Shuangyan Lu, MSc¹

Yan Xie, PhD¹

Kun Lin, MSc¹

Siwei Li, PhD¹

Yidan Zhou, undergraduate student²

Pei Ma, MSc¹

Zhibua Lv, MSc¹

Xin Zhou, Prof.¹

¹ Center for Gene Diagnosis, Zhongnan Hospital, Wuhan University

² School of Life Sciences, Jiangnan University, Hubei, Wuhan 430056, P.R.China.

Genome-Wide Association Studies-derived susceptibility loci in Type 2 Diabetes: confirmation in a Chinese population

Abstract

Purpose: Several novel genetic variants for type 2 diabetes mellitus (T2DM) have been identified through genome-wide association studies (GWAS). A case-control study was performed to investigate the association of five new European or South Asian GWAS-derived susceptibility loci with T2DM in a Chinese population.

Methods: Five single nucleotide polymorphisms (SNPs) were genotyped: rs3923113 near GRB14, rs16861329 in ST6GAL1, rs1802295 in VPS26A, rs7178572 in HMG20A, and rs231362 near KCNQ1, by high-resolution melting (HRM) of small amplicons. The association between T2DM and related quantitative traits in a total of 900 Chinese individuals, including 498 type 2 diabetic patients and 402 ethnically matched control subjects, were examined.

Results: After adjusting for age and gender, rs1802295 (OR 5.724, $P=0.03$) and rs231362 (OR=5.683, $P=0.016$) were found to be associated with T2DM. Triglyceride levels were higher in TT and CT carriers for rs16861329 (1.05 (0.8-1.34) mmol/l) than in CC carriers (0.91 (0.73-1.23) mmol/l) with $P=0.008$ and that high-density lipoprotein cholesterol (HDL-C) was lower in TT and CT carriers (1.17 (1.02-1.33) mmol/l) than in CC carriers (1.21 (1.05-1.41) mmol/l), with $P=0.034$. For rs3923113, the HDL-C levels were lower in the GG carriers (1.08 (0.90-1.18) mmol/l) than in the GT+TT carriers (1.21 (1.04-1.38) mmol/l), with $P=0.018$. Ours is the first report of this association.

Conclusion: rs231362-KCNQ1 and rs1802295-VPS26A are associated with T2DM in the Chinese population. The remaining three SNPs are associated with other aspects of lipid metabolism.

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Correspondence to:

Xin Zhou

Center for Gene Diagnosis

Zhongnan Hospital of Wuhan University

Donghu Road 169, Wuhan 430071, P.R.China

tel: +86 027 62170685, 86 13707103243

email: zhouxjyk@163.com

Type 2 diabetes (T2DM) is a complicated and chronic metabolic disease that is characterized by insulin resistance and pancreatic beta-cell dysfunction [1]. T2DM is a major public health problem with a rapidly rising global prevalence [2]. Though unhealthy diet, obesity and physical inactivity contribute to T2DM, genetic factors are considered to play an important role in its pathogenesis [3]. To date, genome-wide association studies (GWAS) have identified approximately 56 loci associated with susceptibility to T2DM [4-6]. One European [7] and two Japanese GWAS [8, 9] have identified an association of the potassium voltage-gated channel, KQT-like subfamily member 1 (KCNQ1), locus with T2DM. In addition, a GWAS of individuals of South Asian ancestry identified six new T2D Msusceptibility loci [4].

Growth factor receptor-binding protein 14 (Grb14) is an adaptor protein that may down-regulate insulin signaling [10]. Grb14 inhibits insulin receptor (IR) catalytic activity through interaction with the BPS domain and impedes peptide substrate binding [11]. The expression of GRB14 in adipose tissue may play a role in insulin sensitivity. ST6 beta-galactosamide alpha-2,6-sialyltransferase 1 (ST6GAL1), which is the major α -2,6-sialyltransferase responsible for the broad synthesis of glycoproteins and glycolipids, is associated with pancreatic beta-cell function. Vacuolar protein sorting 26 homolog A (VPS26A), whose encoded protein is a component of the large, multimeric retromer complex, is involved in the retrograde transport of proteins from endosomes to the trans-Golgi network. Finally, high mobility group 20A (HMG20A), which activates REST (RE-1 silencing transcription factor)-responsive genes, plays a key role in the initiation of neuronal differentiation [12].

In this study, the potential association of these new susceptibility loci, which were identified in European and South Asian GWAS, with T2DM were investigated in Chinese individuals.

Materials and Methods

Study population

A total of 900 individuals were included in this study. There were 498 unrelated patients with T2DM who were admitted to the Endocrinology Department at Zhongnan Hospital of Wuhan University and the Center Hospital of Xiangyang from February 2011 to February 2012. T2DM was diagnosed on the basis of the 1999 World Health Organization criteria (fasting plasma glucose ≥ 7.0 mmol/l and/or 2 h plasma glucose ≥ 11.1 mmol/l). Individuals with other forms of diabetes, serious cardiovascular disease, thyroid gland dysfunction, or tumors

TABLE 1. Clinical characteristics of the two study groups

	T2DM(498)	Control(402)
Gender n (male/female)	498(297/201)	402(267/135)*
Age (years)	56 $\pm$ 7	49 $\pm$ 9*
BMI (kg/m ²)	25.22 $\pm$ 3.19	25.11 $\pm$ 3.19
Fasting plasma glucose (mmol/l)	7.5(5.73-10.4)	4.74(4.42-5.09)*
HbA1C (%)	7.5(6.5-9.4)	NA
2h Plasma glucose (mmol/l)	14.1(9.81-18.2)	NA
Total cholesterol (mmol/l)	4.95(4.14-5.74)	4.33(3.95-4.71) *
Triglycerides (mmol/l)	1.46(1.03-2.33)	0.95(0.74-1.26)*
HDL- C (mmol/l)	1.17(0.96-1.46)	1.2(1.03-1.37)
LDL- C (mmol/l)	2.79(2.27-3.47)	2.54(2.3-2.74)*

Data are mean $\pm$ standard deviation or median (interquartile range); NA, not applicable; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; *, $P < 0.05$.

were excluded from this study. There were also 402 "healthy" individuals recruited from the Zhongnan Hospital with the following inclusion criteria: (1) fasting plasma glucose < 6.1 mmol/l, (2) no family history of T2DM or other serious disease, and (3) total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) all within the normal range. All participants were between the ages of 35 to 70 years. Informed consent was obtained from all participants. The study protocol was approved by the ethics committee of the Wuhan University Medical School. The clinical characteristics of the subjects are summarized in Table 1.

All participants were examined in the morning after a 12 hour overnight fast. Blood samples were collected to measure the fasting plasma glucose, perform a 75 g oral glucose tolerance test (OGTT), and measure blood lipids, including TC, TG, HDL-C and LDL-C.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes (EDTA anticoagulated) using the sodium iodide extraction technique [13]. The DNA concentration was measured using a NanoDrop 2000C (NanoDrop Technologies, DE, USA).

TABLE 2. Details of HRM of small amplicon genotyping

SNPs	Forward primer	Reverse primer	Product (bp)
rs3923113	TGAGAAAGCAACTGTGT	CAATGAATGGATTTTTTATTTTAGC	52
rs16861329	TCTCACTGGTCTCTCTGCTATAC	GCTGAGGCTGAGCCAAA	47
rs1802295	ACATGCAAAAAATTAAATATTTTGTGTTCA	GACACAAGAGGCCACTCA	65
rs7178572	GAGAATTAAGAGAGCTGAAATGATAGG	CCTCATACCCAAAAATCTCTTACCA	58
rs231362	TTACCATGGTCCTCTTCCCTCG	CTGTGTAGCTCACCTGCCTTTG	61

TABLE 3. Association of genotype with type 2 diabetes in Chinese population

Gene/SNP	genotype	control	T2DM	HWE	P	OR(95%CI)
GRB14 rs3923113 G/ T [*]	GG	14	19	0.41		
	GT	109	104		0.673	0.843(0.381-1.866)
	TT	278	370		0.749	1.132(0.53-2.419)
ST6GAL1 rs16861329 C/ T [*]	CC	271	331	0.23		
	TC	113	143		0.999	1.0(0.731-1.368)
	TT	17	21		0.981	0.991(0.489-2.009)
VPS26A rs1802295 C/ T [*]	CC	318	383	0.18		
	CT	82	102		0.787	1.049(0.741-1.486)
	TT	2	11		0.03*	5.724(1.186-27.628)
HMG20A rs7178572 A/ G [*]	AA	159	199	0.15		
	AG	198	240		0.508	0.904(0.67-1.2)
	GG	45	55		0.414	1.225(0.752-1.996)
KCNQ1 rs231362 T/ C [*]	TT	9	3	0.85		
	TC	105	86		0.103	3.312(0.785-13.971)
	CC	285	402		0.016*	5.683(1.378-23.446)

P was adjusted for age and gender; * P<0.05; ^{*} risk allele; HWE, Hardy-Weinberg

SNP genotyping

Five SNPs from five genes were selected to test for an association with T2DM in Chinese individuals: a single nucleotide polymorphism (SNP), near KCNQ1, based on previous reports, rs231362 [7], and four new SNPs from the loci identified through the South Asian GWAS [4] (rs3923113 near GRB14, rs16861329 in ST6GAL1, rs1802295 in VPS26A, and rs7178572 in HMG20A). PCR with LCGreen saturation fluorescent dye, was performed and the melting curves were separated by an automated analysis of the LightScanner 32

using the high-resolution melting of a small amplicon [14-16]. The primer details are available in Table 2. The product length ranged from 47 bp to 65 bp. The melting curves are presented in Figure 1. Homozygote has only one melting peak, and heterozygote has two melting peaks. The genotyping results were confirmed by DNA sequencing. The genotyping quality control measures for the SNPs mainly depended upon the DNA sequencing. Ninety six samples were amplified each time for each SNP, and if the melting temperatures were highly consistent, then one sample was selected from each genotype for se-

quencing; therefore, 30 samples were sequenced for each SNP. The genotype frequencies in GRB14 (rs3923113), ST6GAL1 (rs16861329), VPS26A (rs1802295), HMG20A (rs7178572) and KCNQ1 (rs231362) were consistent with the Hardy-Weinberg equilibrium (Table 3). Their minor allele frequencies (MAF) were comparable with those found in the HapMap CHB sample (www.hapmap.org). Their call rates were >98%, as shown in Table 4.

Statistical analysis

All statistical analyses were performed in SPSS 13.0 for Windows and PASS 11. Quantitative trait data are shown as the mean $\pm$ standard deviation (for data with normal distributions) or the median plus an interquartile range (for data with skewed distributions), and the differences between T2DM patients and control subjects were assessed using an independent t test or Mann-Whitney U tests. The distributions of the genotype for all SNPs were analyzed for deviations from Hardy-Weinberg equilibrium (HWE) using a chi-square test. The association of each SNP with the quantitative traits was analyzed using the Kruskal-Wallis test. The genotype and allele frequency distributions in the case and control groups were analyzed by a chi-square test and a binary logistic regression analysis, with the wild-type genotype as the reference factor. P values below 0.05 were considered to be statistically significant. The statistical power and sample size were calculated using PASS 11.

Results

Table 1 summarizes the clinical characteristics of the participants. Figure 1 shows the high-resolution melting curves of the small amplicons. The genotype distributions for all of the SNPs were in Hardy-Weinberg equilibrium, with $P > 0.05$ in the control subjects (Table 3). The association analysis with T2DM revealed that the C risk allele of rs231362 (KCNQ1) was significantly associated with T2DM ($p < 0.001$, OR=1.071, 95%CI: 1.034-1.111) (Table 4). After adjusting for age and gender, the CC carriers of rs231362 (KCNQ1) had a higher risk of the disease ($P=0.016$, OR=5.683, 95%CI: 1.378-23.446), and rs1802295 was associated with T2DM ($P=0.03$, OR =5.724, 95%CI: 1.186-27.628) (Table 3). The distributions of rs3923113, rs16861329 and rs7178572 were not significantly different between the two groups.

The clinical parameter characteristics of the two groups were then compared further (Table 5). For rs16861329, the triglycerides in the TT and CT carriers, 1.05 (0.8-1.34) mmol/l, was higher than in the CC carriers, 0.91 (0.73-1.23) mmol/l ($P=0.008$), and the HDL-C in TT and CT carriers, 1.17 (1.02-1.33) mmol/l, was lower than in the CC carriers, 1.21 (1.05-1.41) mmol/l ($P=0.034$). For rs3923113, the HDL-C in the GG carriers, 1.08(0.90-1.18) mmol/l, was lower than in the GT+TT carriers, 1.21 (1.04-1.38) mmol/l ($P=0.018$). Ours is the first report of this association.

TABLE 4. Association of alleles with type 2 diabetes in Chinese population

SNP	Allele	Control	T2DM	Call rate(%)	P	OR(95%CI)
rs3923113	G	137	142	99.3	0.069	1.032(0.991-1.075)
	T*	665	844			
rs16861329	C	655	805	99.6	0.448	1.02(0.838-1.24)
	T*	147	185			
rs1802295	C	718	868	99.7	0.134	1.169(0.902-1.514)
	T*	86	124			
rs7178572	A	516	638	99.6	0.45	0.989(0.873-1.121)
	G*	288	350			
rs231362	T	123	92	98.8	<0.001*	1.071(1.034-1.111)
	C*	675	890			

*, risk allele, *, $P < 0.05$.

TABLE 5. Association of SNPs with quantitative traits in control group

SNP	Genotype (n)	FPG(mmol/l)		Total cholesterol(mmol/l)		Triglycerides(mmol/l)		HDL-cholesterol (mmol/l)		LDL-cholesterol (mmol/l)	
		Median(IQR)	p	Median(IQR)	p	Median(IQR)	p	Median(IQR)	p	Median(IQR)	p
rs3923113	GG(14)	4.585(4.303-5.43)		4.02(3.44-4.46)		1.09(0.77-1.43)		1.08(0.90-1.18)		2.38(2.14-2.7)	
	GT+TT(387)	4.75(4.42-5.08)	0.961	4.34(3.99-4.72)	0.34	0.94(0.74-1.26)	0.337	1.21(1.04-1.38)	0.018*	2.54(2.31-2.75)	0.158
rs16861329	CC (271)	4.78(4.45-5.17)		4.3(3.97-4.65)		0.91(0.73-1.23)		1.21(1.05-1.41)		2.51(2.3-2.72)	
	CT+TT(130)	4.64(4.34-5.01)	0.058	4.41(3.92-4.80)	0.137	1.05(0.8-1.34)	0.008*	1.17(1.02-1.33)	0.034*	2.57(2.31-2.81)	0.072
rs1802295	CC(318)	4.76(4.44-5.1)		4.32(3.92-4.72)		0.93(0.74-1.25)		1.21(1.05-1.38)		2.54(2.3-2.76)	
	CT+TT(84)	4.63(4.22-5.03)	0.051	4.35(4.02-4.63)	0.997	1.06(0.74-1.33)	0.106	1.17(1.01-1.35)	0.194	2.55(2.32-2.73)	0.8
rs7178572	AA(159)	4.69(4.38-5.04)		4.40(4.02-4.74)		0.96(0.73-1.26)		1.19(1-1.41)		2.54(2.34-2.77)	
	AG+GG(243)	4.78(4.45-5.14)	0.112	4.3(3.93-4.67)	0.277	0.94(0.75-1.26)	0.999	1.2(1.05-1.36)	0.916	2.52(2.29-2.73)	0.28
rs231362	TT(9)	4.86(4.21-5.21)		4.35(4.11-4.84)		1.00(0.66-1.22)		1.19(1.07-1.37)		2.45(2.28-2.80)	
	TC+CC(390)	4.75(4.42-5.08)	0.896	4.33(3.94-4.71)	0.574	0.95(0.75-1.26)	0.808	1.20(1.03-1.37)	0.992	2.54(2.30-2.75)	0.936

*, P<0.05, n=402

Discussion

This is the first study to examine the association of these five SNPs in patients with T2DM in a Chinese population. GWAS conducted in European, South Asian and East Asian populations have revealed many T2DM risk loci, and some of them have been confirmed and shown to be common across different populations [17]. In this study, a significant association of rs231362 (KCNQ1) and rs1802295 (VPS26A) were identified in patients with T2DM in a Chinese population.

KCNQ1 has previously been reported to be associated with reduced beta-cell function [7] and the KCNQ1 gene is believed to be a risk locus for T2DM in Chinese individuals [18, 19]; however, rs231362 was not studied in China. Consistent with our study (OR=1.071), a European GWAS by Voight (OR= 1.11) [7], a South Asian study by Been (OR=1.24) [20], and a Japanese study by Ohshige (OR= 1.18) [21] showed that rs231362 is associated with T2DM, suggesting that this locus may be a common susceptibility locus for T2DM across different ethnic groups. VPS26A is expressed in pancreatic, adipose and other tissues, but the reason for this association was not clear.

No associations were detected for several other SNPs, identified through a South Asian GWAS, including rs3923113, rs16861329, and rs7178572. This may be attributed to different ethnicities or to the relatively small sample size. According to the results of a study by Kooner [4], to find an association

with rs3923113 (OR=1.07, MAF=0.26), rs16861329 (OR=1.07, MAF=0.25), rs7178572 (OR=1.08, MAF=0.48), the sample sizes should be enlarged to 23484, 24081, and 14200, respectively.

The analyses of related quantitative traits revealed that the TT carriers of rs3923113-GRB14 have higher HDL-C in control subjects. This result may be due to central adiposity and the expression of GRB14 in adipose tissue [21]. The gene GRB14 is thought to inhibit receptors in the insulin receptor class. In a study by Ridker, the association of GRB14 with HDL-C was significant [22], and GRB14 was thought to have a potentially gender-specific effect. In our study, however, gender specificity was not observed. Our study also identified rs16861329-ST6GAL1 to be associated with triglyceride and HDL-C levels in the control group. rs16861329 is near the gene ADIPOQ, which may be associated with adiponectin levels, obesity and T2DM [24]. In a study by Gong, it was suggested that the down regulation of the hepatic ST6Gal1 gene leads to the defective glycosylation of lipid-carrying apolipoproteins, such as apo E and apo J, resulting in defective intracellular lipid and lipoprotein transport [25], which may be related to the change in the triglycerides and HDL-C found in our study.

In conclusion, rs231362-KCNQ1 and rs1802295-VPS26A were associated with T2DM in a Chinese population. The remaining three SNPs are associated with other aspects of lipid metabolism.

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