384	0.07	0.528
WHR	0.88 ± 0.05	0.90 ± 0.06	0.89 ± 0.05	0.323	0.622	0.555
BMI (kg/m^2)	25.21 ± 2.98	27.33 ± 2.93	25.58 ± 3.45	0.028*	0.56	0.107
CHOL (mmol/l)	4.85 ± 0.85	4.66 ± 0.65	4.89 ± 0.90	0.462	0.848	0.447
TG (mmol/l)	1.59 ± 0.33	1.56 ± 0.89	1.52 ± 0.92	0.937	0.761	0.916
HDL (mmol/l)	1.52 ± 0.33	1.56 ± 0.41	1.51 ± 0.28	0.701	0.901	0.682
LDL (mmol/l)	3.00 ± 0.69	2.86 ± 0.66	3.05 ± 0.80	0.536	0.708	0.446

Note: BMI: body mass index; WHR: Waist-hip Ratio; CHOL: total cholesterol; TG: triglycerides; HDL high-density lipoprotein; from: LDL: low density lipoprotein. *$p \leq 0.05$.

Table VIII. Association between the genotype frequencies or allele frequencies of SHIP2 (+1893CC/AA) and the hypertension in T2DM patients.

Groups	Genotype frequencies			Allele frequencies	
	CC	CA	AA	C	A
Hypertension	195 (88.6%)	24 (10.9%)	1 (0.5%)	414 (94.1%)	2 (5.9%)
Non-hypertension	142 (91.0%)	12 (7.7%)	2 (1.3%)	296 (94.9%)	16 (5.1%)
χ^2		1.828		0.211	
p		0.401		0.646	

SHIP2 (+1893CC/AA) locus in the hypertension patients was 88.6% (195 of 220) of CC type, 10.9% (24 of 220) of CA type and 0.5% (1 of 220) of AA type, respectively. The distribution of SHIP2 (+1893CC/AA) locus in the non-hypertension patients was 91.0% (142 of 156) of CC type, 12% (12 of 156) of CA type and 2% (2 of 156) of AA type, respectively. There was no significant difference among these three genotypes ($\chi^2 = 1.828$, $p = 0.401$). The C allele and A allele distribution was 94.1% (414 of 416) and 5.9% (2/416) in the hypertension patients, respectively. The C allele and A allele distribution was 94.9% (296 of 312) and 5.1% (16/312) in the non-hypertension patients, respectively. The allele distribution was no significant difference between these two groups ($\chi^2 = 0.211$, $p = 0.646$).

As shown in Table IX, the distribution of SHIP2 (+2945A/G) locus in the hypertension patients was 88.18% (194 of 220) of GG type, 8.63% (19 of 376) of AG type and 3.19% (7 of 220) of AA type, respectively. The distribution of

Table IX. Association between the genotype frequencies or allele frequencies of SHIP2 (+2945A/G) and the hypertension in T2DM patients.

Groups	Genotype frequencies			Allele frequencies	
	GG	AG	AA	G	A
Hypertension	194 (88.18%)	19 (8.63%)	7 (3.19%)	407 (92.5%)	33 (7.5%)
Non-hypertension	120 (76.92%)	27 (17.3%)	9 (5.78%)	267 (85.57%)	45 (14.43%)
χ^2		8.431		9.412	
p		0.015		0.002	

SHIP2 (+2945A/G) locus in the non-hypertension patients was 76.92% (120 of 156) of GG type, 17.3% (27 of 156) of AG type and 5.78% (9 of 156) of AA type, respectively. The AA type distribution was significantly lower in the hypertension patients as compared with the no-hypertension patients (χ^2 = 8.431, p = 0.015). The G allele and A allele distribution was 92.5% (407 of 440) and 7.5% (33/312) in the hypertension patients, respectively. The G allele and A allele distribution was 85.57% (267 of 440) and 14.43% (45/312) in the non-hypertension patients, respectively. The G allele distribution was significantly higher in the hypertension patients as compared with the no-hypertension patients (χ^2 = 9.412, p = 0.002).

Discussion

The metabolic syndrome including T2DM, hypertension, central obesity, and dyslipidemia, occurs at a high rate globally[21,22]. Insulin resistance is the focal component of the metabolic syndrome, and both genetic and environmental factors contribute to its development[23]. Genetic susceptibility to T2DM involves many genes, most of which are still unknown.

SHIP2 is a negative regulator of insulin signaling, and has been proved to be associated with T2DM, hypertension, and insulin resistance[24-26]. Some polymorphisms of SHIP2 have been reported to be associated with the metabolic syndrome including T2DM and hypertension in British and French T2DM population[18]. SHIP2 gene polymorphism was also associated with T2DM in Japanese population[19]. It is unclear whether SHIP2 phenotype is associated with T2DM in Chinese population.

In the present study, we investigated the relation between single nucleotide polymorphisms (SNPs) on SHIP2 gene and the pathogenesis of T2DM in Chinese Han population. Our results showed that the SHIP2 (+1893CC/AA) (+2945A/G) genotype distribution and allele frequency in both T2DM group and the control group were in accordance with Hardy-Weinberg genetic equilibrium. The distribution of SHIP2 (+1893CC/AA) locus genotype was significantly different between the T2DM group and the control group. The allele frequency of SHIP2 (+1893CC/AA) locus was also significantly different between the T2DM group and the control group. However, the distribution and allele frequency of SHIP2 (+2945A/G) locus genotype were not significantly different between the T2DM group and the control group. These results suggested that the distribution and allele frequency of SHIP2 (+1893CC/AA) were correlated with T2DM.

We did not find any correlation between SHIP2 (+1893CC/AA) and SHIP2 (+2945A/G) genotypes with the clinical features of T2DM patients, such as the patient's age, WHR, BMI, CHOL, TG, HDL, LDL. We also did not find any correlation between SHIP2 (+1893CC/AA) and SHIP2 (+2945A/G) genotypes with the clinical features in the control group.

It has been reported that hypertension is the high risk factor for the T2DM. At least one third of diabetic patients showed hypertension. In the present study, we analyzed the correlation between the SHIP2 (+1893CC/AA) or (+2945A/G) genotype and the hypertension in T2DM patients. Totally, 220 of 376 T2DM patients showed hypertension in our present study. No significant difference was found for the distribution of SHIP2 (+1893CC/AA) locus between hypertension patients and non-hypertension patients. However, for SHIP2 (+2945A/G) genotype, the AA type distribution was significantly lower in the hypertension patients as compared with the no-hypertension patients. In addition, the G allele distribution was significantly higher in the hyper-

tension patients as compared with the no-hypertension patients. These results suggested that the G allele seemed to increase the susceptibility to hypertension for T2DM patients.

Conclusions

Our results confirmed the role of SHIP2 (+1893CC/AA) and (+2945A/G) genotype in the pathogenesis of T2DM in Chinese Han population. Our study indicated that the genotype and allele frequency of SHIP2 (+1893CC/AA) locus in T2DM patients showed significantly different from between the healthy control population. In addition, the G allele of SHIP2 (+2945A/G) seemed to increase the susceptibility to hypertension for T2DM patients.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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