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Microsatellite and Single Nucleotide Polymorphisms in the *Insulin-Like Growth Factor 1* Promoter with Insulin Sensitivity and Insulin Secretion

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCE 1,2 Ruyao Wang
BC 2 Dandan Xu
A 2 Rui Liu
B 2 Lijie Zhao
D 2 Liling Hu
F 2 Ping Wu

1 Graduate School, Tianjin Medical University, Tianjin, P.R. China
2 Department of Clinical Laboratory, Tianjin Union Medical Center, Tianjin, P.R. China

Corresponding Author: Rui Liu, e-mail: lr3596@163.com
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Background: To investigate associations of the CA microsatellite and rs35767, rs5742612, and rs2288377 polymorphisms and the single nucleotide polymorphism (SNP) haplotypes with and without the CA microsatellite in the *IGF1* promoter with insulin sensitivity and secretion.

Material/Methods: The CA microsatellite and SNPs were genotyped in 389 type 2 diabetes mellitus (T2DM) patients. A 75 g oral glucose tolerance test (OGTT) was given to all the participants. Associations of the genotypes and haplotypes with insulin sensitivity, insulin secretion, glucose tolerance, and insulin-like growth factor 1 (IGF1) were analyzed by ANCOVA (general linear model) and multiple linear regression, after controlling for gender, age, and BMI.

Results: The CA microsatellite, rs35767 polymorphisms, and SNP haplotypes with or without CA showed no significant association with metabolic parameters. The C allele of rs5742612 was found to be associated with decreased insulin sensitivity (HOMA-S index, β=–0.131, P=0.008; fasting insulin level, β=0.022, P=0.006) and increased insulin secretion (HOMA-B index, β=0.099, *P*=0.008; insulin AUC, β=0.112, *P*=0.012). The linear regression model also indicated that the A allele of rs2288377 was associated with decreased insulin sensitivity (HOMA-S index, β=–0.159, *P*=0.001; fasting insulin, β=0.143, *P*=0.001) and increased insulin secretion (HOMA-B index, β=0.114, *P*=0.017; insulin AUC, β=0.042, *P*=0.002).

Conclusions: The CA microsatellite and rs35767 have no genotype-related difference in insulin sensitivity or secretion. The rs5742612 and rs2288377 polymorphisms are significantly associated with insulin biology, with the TT genotype exhibiting higher insulin sensitivity and lower insulin secretion compared with carriers of the C allele and A allele, respectively, mostly attributed to the direct functional roles of the two *loci*.

MeSH Keywords: Insulin Resistance • Insulin-Like Growth Factor I • Microsatellite Repeats • Polymorphism, Single Nucleotide

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Background

Type 2 diabetes mellitus (T2DM) is a complex condition of hyperglycemia caused by impaired insulin sensitivity and deficient β cell function [1]. Sets of susceptibility *loci* involved in the process of insulin sensitivity and/or secretion have been proposed [2]. A meta-analysis conducted by the genome-wide association studies identified two *loci* associated with fasting insulin and insulin resistance and 16 *loci* associated with fasting glucose and β cell function [3]. Meanwhile, the genome-wide association studies elucidated 60 T2DM- and glycemic trait-related *loci* [4]. Another more recent study of the association of genetic scores with a range of metabolic measures in up to 18,565 individuals also validated the role of genetic variants as instruments for insulin resistance and secretion [5].

Insulin-like growth factor 1 (IGF1) has been reported to play important roles in regulating glucose homeostasis and insulin sensitivity [6]. There is evidence that *IGF1* gene deletion is associated with reduced insulin sensitivity [7], and IGF1 treatment results in positive effects on insulin sensitivity [8–10]. A CA repeat microsatellite located on the 979 bp upstream of *IGF1* is often reported to correlate with insulin sensitivity and/or risk of T2DM. However, there are controversial hypotheses and observations regarding such an association. Some studies suggested a positive association between the CA19 (with 19 CA repeats) allele, which is the most common one, and increased risk of T2DM [11], whereas other studies observed an opposite association [12], implying that the microsatellite may not be the sole regulatory unit in the promoter region. Moreover, a recent study of the short- and long-term metabolic response to recombinant human growth hormone (GH) in GH-deficient adults suggested that the impact showed obvious gene specificity and after 1 year of GH, a significant worsening of insulin sensitivity was recorded only in homozygous CA19 repeats subjects [13]. Besides, it has been shown that a functional single-nucleotide polymorphism (SNP), rs35767: T>C (NM 000618.3: c.-1410T>C), was also associated with glucose homeostasis and insulin sensitivity in which carriers of the GG genotype exhibited lower insulin sensitivity and IGF1 concentrations compared with subjects carrying the A allele [14]. In this way, both the microsatellite and SNPs may have functional roles in the regulation of insulin sensitivity and insulin secretion.

In a systematic survey of human variations in the cis-regulatory regions, *IGF1* was 1 of the 23 genes that show consistent allelic imbalance, implying that certain functional elements lie within *IGF1* regulatory region among individuals [15]. This was confirmed in another study. Chen et al. revealed that there was strong linkage disequilibrium (LD) between the CA microsatellite and SNPs in the promoter of the *IGF1* gene, which constituted specific haplotype patterns in the population [16] The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China.</auth-address><titles><title>Haplotype effect in the IGF1 promoter accounts for the association between microsatellite and serum IGF1 concentration</title><secondary-title>Clin Endocrinol (Oxf. As the CA microsatellite and SNPs are in close proximity with each other, and the existence of LD, in this study, we aimed to investigate the role of the CA microsatellite and each SNP in the regulation of insulin sensitivity and insulin secretion and to examine if the microsatellite and SNPs affected each other by functional genetic approaches in this procedure.

Material and Methods

Study subjects

This study included 389 Tianjin native patients of the Han nationality with T2DM, treated in Tianjin Union Medical Center from November 2012 to June 2014. T2DM was defined according to the 1999 clinical practice recommendations by the American Diabetes Association. We excluded individuals with Cushing syndrome, hyperthyroidism, or hypothyroidism; pregnancy; or history of cardiovascular disease or cancer. A simplified questionnaire for sex, age, and family history of T2DM and anthropometrical measurements of body mass index (BMI) was administered by trained nurses to all the participants. Blood samples were collected in the early morning after hospitalization and 8 hours of fasting. Venous blood samples were collected using tubes with coagulant. Participants were then given a standard 75 g oral glucose tolerance test (OGTT) with 60- and 120-min blood samples drawn. Serum glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL cholesterol), and low-density lipoprotein cholesterol (LDL cholesterol) were measured by enzymatic methods using an automatic biochemical analyzer ARCHITECT CI16000 (Abbott, Abbott Park, Illinois, USA) and a reagent kit provided by Roche, Switzerland. Serum insulin and peptide C concentrations were determined by the electrochemiluminescence method (Cobas e411, Roche, Switzerland) and the matched reagents. Serum levels of IGF1 were measured by the electrochemiluminescence method using Immulite 2000 (Siemens, Germany), with reagents from Diagnostic Products Corp. Hemoglobin A1c (HbA1c) was measured by high performance liquid chromatography using Hb9210 (Premier, China) with the matched reagents.

tagSNPs selection

According to previous studies, the evolutionarily conserved region (ECR) was defined by >75% identity between human and three other species [17, 18]. Three polymorphisms, rs35767: T>C (NM 000618.3: c.-1410T>C), rs5742612: T>C (NM000618.3: c.-705T>C), and rs2288377: T>A (NM 000618.3: c.-603T>A),

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were selected as tagSNPs, as they covered 100% variance in the ECR of the IGF1 promoter. The SNP information was obtained from the National Center for Biotechnology Information (NCBI) dbSNP. Common haplotype was defined as haplotype with a frequency of >5% in the population.

DNA analysis

Genomic DNA was extracted from blood leukocytes using the High Pure Polymerase Chain Reaction (PCR) Template Preparation Kit (Tiangen Biotech, Beijing, China), according to the protocol of the manufacturer. DNA samples were stored at –80°C until use.

The CA microsatellite of the *IGF1* gene located at 979 bp upstream from the transcription start site was determined by PCR and fragment analysis using the ABI-3730XL DNA Sequencer (Applied Biosystems, Foster City, California, USA). PCR primers were 5'- AGAAAACACACTCTGGCACAC-3' and 5'-GCAAAGACTCTGCCGAGCTG-3' (Invitrogen, Shanghai, China). The forward primer was 5' labeled with a fluorescent dye (FAM). The PCR reaction was performed in a final volume of 50 µL containing 10 ng of DNA, 10× PCR buffer 5 µL, 50 mm $MgCl_2$ 1.6 µL, 2 mm dNTPs 4 µL, 5 µm each of the primers 2 µL, and 1.5 units of Platinum® Taq DNA polymerase (Invitrogen Trading, Shanghai, China). The amplified conditions were 95°C/5 min followed by 35 thermal cycles (95°C/30 s, 60°C/30 s, and 72°C/30 s) and a final extension at 72°C/5 min. The length of amplified fragments was determined by GeneScan 3.0 and GeneMapper analysis software (Applied Biosystems, Foster City, California, USA). The fragments ranged in size from 227 to 257 bp, depending on the number of CA repeats.

Genotyping for rs35767, rs5742612, and rs2288377 in the promoter region was done by PCR followed by gene sequencing (ABI-3730XL, Applied Biosystems, Foster City, California, USA). The sequencing results were compared with reference sequences in NCBI. The sequence of forward and reverse primers of the three SNPs was designed according to NCBI. Primers for rs35767 were 5'-TTGGGCACATAGTAGAGCTCAC-3' and 5'-CAAAAGCCCAGAGCAGACAT- 3' (405 bp); primers for rs5742612 and rs2288377 were 5' -GGTTTTACAGCTCGGCATAGTC-3' and 5'-TCTGCTGGGCATGAAGACAC- 3' (359 bp).

Calculations

The area under the curve (AUC) for insulin and glucose was calculated by the trapezoidal method. The homeostasis model assessment-sensitivity (HOMA-S) index was calculated as 22.5/(fasting insulin×fasting glucose). The homeostasis model assessment-beta (HOMA-B) index was calculated as (20×fasting insulin)/(fasting glucose–3.5).

Statistical analysis

Categorical variables were analyzed using a χ^2 test. For intergroup comparisons, continuous normal distribution variables were analyzed by analysis of covariance (ANCOVA), general linear model, after adjusting for gender, age, and BMI, and expressed as mean ± standard deviations. Continuous non-normal distribution variables were analyzed by the Kruskal-Wallis H test and expressed as median (25%, 75%) percentiles. The Hardy-Weinberg equilibriums among the genotypes were in Hardy-Weinberg equilibrium by the χ^2 test (all P>0.001). Associations between the CA microsatellite, SNPs, haplotypes of the ECR, and continuous traits were estimated by multiple linear regression analysis controlling for gender, age, and BMI, and expressed as β and SE per allele, with non-normal distribution variables log-transformed first. The analysis was conducted according to both an additive and a dominant genetic model. All statistical analyses were performed according to SPSS 19.0 software. A two-sided $P<0.05$ was considered as statistically significant.

Ethics

The protocol for this study was compatible with the local ethical guidelines. The study was approved by the Academic Committees in Tianjin Union Medical Center. Informed consent was obtained from all patients.

Results

The study included 389 patients with T2DM. Clinical characteristics according to the CA microsatellite, SNPs, and haplotypes of the ECR by ANCOVA and associations of the CA microsatellite, SNPs, and haplotypes with continuous traits by multiple linear regression analysis are shown in Tables 1–6 separately.

CA microsatellite

We compared other genotypes with the CA19/CA19 homozygote as it is the most common one. The CA microsatellite genotype did not show any significant association with gender, family history of T2DM, anthropometric or metabolic parameters, or IGF1 levels. However, we observed a significant difference in age, total cholesterol, and LDL cholesterol among subjects with different CA microsatellites. The linear regression analysis found no significant contribution of the CA microsatellite to metabolic parameters, after adjusting for gender, age, and BMI (Table 1).

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Table 1. Clinical characteristics of the 389 study population according to different CA microsatellite and multiple linear regression analysis for association of CA repeat with metastatic traits.

	CA19/CA19	CA19/Other	Other/Other	P	P (non-CA19/19 *vs.* CA19/19)	β	SE	R^2 (%)	P
N(M/F)	34/17	81/57	121/79	0.608	0.362				
Age (years)	62±8	65±10	65±9	0.073	0.006				
Family history of T2DM (Y/N)	23/28	82/56	111/89	0.213	0.131				
Anthropometry									
Height (cm)	166.86±8.21	166.93±8.11	166.81±8.04	0.351	0.997				
BMI (kg/m^2)	24.60±2.89	25.53±3.21	25.13±3.77	0.166	0.185				
Total cholesterol (mmol/l)	3.54±1.29	3.21±1.10	3.10±1.06	0.035	0.016				
Triacylglycerol (mmol/l)	1.50 (0.96, 2.15)	1.35 (0.94, 1.80)	1.32 (0.85, 1.86)	0.284	0.116				
HDL-cholesterol (mmol/l)	0.78 (0.63, 0.98)	0.73 (0.58, 0.92)	0.73 (0.55, 0.91)	0.456	0.236				
LDL-cholesterol (mmol/l)	2.09 (1.68, 2.64)	1.94 (1.51, 2.48)	1.87 (1.44, 2.36)	0.070	0.039				
VLDL-cholesterol (mmol/l)	0.68 (0.44, 0.98)	0.61 (0.43, 0.82)	0.61 (0.39, 0.83)	0.254	0.099				
OGTT									
Fasting insulin (mU/L)	12.46 (8.87, 24.37)	11.19 (6.92, 23.00)	11.35 (7.21, 21.40)	0.809	0.515				
60-min insulin (mU/L)	34.48 (18.10, 61.62)	31.83 (17.96, 66.73)	33.41 (19.44, 51.10)	0.908	1.847				
120-min insulin (mU/L)	36.20 (20.02, 71.71)	37.38 (20.78, 69.90)	35.93 (22.37, 66.12)	0.957	0.791				
Fasting peptide C (ng/ml)	2.56 (1.74, 4.08)	2.70 (2.03, 3.87)	2.62 (1.86, 3.77)	0.447	0.776				
60-min peptide C (ng/ml)	4.31 (3.14, 8.20)	3.29 (4.77, 7.13)	4.71 (3.16, 6.69)	0.893	0.920				
120-min peptide C (ng/ml)	5.91 (4.04, 9.21)	6.07 (4.03, 9.28)	6.03 (3.97, 8.71)	0.916	0.783				
Fasting glucose (mmol/L)	10.25±4.51	10.65±4.03	10.41±4.06	0.705	0.516				
60-min glucose (mmol/L)	16.69±3.99	16.72±4.01	16.88±4.15	0.901	0.771				
120-min glucose (mmol/L)	18.32±5.62	18.64±4.94	18.50±5.33	0.941	0.849				
Metastatic parameters									
Insulin sensitivity									
HOMA-S index[a]	0.22 (0.11, 0.33)	0.20 (0.09, 0.36)	0.20 (0.10, 0.36)	0.951	0.807	–0.010	–	–	0.836
Fasting insulin (mU/L)[a]	12.46 (8.87, 24.37)	11.19 (6.92, 23.00)	11.35 (7.21, 21.40)	0.809	0.515	–0.002	–	–	0.963

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Table 1 continued. Clinical characteristics of the 389 study population according to different CA microsatellite and multiple linear regression analysis for association of CA repeat with metastatic traits.

	CA19/CA19	CA19/Other	Other/Other	P	P (non-CA19/19 *vs.* CA19/19)	β	SE	R^2 (%)	P
Insulin secretion									
HOMA-B index (%)[a]	50.28 (25.39, 102.01)	40.69 (20.70, 98.23)	43.79 (23.21, 88.08)	0.630	0.368	–0.027	–	–	0.585
Insulin AUC (mU/L*h)[a]	65.38 (33.62, 94.36)	59.67 (33.18, 103.89)	61.48 (35.32, 96.12)	0.935	0.802	0.001	–	–	0.979
Glucose tolerance									
Glucose AUC (mmol/L*h)	30.98±8.17	31.37±7.64	31.36±7.99	0.929	0.702	0.010	–	–	0.836
Fasting glucose (mmol/L)	10.25±4.51	10.65±4.03	10.41±4.06	0.705	0.705	0.018	–	–	0.723
120-min glucose (mmol/L)	18.32±5.62	18.64±4.94	18.50±5.33	0.941	0.941	0.010	–	–	0.842
IGF1 (ng/ml)	136.70±71.18	124.94±63.64	135.52±65.40	0.402	0.847	0.003	–	–	0.959
HbA1c (%)	7.84±1.78	8.13±1.79	8.08±1.82	0.675	0.396	0.043	–	–	0.392

T2DM – type 2 diabetes mellitus; IGF1 – insulin like growth factor 1; CA – cytosine-adenosine; BMI – body mass index; OGTT – oral glucose tolerance test; HDL-cholesterol – high-density lipoprotein cholesterol; LDL-cholesterol – low-density lipoprotein cholesterol; HbA1c – hemoglobin A1c; AUC – area under the curve; HOMA-S – homeostasis model assessment-sensitivity; HOMA-B – homeostasis model assessment-beta. [a] Log-transformed before multiple linear regression analysis.

Table 2. Clinical characteristics of the 389 study population according to SNP rs35767 and multiple linear regression analysis for association of rs35767 with metastatic traits.

	CC	CT	TT	P	P (CT+TT *vs.* CC)	β	SE	R^2 (%)	P
N (M/F)	92/61	109/70	35/22	0.983	0.195				
Age (years)	65±9	65±9	67±10	0.370	0.838				
Family history of T2DM (Y/N)	84/69	102/77	30/27	0.831	0.917				
Anthropometry									
Height (cm)	166.68±8.00	166.75±7.98	167.68±8.61	0.574	0.725				
BMI (kg/m^2)	24.92±3.25	25.60±3.27	24.71±4.49	0.102	0.194				
Total cholesterol (mmol/l)	3.40±1.21	3.07±1.04	3.01±1.01	0.015	0.004				
Triacylglycerol (mmol/l)	1.42 (0.91, 2.02)	1.30 (0.92, 1.80)	1.25 (0.82, 1.94)	0.257	0.115				
HDL-cholesterol (mmol/l)	0.72 (0.58, 0.91)	0.75 (0.58, 0.96)	0.75 (0.60, 0.89)	0.834	0.760				
LDL-cholesterol (mmol/l)	2.03 (1.57, 2.63)	1.82 (1.46, 2.34)	1.93 (1.37, 2.30)	0.028	0.008				
VLDL-cholesterol (mmol/l)	0.65 (0.42, 0.92)	0.59 (0.42, 0.82)	0.55 (0.36, 0.81)	0.141	0.095				

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Table 2 continued. Clinical characteristics of the 389 study population according to SNP rs35767 and multiple linear regression analysis for association of rs35767 with metastatic traits.

	CC	CT	TT	P	P (CT+ TT *vs.* CC)	β	SE	R^2 (%)	P
OGTT									
Fasting insulin (mU/L)	10.95 (6.49, 20.86)	12.16 (7.63, 23.01)	13.86 (8.27, 26.35)	0.241	0.104				
60-min insulin (mU/L)	30.50 (18.28, 53.58)	33.48 (18.80, 59.72)	34.93 (19.29, 51.16)	0.533	0.262				
120-min insulin (mU/L)	35.13 (19.70, 58.05)	37.28 (22.61, 69.34)	40.64 (21.91, 79.37)	0.222	0.083				
Fasting peptide C (ng/ml)	2.56 (1.78, 3.80)	2.70 (2.02, 3.93)	2.67 (1.73, 3.50)	0.457	0.350				
60-min peptide C (ng/ml)	4.53 (3.21, 6.25)	4.85 (3.14, 7.47)	4.72 (3.31, 6.23)	0.495	0.358				
120-min peptide C (ng/ml)	5.77 (4.02, 7.97)	6.35 (4.03, 9.69)	6.50 (3.75, 8.53)	0.434	0.253				
Fasting glucose (mmol/L)	10.47±4.34	10.69±4.18	9.80±3.04	0.372	0.973				
60-min glucose (mmol/L)	16.72±4.19	16.87±3.86	16.80±4.45	0.915	0.983				
120-min glucose (mmol/L)	18.51±5.39	18.38±5.25	19.04±4.72	0.802	0.847				
Insulin sensitivity									
HOMA-S index[a]	0.22 (0.11, 0.42)	0.20 (0.08, 0.33)	0.18 (0.09, 0.26)	0.195	0.100	–0.080	–	–	0.110
Fasting insulin(mU/L)[a]	10.95 (6.49, 20.86)	12.16 (7.63, 23.01)	13.86 (8.27, 26.35)	0.241	0.104	0.087	–	–	0.081
Insulin secretion									
HOMA-B index (%)[a]	42.65 (21.13, 89.05)	46.37 (23.00, 96.67)	42.99 (22.51, 102.61)	0.544	0.327	0.071	–	–	0.159
Insulin AUC (mU/L*h)[a]	56.93 (31.40, 88.76)	62.47 (37.95, 115.87)	61.70 (35.39, 105.96)	0.411	0.182	0.071	–	–	0.156
Glucose tolerance									
Glucose AUC (mmol/L*h)	31.24±8.22	31.40±7.75	31.21±7.88	0.949	0.798	0.008	–	–	0.869
Fasting glucose (mmol/L)	10.47±4.34	10.69±4.18	9.80±3.04	0.372	0.973	–0.006	–	–	0.904
120-min glucose (mmol/L)	18.51±5.39	18.38±5.25	19.04±4.72	0.802	0.847	0.004	–	–	0.945
IGF1 (ng/ml)	125.88±63.47	134.28±68.65	140.74±60.91	0.195	0.098	0.073	–	–	0.127
HbA1c (%)	8.10±1.92	8.01±1.73	8.15±1.74	0.852	0.798	–0.013	–	–	0.796

T2DM – type 2 diabetes mellitus; IGF1 – insulin like growth factor 1; CA – cytosine-adenosine; BMI – body mass index; OGTT – oral glucose tolerance test; HDL-cholesterol – high-density lipoprotein cholesterol; LDL-cholesterol – low-density lipoprotein cholesterol; HbA1c – hemoglobin A1c; AUC – area under the curve; HOMA-S – homeostasis model assessment-sensitivity; HOMA-B – homeostasis model assessment-beta. [a] Log-transformed before multiple linear regression analysis.

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Table 3. Clinical characteristics of the 389 study population according to SNP rs5742612 and multiple linear regression analysis for association of rs5742612 with metastatic traits.

	TT	CT	CC	P	P (CT+ CC *vs.* TT)	β	SE	R^2 (%)	P
N (M/F)	126/85	88/55	22/13	0.907	0.679				
Age (years)	65±9	64±10	66±11	0.429	0.397				
Family history of T2DM (Y/N)	115/96	79/64	22/13	0.652	0.683				
Anthropometry									
Height (cm)	166.15±8.23	167.59±7.71	168.11±8.30	0.090	0.060				
BMI (kg/m^2)	24.80±3.56	25.85±3.34	25.02±3.22	0.026	0.012				
Total cholesterol (mmol/l)	3.38±1.17	2.95±1.02	3.04±0.95	0.002	0.001				
Triacylglycerol (mmol/l)	1.42 (0.90, 1.96)	1.24 (0.88, 1.80)	1.34 (0.86, 1.97)	0.246	0.099				
HDL-cholesterol (mmol/l)	0.74 (0.61, 0.94)	0.70 (0.53, 0.91)	0.75 (0.61, 0.93)	0.413	0.227				
LDL-cholesterol (mmol/l)	2.02 (1.56, 2.57)	1.76 (1.38, 2.28)	1.97 (1.44, 2.27)	0.006	0.002				
VLDL-cholesterol (mmol/l)	0.65 (0.41, 0.88)	0.59 (0.40, 0.82)	0.57 (0.37, 0.88)	0.188	0.076				
OGTT									
Fasting insulin (mU/L)	10.99 (6.72, 19.74)	13.20 (7.71, 28.72)	14.23 (7.83, 21.83)	0.070	0.022				
60-min insulin (mU/L)	29.67 (18.10, 48.02)	37.52 (20.77, 72.25)	38.74 (16.30, 47.73)	0.037	0.010				
120-min insulin (mU/L)	35.13 (20.02, 59.49)	42.09 (22.43, 73.47)	42.00 (24.37, 76.26)	0.090	0.029				
Fasting peptide C (ng/ml)	2.47 (1.79, 3.64)	2.86 (2.07, 4.24)	2.67 (1.71, 3.50)	0.031	0.024				
60-min peptide C (ng/ml)	4.52 (3.07, 6.24)	4.97 (3.57, 7.90)	4.83 (3.36, 6.46)	0.070	0.028				
120-min peptide C (ng/ml)	5.69 (3.95, 8.23)	6.61 (4.06, 9.69)	7.05 (4.34, 8.57)	0.221	0.082				
Fasting glucose (mmol/L)	10.35±4.07	10.94±4.30	9.27±3.22	0.073	0.528				
60-min glucose (mmol/L)	16.66±3.97	16.91±4.16	17.20±4.40	0.633	0.386				
120-min glucose (mmol/L)	18.63±5.23	18.14±5.31	19.49±4.77	0.449	0.882				
Insulin sensitivity									
HOMA-S index[a]	0.23 (0.11, 0.38)	0.17 (0.07, 0.32)	0.19 (0.10, 0.34)	0.037	0.018	–0.131	0.049	5.8	0.008
Fasting insulin(mU/L)[a]	10.99 (6.72, 19.74)	13.20 (7.71, 28.72)	14.23 (7.83, 21.83)	0.070	0.022	0.120	0.044	6.0	0.006

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Table 3 continued. Clinical characteristics of the 389 study population according to SNP rs5742612 and multiple linear regression analysis for association of rs5742612 with metastatic traits.

	TT	CT	CC	P	P (CT+ CC *vs.* TT)	β	SE	R^2 (%)	P
Insulin secretion									
HOMA-B index (%)[a]	41.99 (21.34, 85.51)	47.54 (23.00, 102.88)	52.85 (30.06, 100.11)	0.198	0.092	0.099	0.048	3.8	0.008
Insulin AUC (mU/L*h)[a]	55.03 (31.50, 86.13)	68.71 (40.04, 129.00)	71.04 (35.26, 93.00)	0.047	0.013	0.112	0.042	6.1	0.012
Glucose tolerance									
Glucose AUC (mmol/L*h)	31.17±7.78	31.45±8.10	31.59±7.70	0.851	0.573	0.022	–	–	0.662
Fasting glucose (mmol/L)	10.35±4.07	10.94±4.30	9.27±3.22	0.073	0.528	0.029	–	–	0.564
120-min glucose (mmol/L)	18.63±5.23	18.14±5.31	19.49±4.77	0.449	0.882	0.019	–	–	0.706
IGF1 (ng/ml)	132.12±69.10	128.82±61.49	143.44±60.44	0.448	0.914	–0.015	–	–	0.748
HbA1c (%)	8.10±1.82	7.98±1.74	8.20±1.97	0.762	0.729	–0.018	–	–	0.720

T2DM – type 2 diabetes mellitus; IGF1 – insulin like growth factor 1; CA – cytosine-adenosine; BMI – body mass index; OGTT – oral glucose tolerance test; HDL-cholesterol – high-density lipoprotein cholesterol; LDL-cholesterol – low-density lipoprotein cholesterol; HbA1c – hemoglobin A1c; AUC – area under the curve; HOMA-S – homeostasis model assessment-sensitivity; HOMA-B – homeostasis model assessment-beta. [a] Log-transformed before multiple linear regression analysis.

Table 4. Clinical characteristics of the 389 study population according to SNP rs2288377 and multiple linear regression analysis for association of rs2288377 with metastatic traits.

	TT	AT	AA	P	P (AT+ AA *vs.* TT)	β	SE	R^2 (%)	P
N (M/F)	124/81	87/52	25/20	0.701	1.000				
Age (years)	66±9	65±10	64±11	0.251	0.142				
Family history of T2DM (Y/N)	112/93	75/64	29/16	0.437	0.759				
Anthropometry									
Height (cm)	166.25±8.28	167.56±7.73	167.47±8.07	0.131	0.116				
BMI (kg/m^2)	24.75±3.59	25.84±3.34	25.29±3.11	0.021	0.007				
Total cholesterol (mmol/l)	3.36±1.11	2.94±1.03	3.18±1.23	0.005	0.002				
Triacylglycerol (mmol/l)	1.41 (0.90, 1.92)	1.24 (0.86, 1.80)	1.47 (0.93, 2.03)	0.283	0.195				
HDL-cholesterol (mmol/l)	0.74 (0.61, 0.95)	0.69 (0.52, 0.91)	0.73 (0.62, 0.91)	0.416	0.215				
LDL-cholesterol (mmol/l)	2.02 (1.56, 2.57)	1.75 (1.35, 2.28)	2.00 (1.52, 2.33)	0.007	0.003				
VLDL-cholesterol (mmol/l)	0.65 (0.41, 0.86)	0.59 (0.40, 0.82)	0.61 (0.41, 0.89)	0.354	0.159				

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Table 4 continued. Clinical characteristics of the 389 study population according to SNP rs2288377 and multiple linear regression analysis for association of rs2288377 with metastatic traits.

	TT	AT	AA	P	P (AT+ AA *vs.* TT)	β	SE	R^2 (%)	P
OGTT									
Fasting insulin (mU/L)	10.87 (6.59, 19.14)	13.20 (7.66, 29.69)	15.97 (9.58, 25.98)	0.009	0.003				
60-min insulin (mU/L)	29.35 (17.90, 47.24)	38.85 (21.02, 73.19)	38.74 (19.51, 51.16)	0.009	0.002				
120-min insulin (mU/L)	34.86 (19.98, 59.21)	42.09 (23.27, 74.23)	42.43 (23.36, 68.43)	0.035	0.010				
Fasting peptide C (ng/ml)	2.43 (1.78, 3.59)	2.94 (2.06, 4.24)	2.68 (1.85, 3.60)	0.011	0.003				
60-min peptide C (ng/ml)	4.48 (2.99, 6.15)	5.01 (3.62, 7.99)	4.91 (3.33, 7.10)	0.018	0.005				
120-min peptide C (ng/ml)	5.63 (3.90, 8.00)	6.64 (4.11, 10.04)	6.99 (4.17, 8.57)	0.092	0.030				
Fasting glucose (mmol/L)	10.29±4.06	10.84±4.25	10.16±3.87	0.294	0.406				
60-min glucose (mmol/L)	16.63±4.00	16.82±4.16	17.48±4.14	0.502	0.368				
120-min glucose (mmol/L)	18.70±5.21	18.04±5.26	19.26±5.11	0.424	0.690				
Insulin sensitivity									
HOMA-S index[a]	0.23 (0.12, 0.39)	0.17 (0.06, 0.32)	0.16 (0.07, 0.26)	0.006	0.002	–0.159	0.049	6.6	0.001
Fasting insulin(mU/L)[a]	10.87 (6.59, 19.14)	13.20 (7.66, 29.69)	15.97 (9.58, 25.98)	0.009	0.003	0.143	0.043	6.8	0.001
Insulin secretion									
HOMA-B index (%)[a]	39.89 (21.00, 84.92)	48.94 (25.68, 105.11)	52.85 (30.60, 94.80)	0.095	0.037	0.114	0.048	4.2	0.017
Insulin AUC (mU/L*h)[a]	53.58 (31.16, 86.07)	69.06 (40.07, 133.36)	72.10 (36.36, 94.17)	0.011	0.003	0.042	0.030	6.7	0.002
Glucose tolerance									
Glucose AUC (mmol/L*h)	31.15±7.83	31.26±8.03	32.19±7.73	0.762	0.583	0.022	–	–	0.662
Fasting glucose (mmol/L)	10.29±4.06	10.84±4.25	10.16±3.87	0.294	0.406	0.042	–	–	0.407
120-min glucose (mmol/L)	18.70±5.21	18.04±5.26	19.26±5.11	0.424	0.690	–0.034	–	–	0.503
IGF1 (ng/ml)	134.080±68.88	130.300±59.80	127.070±68.44	0.735	0.452	–0.046	–	–	0.333
HbA1c (%)	8.12±1.84	7.92±1.64	8.27±2.11	0.497	0.553	–0.031	–	–	0.543

T2DM – type 2 diabetes mellitus; IGF1 – insulin like growth factor 1; CA – cytosine-adenosine; BMI – body mass index; OGTT – oral glucose tolerance test; HDL-cholesterol – high-density lipoprotein cholesterol; LDL-cholesterol – low-density lipoprotein cholesterol; HbA1c – hemoglobin A1c; AUC – area under the curve; HOMA-S – homeostasis model assessment-sensitivity; HOMA-B – homeostasis model assessment-beta. [a] Log-transformed before multiple linear regression analysis.

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Table 5. Clinical characteristics of the 389 study population according to SNP haplotype and multiple linear regression analysis for association of SNP haplotype with metastatic traits.

	CTT	TCA	TTT	P	P (AT+ AA *vs.* TT)	β	SE	R^2 (%)	P
N (M/F)	293/192	132/81	47/33	0.866					
Age (years)	65±9	65±10	67±9	0.163					
Family history of T2DM (Y/N)	270/215	123/90	39/41	0.384					
Anthropometry									
Height (cm)	166.71±7.97	167.77±7.88	165.38±8.87	0.061					
BMI (kg/m^2)	25.17±3.27	25.57±3.30	24.41±4.80	0.035					
Total cholesterol (mmol/l)	3.28±1.16	2.98±1.00	3.23±1.07	0.007					
Triacylglycerol (mmol/l)	1.40 (0.91, 0.58)	1.29 (0.87, 1.82)	1.28 (0.87, 1.80)	0.257					
HDL-cholesterol (mmol/l)	0.73 (0.58, 0.93)	0.72 (0.58, 0.92)	0.81 (0.62, 0.96)	0.324					
LDL-cholesterol (mmol/l)	1.96 (1.53, 2.51)	1.81 (1.40, 2.27)	1.95 (1.47, 2.49)	0.021					
VLDL-cholesterol (mmol/l)	0.64 (0.42, 0.85)	0.57 (0.39, 0.82)	0.58 (0.40, 0.82)	0.122					
OGTT									
Fasting insulin (mU/L)	11.23 (6.87, 21.58)	13.72 (7.79, 27.19)	11.63 (7.56, 17.95)	0.111					
60-min insulin (mU/L)	31.66 (18.37, 55.66)	38.74 (20.46, 67.73)	28.61 (17.75, 43.33)	0.019					
120-min insulin (mU/L)	35.46 (20.30, 65.78)	42.00 (22.94, 73.85)	35.07 (21.36, 66.40)	0.101					
Fasting peptide C (ng/ml)	2.62 (1.87, 3.83)	2.79 (1.99, 3.92)	2.37 (1.81, 2.34)	0.123					
60-min peptide C (ng/ml)	4.63 (3.20, 6.81)	4.91 (3.39, 7.53)	4.36 (2.76, 6.24)	0.059					
120-min peptide C (ng/ml)	5.92 (4.03, 8.53)	6.73 (4.29, 9.48)	5.37 (3.58, 8.67)	0.178					
Fasting glucose (mmol/L)	10.59±4.25	10.39±4.04	10.20±3.07	0.724					
60-min glucose (mmol/L)	16.77±4.07	17.01±4.22	16.40±3.69	0.452					
120-min glucose (mmol/L)	18.461±5.32	18.58±5.15	18.77±4.77	0.900					
Insulin sensitivity									
HOMA-S index[a]	0.21 (0.10, 0.37)	0.18 (0.07, 0.32)	0.21 (0.13, 0.31)	0.157	–0.033	–	–	0.355	
Fasting insulin(mU/L)[a]	11.23 (6.87, 21.58)	13.72 (7.79, 27.19)	11.63 (7.56, 17.95)	0.111	0.040	–	–	0.254	

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Table 5 continued. Clinical characteristics of the 389 study population according to SNP haplotype and multiple linear regression analysis for association of SNP haplotype with metastatic traits.

	CTT	TCA	TTT	P	P (AT+ AA *vs.* TT)	β	SE	R^2 (%)	P
Insulin secretion									
HOMA-B index (%)[a]	43.15 (22.72, 92.18)	48.94 (25.76, 100.71)	36.63 (21.34, 82.96)	0.159	0.034	–	–	0.339	
Insulin AUC (mU/L*h)[a]	60.79 (32.92, 95.18)	69.93 (37.52, 120.18)	51.55 (34.00, 92.10)	0.040	0.018	–	–	0.616	
Glucose tolerance									
Glucose AUC (mmol/L*h)	31.30±8.03	31.49±7.93	30.89±6.69	0.747	–0.007	–	–	0.842	
Fasting glucose (mmol/L)	10.59±4.25	10.39±4.04	10.20±3.07	0.724	–0.027	–	–	0.440	
120-min glucose (mmol/L)	18.461±5.32	18.58±5.15	18.77±4.77	0.900	0.016	–	–	0.647	
IGF1 (ng/ml)	120.00 (82.50, 169.00)	134.00 (93.25, 171.50)	130.50 (96.10, 205.00)	0.158	0.026	0.013	11.8	0.042	
HbA1c (%)	8.07±1.85	8.05±1.81	8.10±1.48	0.994	–0.004	–	–	0.907	

T2DM – type 2 diabetes mellitus; IGF1 – insulin like growth factor 1; CA – cytosine-adenosine; BMI – body mass index; OGTT – oral glucose tolerance test; HDL-cholesterol – high-density lipoprotein cholesterol; LDL-cholesterol – low-density lipoprotein cholesterol; HbA1c – hemoglobin A1c; AUC – area under the curve; HOMA-S – homeostasis model assessment-sensitivity; HOMA-B – homeostasis model assessment-beta. [a] Log-transformed before multiple linear regression analysis.

Table 6. Clinical characteristics of the 389 study population according to SNP&CA haplotype and multiple linear regression analysis for association of SNP&CA haplotype with metastatic traits.

	C19TT	C17TT	C18TT	T21CA	P	β	SE	R^2 (%)	P
N (M/F)	133/81	47/28	82/58	87/62	0.915				
Age (years)	64±10	66±9	66±9	65±10	0.559				
Family history of T2DM (Y/N)	113/101	43/32	83/57	85/64	0.759				
Anthropometry									
Height (cm)	167.16±8.13	166.91±8.46	166.33±7.61	167.77±8.04	0.398				
BMI (kg/m^2)	25.14±3.18	24.88±3.20	25.39±3.27	25.63±3.35	0.349				
Total cholesterol (mmol/l)	3.33±1.21	3.31±1.12	3.23±1.08	2.99±0.98	0.045				
Triacylglycerol (mmol/l)	1.41 (0.94, 1.91)	1.30 (0.84, 1.72)	1.52 (1.03, 2.03)	1.33 (0.91, 1.87)	0.040				
HDL-cholesterol (mmol/l)	0.74 (0.61, 0.95)	0.74 (0.56, 0.91)	0.73 (0.54, 0.92)	0.58 (0.71, 0.92)	0.580				
LDL-cholesterol (mmol/l)	1.98 (1.59, 2.51)	1.99 (1.47, 2.63)	1.94 (1.55, 2.47)	1.87 (1.40, 2.28)	0.119				
VLDL-cholesterol (mmol/l)	0.64 (0.43, 0.86)	0.60 (0.40, 0.78)	0.69 (0.48, 0.92)	0.60 (0.42, 0.84)	0.032				

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Table 6 continued. Clinical characteristics of the 389 study population according to SNP&CA haplotype and multiple linear regression analysis for association of SNP&CA haplotype with metastatic traits.

	C19TT	C17TT	C18TT	T21CA	P	β	SE	R^2 (%)	P
OGTT									
Fasting insulin (mU/L)	12.21 (7.58, 24.37)	10.12 (6.65, 17.03)	10.71 (6.58, 21.64)	13.30 (7.79, 25.82)	0.127				
60-min insulin (mU/L)	31.59 (18.28, 61.67)	33.34 (17.55, 53.73)	30.28 (18.34, 57.96)	38.85 (20.03, 60.19)	0.475				
120-min insulin (mU/L)	36.25 (20.08, 66.42)	34.86 (19.16, 58.55)	36.90 (21.68, 69.72)	42.00 (22.09, 69.96)	0.531				
Fasting peptide C (ng/ml)	2.67 (1.93, 3.91)	2.47 (1.41, 3.59)	2.63 (1.94, 3.80)	2.82 (1.99, 3.78)	0.326				
60-min peptide C (ng/ml)	4.53 (3.27, 7.24)	4.72 (2.99, 6.32)	4.46 (3.03, 6.82)	4.91 (3.40, 7.12)	0.747				
120-min peptide C (ng/ml)	5.91 (4.08, 8.60)	5.69 (3.78, 8.49)	5.86 (4.02, 8.42)	6.64 (3.96, 9.00)	0.808				
Fasting glucose (mmol/L)	10.64±4.31	10.23±4.42	10.79±4.20	10.47±4.19	0.751				
60-min glucose (mmol/L)	16.72±4.00	17.12±3.95	16.78±4.31	16.84±4.06	0.951				
120-min glucose (mmol/L)	18.48±5.24	19.00±5.10	18.35±5.74	18.35±5.07	0.919				
Insulin sensitivity									
HOMA-S index[a]	0.20 (0.09, 0.34)	0.26 (0.12, 0.43)	0.20 (0.09, 0.37)	0.19 (0.07, 0.33)	0.132	–0.014	–	–	0.696
Fasting insulin(mU/L)[a]	12.21 (7.58, 24.37)	10.12 (6.65, 17.03)	10.71 (6.58, 21.64)	13.30 (7.79, 25.82)	0.127	0.019	–	–	0.588
Insulin secretion									
HOMA-B index (%)[a]	42.04 (22.45, 99.63)	44.01 (20.21, 89.23)	42.70 (21.38, 91.99)	52.85 (25.37, 101.49)	0.554	0.016	–	–	0.651
Insulin AUC (mU/L*h)[a]	61.39 (33.58, 95.35)	61.59 (30.47, 77.72)	56.14 (31.41, 120.03)	69.93 (37.52, 117.41)	0.501	0.022	–	–	0.528
Glucose tolerance									
Glucose AUC (mmol/L*h)	31.28±7.94	31.74±7.93	31.35±8.47	31.25±7.87	0.989	–0.009	–	–	0.797
Fasting glucose (mmol/L)	10.64±4.31	10.23±4.42	10.79±4.20	10.47±4.19	0.751	–0.027	–	–	0.440
120-min glucose (mmol/L)	18.48±5.24	19.00±5.10	18.35±5.74	18.35±5.07	0.919	0.001	–	–	0.988
IGF1 (ng/ml)	119.50 (80.90, 173.25)	134.00 (77.90, 155.00)	129.00 (82.88, 189.25)	137.00 (93.60, 175.50)	0.742	0.044	–	–	0.190
HbA1c (%)	7.99±1.74	8.46±2.02	8.04±1.86	8.06±1.80	0.368	–0.009	–	–	0.791

T2DM – type 2 diabetes mellitus; IGF1 – insulin like growth factor 1; CA – cytosine-adenosine; BMI – body mass index; OGTT – oral glucose tolerance test; HDL-cholesterol – high-density lipoprotein cholesterol; LDL-cholesterol – low-density lipoprotein cholesterol; HbA1c – hemoglobin A1c; AUC – area under the curve; HOMA-S – homeostasis model assessment-sensitivity; HOMA-B – homeostasis model assessment-beta. [a] Log-transformed before multiple linear regression analysis.

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rs35767

No significant rs35767 genotype-related differences in gender, age, family history of T2DM, or anthropometric or metabolic parameters were observed, nor in IGF1 level. However, the CC homozygote subjects exhibited higher levels of total cholesterol and LDL cholesterol than the CT/TT carriers. The linear regression analysis showed no significant correlation of metabolic parameters with rs35767 polymorphism (Table 2).

rs5742612

Subjects with the rs5742612 genotypes showed significantly different BMI, total cholesterol, and LDL cholesterol, but no significant differences in gender, age, or family history of T2DM. Additionally, a significant difference between the TT and CT/CC genotypes was observed in fasting insulin (10.99 [6.72, 19.74] *vs.* 13.30 [7.73, 27.74] mU/L, *P*=0.022); 60-min insulin (29.67 [18.10, 48.02] *vs.* 38.13 [20.61, 70.42] mU/L, *P*=0.010); 120-min insulin (35.13 [20.02, 59.49] *vs.* 42.05 [22.57, 73.66] mU/L, *P*=0.029); fasting peptide C (2.47 [1.79, 3.64] *vs.* 2.81 [2.02, 3.93] ng/mL, *P*=0.024); 60-min peptide C (4.52 [3.07, 6.24] *vs.* 4.93 [3.41, 7.76] ng/mL, *P*=0.028) during OGTT; and HOMA-S index (0.23 [0.11, 0.38] *vs.* 0.17 [0.07, 0.32], *P*=0.018); and insulin AUC (55.03 [31.50, 86.13] *vs.* 69.49 [37.85, 120.57] mU/L*h, *P*=0.013). However, a difference between the genotypes with regard to the glucose levels during the OGTT, glucose AUC, or IGF1 level was not observed. In the meantime, the C allele of rs5742612 was found to be associated with decreased insulin sensitivity (HOMA-S index, β=–0.131, P=0.008; fasting insulin level, β=0.022, P=0.006) and increased insulin secretion (HOMA-B index, β=0.099, *P*=0.008; insulin AUC, β=0.112, *P*=0.012), after controlling for the effects of gender, age, and BMI according to the multiple linear regression model (Table 3).

rs2288377

Subjects with the rs2288377 genotype showed significantly different BMI, total cholesterol, and LDL cholesterol, but no significant differences in gender, age, or family history of T2DM. Moreover, we confirmed significant differences in fasting insulin (10.87 [6.59, 19.14] *vs.* 13.98 [7.84, 28.48] mU/L, *P*=0.003); 60-min insulin (29.35 [17.90, 47.24] *vs.* 38.80 [21.14, 71.23] mU/L, *P*=0.002); 120-min insulin (34.86 [19.98, 59.21] *vs.* 42.26 [23.40, 74.04] mU/L, *P*=0.010); fasting peptide C (2.43 [1.78, 3.59] *vs.* 2.88 [2.03, 4.16] ng/mL, *P*=0.003); 60-min peptide C (4.48 [2.99, 6.15] *vs.* 4.96 [3.44, 7.89] ng/mL, *P*=0.005); and 120-min peptide C (5.63 [3.90, 8.00] *vs.* 6.74 [4.15, 9.67] ng/mL, *P*=0.030) during OGTT and HOMA-S index (0.23 [0.12, 0.39] *vs.* 0.17 [0.06, 0.31], *P*=0.002); HOMA-B index (39.89 [21.00, 84.92] *vs.* 49.84 [25.89, 102.48], *P*=0.037); and insulin AUC (53.58 [31.16, 86.07] *vs.* 71.24 [39.12, 120.57] mU/L*h, *P*=0.003) among carriers of the TT, AT/AA genotype. Nevertheless, no significant difference between the genotypes with regard to the glucose levels during the OGTT, glucose AUC, and IGF1 level was observed. The linear regression model indicated that the A allele of rs2288377 was associated with decreased insulin sensitivity (HOMA-S index, β=–0.159, *P*=0.001; fasting insulin, β=0.143, *P*=0.001) and increased insulin secretion (HOMA-B index, β=0.114, *P*=0.017; insulin AUC, β=0.042, *P*=0.002), independent of gender, age, and BMI (Table 4).

SNP haplotype

Subjects with the SNP haplotype showed significantly different BMI, total cholesterol, and LDL cholesterol, but no significant differences in gender, age, or family history of T2DM. We also observed a significant difference between the CTT, TCA, and TTT haplotypes in 60-min insulin (31.66 [18.37, 55.66] *vs.* 38.74 [20.46, 67.73] *vs.* 28.61 [17.75, 43.33], *P*=0.019); insulin AUC (60.79 [32.92, 95.18] *vs.* 69.93 [37.52, 120.18] *vs.* 51.55 [34.00, 92.10], *P*=0.040); and IGF1 (128.98±65.42 *vs.* 133.62±61.25 *vs.* 145.23±75.80, *P*=0.049). The linear regression analysis showed no significant correlation between metabolic parameters and rs35767 polymorphism, except IGF1 (β=7.631, *P*=0.020), independent of gender, age, and BMI (Table 5).

SNP&CA haplotype

Four common haplotypes were compared in our study. We observed a significant difference in BMI, total cholesterol, and LDL cholesterol among subjects with different SNP&CA haplotypes. However, there were no significant SNP&CA haplotype-related differences in gender, age, family history of T2DM, or metabolic parameters, nor in IGF1 level. The linear regression analysis also showed no significant correlation between metabolic parameters and the SNP&CA haplotype (Table 6).

Discussion

Diabetes turns up when elevation in insulin secretion is insufficient to overcome tissue insulin resistance. Insulin resistance is thought to result mainly from environmental factors, such as obesity. However, there is compelling evidence that both insulin sensitivity and insulin secretion also have a genetic component [19]. IGF1 is a growth factor of significant homology with insulin and has been widely reported to elevate insulin sensitivity [20–22]. The gene coding IGF1 is the *IGF1* gene (NG 011713.1GI: 225735562), the CA microsatellite and SNPs of which have been reported to play different roles in insulin sensitivity and insulin secretion. In our present study, we investigated the relationship of the CA microsatellite, three SNPs, and for the first time, the SNP with or without the CA haplotype in the ECR of the *IGF1* promoter with insulin sensitivity and insulin secretion. Multiple regression showed no

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statistical contribution to insulin sensitivity or insulin secretion of different CA microsatellite or rs35767 polymorphisms, nor of the SNP haplotype only or the SNP with CA microsatellite haplotype. We also confirmed that the rs5742612 and rs2288377 polymorphisms were associated with insulin sensitivity and insulin secretion, with carriers of the TT genotype exhibiting higher insulin sensitivity and lower insulin secretion, respectively, independent of gender, age, and BMI. However, correlation of the studied microsatellite, SNPs, and haplotypes with circulating IGF1 concentration was observed only in the SNP haplotype without the CA microsatellite.

Studies for correlation of the CA microsatellite with risk of type 2 diabetes and insulin sensitivity have led to controversial conclusions. Prior studies examining the role of the CA microsatellite in *IGF1* regarding predisposition to common human disease have found the variant inconsistently, with the same allele associated with high [12] and low [23] risk of T2DM, respectively. Different from the CA microsatellite, research on the SNPs came to a consistent conclusion. A recent meta-analysis of a genome-wide association study of β cell function and insulin resistance in an East Asian population showed weak association between the rs35767 polymorphisms and insulin resistance with 0.009 HOMA-IR rise per G allele [24], while a previous meta-analysis revealed a 0.010 pmol/L per G allele increase in fasting insulin level [3]. Similarly, Mannino et al. proposed that the rs35767 was associated with circulating insulin sensitivity and IGF1 levels, with carriers of the GG genotype exhibiting lower insulin sensitivity and IGF1 concentrations compared with subjects carrying the A allele [14]. However, the correlation of the CA microsatellite and rs35767 polymorphisms with insulin sensitivity and secretion was not observed in our research. The inconsistent and opposite findings of association between *IGF1* gene variation and insulin sensitivity may be due to a number of factors [23]. Differences in environmental factors may contribute to the inconsistent findings, in particular in relation to the selection of study subjects and their demographic, clinical, and metabolic characteristics [25].

In addition, we observed a significant association of metabolic parameters with the rs5742612 and rs2288377 polymorphisms for the first time. Carriers of the TT genotype exhibited higher insulin sensitivity and lower insulin secretion and insulin and peptide C levels during OGTT compared with subjects carrying the C allele and A allele, respectively. In the linear regression analysis, the rs5742612 donated 0.131 HOMA-S reductions and 0.099 HOMA-B rises per C allele, and the rs2288377 polymorphism donated 0.159 HOMA-S reductions and 0.143 HOMA-B rises per A allele, respectively, independently of age, gender, and BMI. However, no significant association was found between the rs5742612 or rs2288377 polymorphisms and serum IGF1 levels, glucose tolerance, or HbA1c levels. We presumed that the associations of the rs5742612 and rs2288377 SNPs with insulin sensitivity and insulin secretion might be attributed to the direct functional roles of the two *loci* or be mediated by circulating IGF1. The first hypothesis is that these SNPs might tag other variants in the *IGF1* gene associated with insulin sensitivity and β cell function. In a recent study, the investigators revealed plenty of rare variants associated with fasting insulin levels that had not been previously described, by further study of the *IGF1* gene [26]. Examination showed that the variants were located near the DNAseI hypersensitive sites and FOXA1 binding sites, indicating that they might have direct function because FOXA1 is a major transcriptional regulator factor in glucose homeostasis and insulin metabolism [27,28]. The rs5742612 and rs2288377 SNPs might tag these variants and have direct function in insulin secretion and glucose homeostasis. Alternatively, the associations may be mediated by circulating IGF1. Low circulating IGF1 levels may result in GH over-secretion by negative feedback on the pituitary and/or hypothalamus [29]. Thereby, inactivation of GH in liver IGF1-deficient mice resulted in decreased blood glucose and increased insulin sensitivity [29]. These results suggest that enhanced GH secretion may be a major mediator of low insulin sensitivity in subjects with low circulating IGF1 levels. To solve this confusion, we investigated the association of SNP-only and SNP with CA haplotypes with insulin sensitivity and secretion for the first time, but no significant difference was observed. However, we did observe that the SNP-only haplotype was associated with circulating IGF1 levels. This potentially can exclude the possibility that the functional role of IGF1 SNP *loci* was mediated by circulating IGF1, nor was it mediated by interaction with the CA microsatellite. It is likely that the difference was attributable to the direct functional roles of the two *loci*. However, the precise molecular mechanism of the effect on insulin sensitivity and secretion by these gene polymorphisms requires further investigations.

There were some limitations in our study. Due to the limitations of the cross-sectional study itself, we couldn't come to a causative conclusion. Studies in human cell culture and animal models will be needed to validate the function of these noncoding variants in insulin biology. Moreover, the β values were small, which indicated our study could only explain a small portion of the insulin biology variation. Finally, our results were based on the Han Chinese; further studies are needed to validate the conclusion in other races.

Conclusions

In conclusion, our study has identified the association of the CA microsatellite and rs35767 polymorphisms and for the first time, the rs5742612 and rs2288377 polymorphisms and SNP haplotypes in the ECR of the *IGF1* promoter with insulin sensitivity and secretion. There was no CA microsatellite or

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rs35767 genotype-related insulin sensitivity or secretion. The rs5742612 and rs2288377 polymorphisms were significantly associated with insulin sensitivity and insulin secretion, with the TT genotype exhibiting higher insulin sensitivity and lower insulin secretion compared with carriers of the C allele and A allele, respectively. We consider that the correlation is attributable to the direct functional roles of the two *loci*. Our results have important significance in understanding the correlation of the CA microsatellite and SNPs in the ECR of the *IGF1* promoter with insulin biology. Further study incorporating a more systematic approach to identify the molecular mechanisms for the association is needed.

References:

1. American Diabetes Association: Diagnosis and classification of diabetes mellitus. Diabetes Care, 2009; 32(Suppl. 1): S62–67
2. Morris AP, Voight BF, Teslovich TM et al: Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet, 2012; 44(9): 981–90
3. Dupuis J, Langenberg C, Prokopenko I et al: New genetic *loci* implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet, 2010; 42(2): 105–16
4. Billings LK, Florez JC: The genetics of type 2 diabetes: What have we learned from GWAS? Ann NY Acad Sci, 2010; 1212: 59–77
5. Scott RA, Fall T, Pasko D et al: Common genetic variants highlight the role of insulin resistance and body fat distribution in type 2 diabetes, independent of obesity. Diabetes, 2014; 63(12): 4378–87
6. Seppa S, Voutilainen R, Tenhola S: Markers of insulin sensitivity in 12-year-old children born from preeclamptic pregnancies. J Pediatr, 2015; 167(1): 125–30
7. Woods KA, Camacho-Hübner C, Bergman RN et al: Effects of insulin-like growth factor I (IGF-I) therapy on body composition and insulin resistance in IGF-I gene deletion. J Clin Endocrinol Metab, 2000; 85(4): 1407–11
8. Succurro E, Andreozzi F, Marini MA et al: Low plasma insulin-like growth factor-1 levels are associated with reduced insulin sensitivity and increased insulin secretion in nondiabetic subjects. Nutr Metab Cardiovasc Dis, 2009; 19(10): 713–19
9. Barner C, Petersson M, Edén Engström B, Höybye C: Effects on insulin sensitivity and body composition of combination therapy with GH and IGF1 in GH deficient adults with type 2 diabetes. Eur J Endocrinol, 2012; 167(5): 697–703
10. de Kerdanet M, Caron-Debarle M, Nivot S et al: Ten-year improvement of insulin resistance and growth with recombinant human insulin-like growth factor 1 in a patient with insulin receptor mutations resulting in leprechaunism. Diabetes Metab, 2015; 41(4): 331–37
11. Frayling TM, Hattersley AT, McCarthy A et al: A putative functional polymorphism in the IGF-I gene: association studies with type 2 diabetes, adult height, glucose tolerance, and fetal growth in U.K. populations. Diabetes, 2002; 51(7): 2313–16
12. Vaessen N, Heutink P, Janssen JA et al: A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. Diabetes, 2001; 50(3): 637–42
13. Giavoli C, Profka E, Sala E et al: Impact of IGF(CA)19 gene polymorphism on the metabolic response to GH therapy in adult GH-deficient patients. Eur J Endocrinol, 2014; 170(2): 273–81
14. Mannino GC, Greco A, De Lorenzo C et al: A fasting insulin-raising allele at IGF1 locus is associated with circulating levels of IGF-1 and insulin sensitivity. PLoS One, 2013; 8(12): e85483
15. Serre D, Gurd S, Ge B et al: Differential allelic expression in the human genome: A robust approach to identify genetic and epigenetic cis-acting mechanisms regulating gene expression. PLoS Genet, 2008; 4(2): e1000006
16. Chen HY, Chan IH, Sham AL et al: Haplotype effect in the IGF1 promoter accounts for the association between microsatellite and serum IGF1 concentration. Clin Endocrinol (Oxf), 2011; 74(4): 520–27
17. Palles C, Johnson N, Coupland B et al: Identification of genetic variants that influence circulating IGF1 levels: A targeted search strategy. Hum Mol Genet, 2008; 17(10): 1457–64
18. Wardrop SL, Brown MA: Identification of two evolutionarily conserved and functional regulatory elements in intron 2 of the human BRCA1 gene. Genomics, 2005; 86(3): 316–28
19. Schäfer SA, Machicao F, Fritsche A et al: New type 2 diabetes risk genes provide new insights in insulin secretion mechanisms. Diabetes Res Clin Pract, 2011; 93(Suppl. 1): S9–24
20. Yakar S, Liu JL, Fernandez AM et al: Liver-specific igf-1 gene deletion leads to muscle insulin insensitivity. Diabetes, 2001; 50(5): 1110–18
21. Moses AC, Young SC, Morrow LA et al: Recombinant human insulin-like growth factor I increases insulin sensitivity and improves glycemic control in type II diabetes. Diabetes, 1996; 45(1): 91–100
22. Hussain MA, Schmitz O, Mengel A et al: Insulin-like growth factor I stimulates lipid oxidation, reduces protein oxidation, and enhances insulin sensitivity in humans. J Clin Invest, 1993; 92(5): 2249–56
23. Frayling TM, Hattersley AT, Smith GD, Ben-Shlomo Y: Conflicting results on variation in the IGFI gene highlight methodological considerations in the design of genetic association studies. Diabetologia, 2002; 45(11): 1605–6
24. Hong K-W, Chung M, Cho SB: Meta-analysis of genome-wide association study of homeostasis model assessment β cell function and insulin resistance in an East Asian population and the European results. Mol Genet Genomics, 2014; 289(6): 1247–55
25. Dunger DB, Ong KKL, Sandhu MS: Serum insulin-like growth factor-I levels and potential risk of type 2 diabetes. Horm Res, 2003; 60(Suppl. 3): 131–35
26. Willems SM, Cornes BK, Brody JA et al: Association of the IGF1 gene with fasting insulin levels. Eur J Hum Genet, 2016; 24(9): 1337–43
27. Gao N, Le Lay J et al: Foxa1 and Foxa2 maintain the metabolic and secretory features of the mature beta-cell. Mol Endocrinol, 2010; 24(8): 1594–604
28. Kaestner KH: The FoxA factors in organogenesis and differentiation. Curr Opin Genet Dev, 2010; 20(5): 527–32
29. Yakar S, Setser J, Zhao H et al: Inhibition of growth hormone action improves insulin sensitivity in liver IGF-1 – deficient mice. J Clin Invest, 2004; 113(1): 96–105

Conflict of interests

The authors have no conflict of interests, and they didn't receive any financial support for this study.

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