Quantitative candidate gene association studies of metabolic traits in Han Chinese type 2 diabetes patients

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ABSTRACT. Recent genome-wide association studies have identified many loci associated with type 2 diabetes mellitus (T2DM), hyperuricemia, and obesity in various ethnic populations. However, quantitative traits have been less well investigated in Han Chinese T2DM populations. We investigated the association between candidate gene single nucleotide polymorphisms (SNPs) and metabolic syndrome-related quantitative traits in Han Chinese T2DM subjects. Unrelated Han Chinese T2DM patients (1975) were recruited. Eighty-six SNPs were genotyped and tested for association with quantitative traits including lipid profiles, blood pressure, body mass index (BMI), serum uric acid (SUA), glycated hemoglobin (HbA1c), plasma glucose [fasting plasma glucose (FPG)], plasma glucose 120 min post-OGTT (P2PG; OGTT = oral glucose tolerance test), and insulin resistance-related traits. We found that *CAMTA1*, *ABI2*, *VHL*, *KAT2B*, *PKHD1*, *ESR1*,

TOX, *SLC30A8*, *SFI1*, and *MYH9* polymorphisms were associated with HbA1c, FPG, and/or P2PG; *GCK*, *HHEX*, *TCF7L2*, *KCNQ1*, and *TBX5* polymorphisms were associated with insulin resistance-related traits; *ABCG2*, *SLC2A9*, and *PKHD1* polymorphisms were associated with SUA; *CAMTA1*, *VHL*, *KAT2B*, *PON1*, *NUB1*, *SLITRK5*, *SMAD3*, *FTO*, *FANCA*, and *PCSK2* polymorphisms were associated with blood lipid traits; *CAMTA1*, *SPAG16*, *TOX*, *KCNQ1*, *ACACB*, and *MYH9* polymorphisms were associated with blood pressure; and *UBE2E3*, *SPAG16*, *SLC2A9*, *CDKAL1*, *CDKN2A/B*, *TCF7L2*, *SMAD3*, and *PNPLA3* polymorphisms were associated with BMI (all P values <0.05). Some of the candidate genes were associated with metabolic and anthropometric traits in T2DM in Han Chinese. Although none of these associations reached genome-wide significance ($P < 5 \times 10^{-8}$), genes and loci identified in this study are worthy of further replication and investigation.

Key words: Type 2 diabetes mellitus; Association study; Candidate genes; Quantitative traits; Insulin resistance

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most important epidemic diseases of this century, and presents a challenging healthcare problem. The prevalence of T2DM has been increasing dramatically worldwide. The World Health Organization predicts that there will be 366 million adults with diabetes by 2030 (de Almeida-Pititto et al., 2015). China has the highest prevalence of diabetes in the world, and its incidence is increasing rapidly, especially in urban areas. The most recent national survey, conducted in 2010, reported that the estimated prevalence of diabetes among a representative sample of Chinese adults was 11.6%, representing an estimated 113.9 million adults with diabetes and 493.4 million with prediabetes in China (Xu et al., 2013).

Metabolic syndrome (MetS) is a common disorder in China. The prevalence of MetS is increasing in China because of lifestyle westernization, which entails a high-fat, high-calorie diet and less physical activity. MetS is a disorder of energy utilization and storage, and it increases the risk of developing cardiovascular disease and diabetes. MetS includes multiple clinical traits including increased plasma glucose, abdominal obesity, dyslipidemia, and high blood pressure (Jeong et al., 2014). All current definitions of MetS include five clinical parameters in different combinations, namely: obesity, hypertriglyceridemia, low levels of high density lipoprotein, hypertension, and elevated levels of fasting glucose (Cohen et al., 2012).

Recent developments in single nucleotide polymorphism (SNP) typing, and the collation of information regarding linkage disequilibrium in the human genome, have facilitated genome-wide association studies (GWASs) investigating genes associated with disease susceptibility across the entire human genome (Bazzi et al., 2014). Recent GWASs have identified many loci in several genes that have been consistently associated with T2DM, hyperuricemia, and obesity, in various ethnic populations. The goal of this study was to identify the association of these loci with quantitative metabolic traits in Han Chinese with T2DM. A total of 1975 T2DM patients were recruited and 86SNPs were genotyped.

MATERIAL AND METHODS

Participants

We recruited 1975 T2DM patients in the city of Tianjin. All the subjects were unrelated Han Chinese receiving treatment at the Metabolic Disease Hospital of Tianjin Medical University, the General Hospital of Tianjin Medical University, the Tianjin People's Hospital, and the Eye Hospital of Tianjin Medical University. Genomic DNA samples were extracted from peripheral whole blood samples using the high-salt method, and were stored at -80°C until required for genotype testing. Genotyping was performed by primer extension of multiplex products with detection by matrix-assisted laser desorption time-of-flight mass spectrometry. Written informed consent was obtained from all participants prior to interview, and the protocol was approved by the Committee on Studies Involving Human Beings at Tianjin Medical University. The study was carried out in accordance with the approved guidelines.

The candidate genes chosen for the study were: 1) genes related to T2DM, obesity, or insulin resistance found by previous GWASs; and 2) genes related to glucose and lipid metabolism, and insulin secretion.

Anthropometric and laboratory measurements

All subjects were measured twice for height and weight (using identical standardized anthropometric scales), and body mass index (BMI) was calculated (kg/m^2). Diabetes mellitus was defined according to World Health Organization criteria [fasting plasma glucose ≥7.0 mM, and/or 2-h oral glucose tolerance test (OGTT) ≥11.1 mM], or the use of hypoglycemic drugs. Fasting blood samples were drawn after 12h of fasting followed by an OGTT (75g glucose) to evaluate glucose tolerance status and OGTT-related insulin release (samples for measurement of plasma glucose and serum insulin were drawn at 0, 30, 90, and 120 min). Serum uric acid (SUA) was measured by enzymatic methods (Chemistry Analyzer Au2700, Olympus Medical Engineering Company, Japan), and blood pressure (BP) was measured by a physician using a mercury sphygmomanometer. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG), and insulin were measured using an enzymatic luminescence technique. Values of estimated glomerular filtration rate (e-GFR; mL/min/1.73 m^2) were calculated using the equation proposed by investigators in the Chronic Kidney Disease Epidemiology (CKD-EPI) collaboration (Levey et al., 2009). Insulin resistance was estimated using homeostasis model assessment index-insulin resistance (HOMA-IR) (Vayá et al., 2014):

$$\text{HOMA-IR} = [\text{fasting insulin } (\mu\text{IU/mL}) \times \text{fasting glucose (mM)}/22.5]$$

Statistical analysis

All the analyses were calculated using the Statistical Package for Social Sciences (SPSS, version 18.0) for Windows. Descriptive data are presented as the mean, median, SD, skewness, kurtosis, minimum, and maximum for continuous variables, and as percentages for categorical variables. Because the distribution of TG levels was not normal, log-transformed TG levels were tested in this study. All phenotypes were documented in a FileMaker Pro database. An association

analysis was performed using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/). Nominal P values less than 0.05 were considered statistically significant.

RESULTS

In this study, 53.5% of participants were male and 46.5% were female, with mean ages (SD) of 56.83 (11.82) and 60.45 (11.78) years, respectively. The clinical characteristics of the participants included in the investigation are summarized in Table 1. Eighty-six SNPs were genotyped from 58 candidate genes. The Hardy-Weinberg equilibrium (HWE) test was performed before the association analysis.

Table 1. Basic characteristics of the type 2 diabetes mellitus (T2DM) subjects.

Characteristic	Means	M	s	Skewness	Kurtosis	Min	Max
Age (year)	58.51	59.00	11.94	-0.40	0.28	16.00	87.00
BMI (kg/m^2)	26.06	25.94	3.77	0.37	0.66	13.62	41.15
SBP (mmHg)	130.39	130.00	14.08	1.00	3.49	85.00	220.00
DBP (mmHg)	76.59	80.00	8.13	0.34	1.10	50.00	110.00
eGFR mL/min/1.73 m^2	96.89	96.35	33.69	0.24	0.16	7.14	229.77
SUA (μM)	310.60	301.40	89.92	0.58	0.38	100.60	653.50
LogTG (mM)	0.22	0.21	0.24	0.36	0.11	-0.44	0.99
TC (mM)	5.26	5.14	1.34	0.64	1.70	0.52	10.79
HDL (mM)	1.38	1.30	0.34	0.81	1.21	0.36	2.80
LDL (mM)	3.27	3.14	1.07	0.90	1.44	0.25	7.92
HbA1c (%)	7.96	7.60	1.79	0.90	0.74	4.00	15.20
FPG (mM)	8.17	7.60	2.66	1.20	1.78	2.16	19.00
P2PG (mM)	17.65	18.06	4.54	0.03	0.32	5.35	33.82
FINS (mIU/L)	12.29	8.50	12.99	2.88	9.95	0.20	82.98
P2INS (mIU/L)	47.04	34.90	38.11	1.82	4.33	2.97	257.60
ISI	-4.17	-4.12	1.00	-0.05	0.41	-7.17	-0.80
HOMA-IR	4.36	2.69	4.89	2.72	8.44	0.10	29.79
QUICKI	0.58	0.56	0.16	1.56	3.69	0.32	1.29

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; SUA = serum uric acid; LogTG = log-transformed plasma levels of triglycerides; TC = total cholesterol; HDL = high-density lipoprotein cholesterol; LDL = low density lipoprotein cholesterol; HbA1c = glycated hemoglobin; FPG = fasting plasma glucose; P2PG = plasma glucose 120 min post-OGTT (oral glucose tolerance test); FINS = fasting serum insulin; P2INS = serum insulin 120 min post-OGTT; ISI = insulinogenic index; HOMA-IR = homeostasis model assessment of insulin resistance; QUICKI = quantitative insulin sensitivity check index.

The 86 SNPs were tested for association with a number of metabolic and anthropometric quantitative phenotypes (Tables 2 and 3). Candidate genes associated with blood lipid traits and anthropometric phenotypes are shown in Table 2. The rs7546903 polymorphism of the *CAMTA1* gene exhibited suggestive pleiotropic associations with LogTG (P = 0.036) and diastolic blood pressure (DBP) (P = 0.044); rs16867321 of *UBE2E3* was associated with BMI (P = 0.015); rs11677793 of *SPAG16* was associated with systolic blood pressure (SBP) (P = 0.026), DBP (P = 0.002), and BMI (P = 0.025); rs1678607 of *VHL* was associated with LogTG (P = 0.015) and TC (P = 0.008); rs2929402 and rs1986917 of *KAT2B* were associated with LDL (P = 0.015) and TC (P = 0.003); rs7660895 and rs1014290 of *SLC2A9* were associated with SUA (P = 0.044 and 0.024, respectively); rs6856526 of *LPHN3* was associated with BMI (P = 0.043); rs2231142 of *ABCG2* was associated with SUA (P = 0.0004); rs10946398 and rs7756992 of *CDKAL1* were associated with BMI (P = 0.008 and 0.040, respectively); rs9395706 of *PKHD1* was associated with SUA (P = 0.027); rs705382 of *PON1* was associated with HDL (P = 0.020); rs7805834 of

NUB1 was associated with TC (P = 0.025); rs17304270 of *TOX* was associated with DBP (P = 0.049); rs10811661 of *CDKN2A*/*B* and rs7903146 of *TCF7L2* were associated with BMI (P = 0.030 and 0.036, respectively); rs2237892 of *KCNQ1* was associated with SBP (P = 0.010); rs2241220 of *ACACB* was associated with DBP (P = 0.040); rs371276 of *SLITRK5* was associated with TC (P = 0.047); rs1498506 of *SMAD3* was associated with HDL (P = 0.023) and BMI (P = 0.050); rs17818920 of *SMAD3* was associated with TC (P = 0.036) and LDL (P = 0.023); rs2239359 of *FANCA* was associated with LogTG (P = 0.026); rs4814615 of *PCSK2* was associated with LogTG (P = 0.022); rs735853 of *MYH9* was associated with SBP (P = 0.031), and rs2269532, rs2071731, and rs739097 of *MYH9* were associated with DBP (P = 0.005,0.013, and 0.028, respectively); and rs738409 of *PCSK2* was associated with BMI (P = 0.008).

Table 2. Single-nucleotide polymorphisms (SNPs) associated with blood lipid traits and anthropometric phenotypes (P values).

GENE	SNP	BP	Chr	HWE (P)	MAF*			Log TG	TC	HDL-C	LDL-C	SUA	SBP	DBP	BMI
					CHB	CEU	Global								
CAMTA1	rs7546903	6936272	1	0.603	0.463	0.226	0.368	**0.036**	0.375	0.263	0.250	0.470	0.251	**0.044**	0.131
UBE2E3	rs16867321	181362379	2	1.000	0.415	0.200	0.271	0.345	0.957	0.871	0.741	0.197	0.340	0.617	**0.015**
SPAG16	rs11677793	214161521	2	0.992	0.200	0.456	0.284	0.266	0.899	0.900	0.514	0.966	**0.026**	**0.002**	**0.025**
VHL	rs1678607	10188428	3	0.768	0.111	0.125	0.208	**0.015**	**0.008**	0.667	0.156	0.192	0.946	0.776	0.690
KAT2B	rs2929402	20096110	3	0.227	0.463	0.372	0.419	0.745	0.144	0.882	**0.007**	0.677	0.924	0.107	0.206
KAT2B	rs1986917	20118522	3	0.987	0.433	0.442	0.389	0.121	**0.003**	0.448	0.218	0.207	0.282	0.145	0.399
SLC2A9	rs7660895	9985445	4	0.317	0.366	0.248	0.352	0.904	0.725	0.396	0.992	**0.044**	0.897	0.474	0.864
SLC2A9	rs1014290	10001861	4	0.892	0.363	0.257	0.308	0.765	0.564	0.548	0.906	**0.024**	0.317	0.152	0.741
LPHN3	rs6856526	61057462	4	0.484	0.073	0.009	0.129	0.932	0.613	0.821	0.821	0.078	0.325	0.642	**0.043**
ABCG2	rs2231142	89052323	4	0.989	0.293	0.111	0.139	0.955	0.697	0.417	0.469	**0.000**	0.652	0.978	0.476
CDKAL1	rs10946398	20661034	6	0.851	0.439	0.336	0.408	0.501	0.461	0.683	0.528	0.299	0.452	0.579	**0.008**
CDKAL1	rs7756992	20679709	6	0.312	0.488	0.279	0.405	0.091	0.156	0.481	0.964	0.242	0.848	0.540	**0.040**
PKHD1	rs9395706	51544360	6	0.989	0.476	0.128	0.296	0.203	0.444	0.733	0.239	**0.027**	0.368	0.756	0.654
PON1	rs705382	94955221	7	0.755	0.415	0.336	0.472	0.326	0.626	**0.020**	0.209	0.425	0.512	0.199	0.083
NUB1	rs7805834	151043272	7	0.694	0.073	0.102	0.140	0.418	**0.025**	0.569	0.324	0.304	0.807	0.944	0.684
TOX	rs17304270	59979034	8	0.130	0.061	0.288	0.390	0.189	0.832	0.544	0.128	0.149	0.176	**0.049**	0.948
SLC30A8	rs13266634	118184783	8	0.930	0.476	0.239	0.282	0.237	0.079	0.367	0.417	0.484	0.295	0.817	0.661
CDKN2A/B	rs10811661	22134094	9	0.764	0.415	0.199	0.206	0.437	0.595	0.934	0.949	0.734	0.359	0.603	**0.030**
TCF7L2	rs7903146	114758349	10	0.236	0.024	0.279	0.218	0.059	0.197	0.452	0.594	0.224	0.497	0.517	**0.036**
KCNQ1	rs2237892	2839751	11	0.287	0.317	0.075	0.170	0.326	0.454	0.481	0.826	0.763	**0.010**	0.301	0.392
ACACB	rs2241220	109675029	12	0.996	0.341	0.142	0.109	0.884	0.486	0.165	0.135	0.500	0.693	**0.040**	0.196
SLITRK5	rs371276	89830501	13	0.615	0.463	0.025	0.244	0.691	**0.047**	0.647	0.450	0.186	0.938	0.113	0.296
SMAD3	rs1498506	67367634	15	0.840	0.433	0.475	0.455	0.946	0.941	**0.023**	0.623	0.871	0.196	0.194	**0.050**
FTO	rs17818920	53871903	16	1.000	0.183	0.250	0.258	0.454	**0.036**	0.378	**0.023**	0.920	0.930	0.735	0.713
FANCA	rs2239359	89849480	16	0.970	0.207	0.416	0.393	**0.026**	0.544	0.496	0.106	0.286	0.932	0.626	0.224
PCSK2	rs4814615	17357573	20	1.000	0.488	0.128	0.293	**0.022**	0.964	0.785	0.806	0.262	0.948	0.878	0.256
MYH9	rs735853	36679215	22	0.991	0.110	0.477	0.273	0.936	0.902	0.699	0.423	0.806	**0.031**	0.886	0.344
MYH9	rs2269532	36718039	22	0.881	0.267	0.358	0.390	0.881	0.190	0.526	0.949	0.890	0.511	**0.005**	0.834
MYH9	rs2071731	36718858	22	0.999	0.280	0.367	0.423	0.571	0.364	0.093	0.462	0.747	0.337	**0.013**	0.947
MYH9	rs739097	36746079	22	0.876	0.268	0.456	0.495	0.794	0.081	0.097	0.646	0.493	0.642	**0.028**	0.785
PNPLA3	rs738409	44324727	22	0.804	0.344	0.233	0.284	0.127	0.519	0.957	0.451	0.909	0.958	0.618	**0.008**

*MAF = minor allele frequencies, taken from dbSNP; CHB = Han Chinese; CEU = European American; HWE = Hardy-Weinberg equilibrium; LogTG = log-transformed plasma levels of triglycerides; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; SUA = serum uric acid; SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index.

We also tested for association with glycated hemoglobin (HbA1c), plasma glucose, and insulin resistance-related traits, and the results are shown in Table 3. The rs7546903 polymorphism of the *CAMTA1* gene was associated with P2PG (P = 0.044); rs62183937, rs11675251, and rs1376877 of *ABI2* were associated with HbA1c (P = 0.015, 0.046, and 0.025, respectively);

rs1678607of *VHL* was associated with P2PG (P = 0.032); rs2929402 of *KAT2B* was associated with P2PG (P = 0.005), insulinogenic index (ISI) (P = 0.019), and quantitative insulin sensitivity check index (QUICKI) (P = 0.032); rs2231142 of *ABCG2* was associated with HbA1c (P = 0.012); rs1165196 of *SLC17A1* was associated with HbA1c (P = 0.040); rs9395706 of *PKHD1* was associated with HbA1c (P = 0.004); rs722208 of *ESR1* was associated with fasting plasma glucose (FPG) (P = 0.045); rs1581498 of *intergenic* was associated with homeostasis model assessment of insulin resistance (HOMA-IR) (P = 0.042); rs1799884 of *GCK* was associated with QUICKI (P = 0.050); rs705382 of *PON1* was associated with serum insulin 120 min post-OGTT (P2INS) (P = 0.017); rs11777927 of *TOX* was associated with HbA1c (P = 0.011); rs13266634 of *SLC30A8* was associated with HbA1c (P = 0.033); rs7923837 of *HHEX* was associated with fasting serum insulin (FINS) (P = 0.039) and QUICKI (P = 0.045); rs7903146 of *TCF7L2* was associated with FINS (P = 0.039), P2INS (P = 0.002), ISI (P = 0.0004), HOMA-IR (P = 0.039), and QUICKI (P = 0.001); rs2237892 of *KCNQ1* was associated with P2INS (P = 0.016); rs7312112 of *IGF1* was associated with P2INS (P = 0.044); rs11067076 of *TBX5* was associated with HOMA-IR (P = 0.041); rs5753669 of *SFI1* was associated with P2PG (P = 0.046); and rs2071731 of *SFI1* was associated with P2PG (P = 0.026). We would like to emphasize that these quantitative trait associations are of nominal significance and therefore are not corrected for multiple testing.

Table 3. Single-nucleotide polymorphisms (SNPs) associated with glycosylated hemoglobin (HbA1c) and insulin resistance-related traits (P values).

GENE	SNP	BP	Chr	HWE (P)	MAF*			HbA1c	FPG	P2PG	FINS	P2INS	ISI	HOMA	QUICKI
					CHB	CEU	Global								
CAMTA1	rs7546903	6936272	1	0.603	0.463	0.226	0.368	0.634	0.271	**0.044**	0.145	0.481	0.129	0.141	0.378
ABI2	rs62183937	204193688	2	1.000	0.475	0.125	0.258	**0.015**	0.807	0.604	0.094	0.707	0.236	0.111	0.738
ABI2	rs11675251	204249399	2	0.303	0.171	0.482	0.381	**0.046**	0.492	0.302	0.086	0.966	0.366	0.338	0.562
ABI2	rs1376877	204272090	2	0.177	0.171	0.455	0.383	**0.025**	0.363	0.340	0.075	0.837	0.319	0.331	0.487
VHL	rs1678607	10188428	3	0.768	0.111	0.125	0.208	0.413	0.540	**0.032**	0.447	0.332	0.713	0.484	0.857
KAT2B	rs2929402	20096110	3	0.227	0.463	0.372	0.419	0.640	0.450	**0.005**	0.469	0.758	**0.019**	0.388	**0.032**
ABCG2	rs2231142	89052323	4	0.989	0.293	0.111	0.139	**0.012**	0.673	0.659	0.449	0.439	0.194	0.621	0.447
SLC17A1	rs1165196	25813150	6	0.205	0.232	0.451	0.260	**0.040**	0.183	0.239	0.585	0.938	0.665	0.126	0.777
PKHD1	rs9395706	51544360	6	0.989	0.476	0.128	0.296	**0.004**	0.889	0.418	0.661	0.931	0.622	0.374	0.212
ESR1	rs722208	152322885	6	0.583	0.500	0.246	0.412	0.532	**0.045**	0.344	0.695	0.821	0.636	0.911	0.481
intergenic	rs1581498	22908243	7	0.362	0.400	0.467	0.378	0.514	0.817	0.378	0.093	0.256	0.577	**0.042**	0.866
GCK	rs1799884	44229068	7	0.084	0.171	0.195	0.188	0.949	0.385	0.263	0.244	0.278	0.078	0.432	**0.050**
PON1	rs705382	94955221	7	0.755	0.415	0.336	0.472	0.866	0.891	0.129	0.071	**0.017**	0.496	0.084	0.969
TOX	rs11777927	59881039	8	0.757	0.356	0.267	0.356	**0.011**	0.250	0.474	0.586	0.287	0.223	0.544	0.154
SLC30A8	rs13266634	118184783	8	0.930	0.476	0.239	0.282	**0.033**	0.865	0.701	0.657	0.399	0.430	0.827	0.394
HHEX	rs7923837	94481917	10	1.000	0.244	0.367	0.427	0.383	0.355	0.268	**0.039**	0.112	0.091	0.203	**0.045**
TCF7L2	rs7903146	114758349	10	0.236	0.024	0.279	0.218	0.056	0.147	0.253	**0.039**	**0.002**	**0.000**	**0.039**	**0.001**
KCNQ1	rs2237892	2839751	11	0.287	0.317	0.075	0.170	0.072	0.803	0.553	0.189	**0.016**	0.191	0.263	0.359
IGF1	rs7312112	103046823	12	0.304	0.500	0.385	0.462	0.702	0.986	0.152	0.803	0.091	0.110	0.208	**0.044**
TBX5	rs11067076	114799863	12	0.586	0.037	0.257	0.193	0.735	0.618	0.169	0.430	0.750	0.102	**0.041**	0.222
SFI1	rs5753669	31905819	22	0.497	0.378	0.283	0.271	0.806	0.136	**0.046**	0.203	0.229	0.536	0.771	0.458
MYH9	rs2071731	36718858	22	0.999	0.280	0.367	0.423	**0.026**	0.900	0.257	0.645	0.942	0.825	0.669	0.796

*MAF = minor allele frequencies, taken from dbSNP; CHB = Han Chinese; CEU = European American; HWE = Hardy-Weinberg equilibrium; HbA1c = glycosylated hemoglobin; FPG = fasting plasma glucose; P2PG = plasma glucose 120 min post-OGTT (oral glucose tolerance test); FINS = fasting serum insulin; P2INS = serum insulin 120 min post-OGTT; ISI = insulinogenic index; HOMA-IR = homeostasis model assessment of insulin resistance; QUICKI = quantitative insulin sensitivity check index.

DISCUSSION

T2DM, which is characterized by insulin resistance and hyperglycemia, is associated with a

marked increase in the risk of cardiovascular and metabolic diseases, such as obesity, hypertension, and dyslipidemia (Go et al., 2014). Although the precise mechanisms underlying the development and progression of T2DM have not been fully elucidated, a combination of multiple genetic and environmental factors is considered to contribute to the pathogenesis of the disease. The heritability of T2DM is relatively strong, with an estimated h^2 of 31-69% (Chang et al., 2014). Genome-wide linkage analysis, GWASs, and candidate gene approaches have been used wildly to decipher the genetic basis of T2DM. Over 30 T2DM susceptibility loci with different effect sizes have been identified and replicated by genetic association studies, and several genome-wide association scans for T2DM have also been carried out recently (Rung et al., 2009; Langberg et al., 2012).

Our findings indicated that *CAMTA1*, *ABI2*, *VHL*, *KAT2B*, *PKHD1*, *ESR1*, *TOX*, *SLC30A8*, *SFI1*, and *MYH9* gene polymorphisms were associated with HbA1c, FPG, and/or P2PG ($P < 0.05$). We also discovered that *GCK*, *HHEX*, *TCF7L2*, *KCNQ1*, and *TBX5* gene polymorphisms were associated with insulin resistance-related traits ($P < 0.05$). *KCNQ1*, located on 11p15.5, encodes the pore-forming α subunit of the $I_{Ks}K^+$ channel. Moreover, mutations in *KCNQ1* have been reported to cause long QT syndrome through the loss of function of the slowly activating K^+ channel in the heart. Unoki et al. (2008) reported that SNPs in the *KCNQ1* gene were significantly associated with T2DM in populations of both East Asian and European descent.

SLC30A8, with the chromosomal locus 8q24.11, encodes zinc transporter protein member 8 (ZnT8), which is a zinc transporter specific to the beta cells of the pancreas that transports zinc into the insulin secretory vesicles of the beta cells. The *SLC30A8* gene is highly expressed in the pancreas, particularly in α-, β-, and pancreatic polypeptide producing (PP) cells of the islets of Langerhans. Increased DNA methylation of the *SLC30A8* gene is associated with T2DM (Seman et al., 2015). Rutter and Chimienti (2015) reported that the common rs13266634 polymorphism is associated with reduced β-cell function and a 14% increase in diabetes prevalence per risk (C) allele. Zinc supplementation appears to differentially affect the early insulin response to glucose in the rs13266634 genotype, and could be beneficial for diabetes prevention (Maruthur et al., 2015). The *TCF7L2* gene product is a high mobility group box-containing transcription factor previously implicated in blood glucose homeostasis. It is one of the most significant diabetes susceptibility genes identified to date in various populations. A previous case-control association study by Lewis et al. (2008) reported a significant association between *TCF7L2* rs7903146 and T2DM in an African American population. Qian et al. (2015) conducted a quantitative trait analysis and showed that the AC genotype of rs1552224 presented higher FBG than the AA/CC genotypes in a control population with normal glucose. Mechanistic studies suggest that *TCF7L2* can impair β-cell function, and down regulate the expression levels of glucagon-like peptide 1 receptor (GLP-1R) and glucose-dependent insulinotropic polypeptide receptor (GIP-R), thereby reducing insulin levels (Zhai et al., 2014).

Serum uric acid (SUA) is the final oxidation product of purine metabolism in the circulation. Hyperuricemia (HUA) is a condition in which the subject has increased serum uric acid levels. Studies have noted that an elevated level of uric acid predicts the development of diabetes, obesity, hypertension, and metabolic syndrome (Wang et al., 2013). Liu et al. (2011) systematically analyzed the prevalence of HUA in a general Chinese population using the meta-analysis method, and reported that the prevalence was 8.6% in women and 21.6% in men. Both environmental and genetic factors play an important role in the etiology of HUA and gout. GWASs have uncovered over 30 common sequence variants that influence SUA concentration and gout (Karns et al., 2012). Our study replicated the associations between SNPs of *ABCG2* and *SLC2A9* and SUA

concentration in a Chinese Han population. We also identified a novel locus suggestive of an association with uric acid levels (*PKHD1*, P = 0.027), although the signals did not reach genome-wide significance. *SLC2A9* and *ABCG2* are the genes most strongly associated with regulating serum urate concentration. Yang et al. (2014) reported that the loci of *ABCG2* and *SLC2A9* could explain 1.09 and 1.03% of the variation in SUA levels, respectively. *ABCG2* (ATP-binding cassette, subfamily G, member 2, 4q22), is a uric acid exporter that mediates urate excretion in the kidney. It is expressed in the brush border membrane of the proximal tubules of the kidneys, and is also abundantly expressed in the apical membrane of epithelial cells in the small intestine and liver. These findings suggest a physiological role for *ABCG2*,not only in renal urate excretion but also gut urate excretion via intestinal and biliary secretion in humans (Matsuo et al., 2011). Dehghan et al. (2008) conducted a genome-wide study in a Framingham cohort and a Rotterdam cohort, and reported that an SNP in the *ABCG2* gene, rs2231142, displayed strong evidence of an association with uric acid levels ($P < 10^{-60}$). A meta-analysis of 39,853 people from four different population-based samples (European Americans, African Americans, Mexican Americans, and American Indians) demonstrated that the functional variant rs2231142 (Q141K) in the *ABCG2* gene was significantly associated with both SUA level and gout (Zhang et al., 2013). The *SLC2A9* gene, which has the chromosomal locus 4p16.1, encodes a protein called solute carrier family 2, facilitated glucose transporter member 9 (SLC2A9), also known as glucose transporter type 9 (GLUT9); it is a glucose transporter and plays a significant role in maintaining glucose homeostasis. SLC2A9 is a transporter for both fructose and urate. Fructose intake can facilitate uric acid formation in the liver via increasing purine breakdown (Vitart et al., 2008). *SLC2A9* is a causative gene for renal HUA and plays a significant role in urate reabsorption on renal proximal tubular cells (Han et al., 2015). Hamajima et al. (2011) found that the effect of *SLC2A9* rs11722228 on mean SUA was larger for females than for males in a Japanese population.

The results of our study also found a number of genes associated with blood lipid traits and anthropometric phenotypes: *CAMTA1*, *VHL*, *KAT2B*, *PON1*, *NUB1*, *SLITRK5*, *SMAD3*, *FTO*, *FANCA*, and *PCSK2* were associated with blood lipid traits (P < 0.05); *CAMTA1*, *SPAG16*, *TOX*, *KCNQ1*, *ACACB*, and *MYH9* were associated with blood pressure (P < 0.05); and *UBE2E3*, *SPAG16*, *SLC2A9*, *CDKAL1*, *CDKN2A/B*, *TCF7L2*, *SMAD3*, and *PNPLA3* were associated with BMI (P < 0.05). Dyslipidemia plays a major role in the development of cardiovascular disease in T2DM patients, in whom lipid abnormalities are characterized by hypertriglyceridemia and reduced levels of HDL cholesterol, present mainly in the form of small, dense HDL particles. Insulin resistance, and possibly hyperinsulinemia, probably underlie the lipid-related changes associated with T2DM (Malhotra et al., 2005). Obesity, hypertension, and dyslipidemia as components of metabolic syndrome were closely related to the prevalence of diabetes. Hypertension and diabetes mellitus increasingly occur together in humans, and when they do, they make patients more vulnerable to other cardiovascular diseases and increase mortality (Mozafari et al., 2015). The protein encoded by the *CDKAL1* gene is a member of the methylthiotransferase family and shares considerable domain and amino acid homology with *CDK5RAP1*, an inhibitor of cyclin-dependent kinase 5 (CDK5) activation. Bao et al. (2012) reported that the C allele of *CDKAL1* rs7754840 was significantly associated with increased FPG levels in lean Han Chinese individuals. The association between *FTO* variants and BMI in T2DM has been independently identified in European populations and South Asian Indians, although this association may not be entirely mediated through BMI. The functional role of the *FTO* gene is not yet understood, nor is it clear how the variants affect body size and predict the risk of T2DM (Dina et al., 2007; Yajnik et al., 2009).

The *CDKN2A* and *CDKN2B* genes, which have chromosomal loci in the 9p21 region, encode $p16^{INK4a}$ and $p15^{INK4b}$ proteins, respectively; $p16^{INK4a}$ and $p15^{INK4b}$ are tumor suppressors that inhibit cyclin-dependent kinase 4 (CDK4) and CDK6, respectively, which are two regulators of pancreatic β-cell replication (Li et al., 2013). Several loci near or in the *CNKN2A/2B* gene are involved in T2DM susceptibility. *CDKN2A/B* polymorphisms are associated with impaired insulin release and impaired glucose tolerance, and the TT genotype is associated with higher 2-h post-load glucose levels (Hribal et al., 2011; Parra et al., 2011). The *MYH9* (non-muscle myosin heavy chain 9) gene encodes non-muscle myosin IIA and is expressed in glomerular podocytes and mesangial cells. Cooke et al. (2012) also found that *MYH9* SNPs rs4821480, rs2032487, rs4281481, and rs3752462 are associated with T2DM end-stage renal disease susceptibility in European Americans. In the present study, we confirmed that four SNPs (rs735853, rs2269532, rs2071731, and rs739097) of the *MYH9* gene are associated with SBP and DBP, but Cheng et al. (2011) arrived at different conclusions: they decided that *MYH9* may play an important role in mediating nephropathy in the context of IgAN, but that there was no genetic association between the SNPs and the clinical characteristics of IgAN, such as SBP, DBP, eGFR, and urinary protein excretion.

In the present study, we investigated some of the genes associated with the metabolic and anthropometric traits of T2DM in Han Chinese. Although we did not identify any genes associated with quantitative traits that reached genome-wide significance, we did report a number of genes and loci that are worthy of further study based on replication of other studies or on quantitative trait loci consistency. This report provides a valuable resource for other investigators in the search for the pathogenic variants of quantitative traits in T2DM.

Conflicts of interest

The authors declare no conflict of interest.

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