Letter to the Editor

Association of *TCF7L2* and *GCG* Gene Variants with Insulin Secretion, Insulin Resistance, and Obesity in New-onset Diabetes*

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This cohort study was designed to evaluate the association of transcription factor 7-like 2 (*TCF7L2*) and proglucagon gene (*GCG*) variants with disordered glucose metabolism and the incidence of type 2 diabetes mellitus (T2DM) in a rural adult Chinese population. A total of 7,751 non-T2DM participants ≥18 years old genotyped at baseline were recruited. The same questionnaire interview and physical and blood biochemical examinations were performed at both baseline and follow-up. During a median 6 years of follow-up, T2DM developed in 227 participants. After adjustment for potential contributory factors, nominally significant associations were seen between TT genotype and the recessive model of *TCF7L2* rs7903146 and increased risk of T2DM [hazard ratio (*HR*)=4.068, 95% confidence interval (*CI*): 1.270-13.026; *HR*=4.051, 95% *CI*: 1.268-12.946, respectively]. The TT genotype of rs7903146 was also significantly associated with higher fasting plasma insulin level and the homeostasis model assessment of insulin resistance in case of new-onset diabetes. In addition, the *TCF7L2* rs290487 TT genotype was associated with abdominal obesity and the *GCG* rs12104705 CC genotype was associated with both general obesity and abdominal obesity in case of new-onset diabetes.

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycemia, different degrees of insulin resistance, and pancreatic β-cell dysfunction[1]. Genome-wide association studies have confirmed a large number of known genetic variants associated with increased risk of T2DM. Among all identified variants, transcription factor 7-like 2 (*TCF7L2*) had the largest effect on risk for T2DM in people of European origin[2]. The proglucagon gene (*GCG*) located on chromosome 2q24.2 can be activated by *TCF7L2* in the *WNT* signaling pathway, and expresses glucagon-like peptide1 (GLP-1) in the intestine. GLP-1 plays an essential role in regulating blood glucose level by stimulating glucose-dependent insulin secretion[3]. Overweight and obesity are important risk factors for the development of diabetes[4]. T2DM is caused by genetic and especially environmental factors[5]. In this study, we selected the tag single nucleotide polymorphisms (SNPs) rs7903146, rs290487, rs11196218 of *TCF7L2,* and rs12104705 of *GCG*, and analyzed the association of these variants with quantitative traits related to the risk for T2DM in a 6-year follow-up study of a rural adult Chinese cohort.

A total of 7,751 participants of Northern Chinese ancestry aged 18 to 74 years old were recruited from July to August 2007 and July to August 2008. Reasons for exclusion were fasting plasma glucose (FPG) level ≥7.0 mmol/L or self-reported diabetes diagnosis, pregnancy, physical handicap, mental disorder, obesity caused by disease, use of certain drugs, or cancer. During follow-up, 315 individuals died and 1,110 were lost to follow-up. Thus, 6,326 individuals (81.62%) were followed up from July to

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August 2013 and July to October 2014; of these, 815 had no data on FPG level and unknown history of T2DM. Finally, 5,511 participants were eligible for analysis. T2DM developed in 227. A standard questionnaire was used to assess demographic characteristics, medical history, T2DM family history, smoking and alcohol status, physical activity level, and other risk factors at baseline and follow-up. Anthropometric and laboratory data were obtained at baseline and follow-up. Insulin resistance and β-cell function were calculated from FPG and insulin using the homeostasis model assessment (HOMA) formula[6].

The clinical and biochemical characteristics of subjects (227 with new-onset diabetes and 5,284 non-T2DM participants) at baseline were analyzed (Table 1). The distribution of differences in age, body mass index (BMI), waist circumference, systolic and diastolic blood pressure, fasting plasma glucose, total cholesterol, triglyceride, and HDL-C level between T2DM and non-T2DM subjects was statistically significant (P<0.05).

For the 5,511 study participants, the genotype frequencies of all SNPs were in accordance with Hardy-Weinberg equilibrium (P>0.05). Genotypic and allelic distributions of *TCF7L2* and *GCG* SNPs are shown in Supplementary Table S1 (in the website of BES, www.besjournal.com). The genotypic but not allelic distributions of rs7903146 differed between T2DM and non-T2DM individuals (P=0.02). Supplementary Table S2 (in the website of BES, www.besjournal.com) shows the association of *TCF7L2* and *GCG* SNPs with the incidence of T2DM in different genetic models according to Cox proportional hazards testing. After adjustment for potential risk factors, nominally significant associations were seen for TT genotype and the recessive model of *TCF7L2* rs7903146 and increased risk of T2DM [hazard ratio (*HR*)=4.068, 95% confidence interval (*CI*): 1.270-13.026, P=0.0181; *HR*=4.051, 95% *CI*: 1.268-12.946, P=0.0183, respectively].

Among 227 participants in whom T2DM developed during follow-up, 110 received a diagnosis in the hospital and 117 were diagnosed by FPG measurement on outpatient follow-up. No new patients had a history of hypoglycemic agent use. Table 2 shows that the TT genotype of rs7903146 was significantly associated with higher fasting plasma insulin and the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) in those with new-onset diabetes (P<0.05). In addition, the rs290487 TT genotype of *TCF7L2* was associated with abdominal obesity and the rs12104705 CC genotype of *GCG* was associated with both general obesity and abdominal obesity in those with new-onset diabetes (P<0.05) (Table 3).

Table 1. Anthropometric and Biochemical Characteristics of the Study Population (n=5,511)

Variable	T2DM (n=227)	Non-T2DM (n=5284)	χ^2/Z Value	P Value
Age (y)	52 (44-60)	49 (41-58)	3.1696	0.0015
Sex, M (%)	97 (42.73)	2088 (39.52)	1.1444	0.2847
Current smokers	69 (30.40)	1450 (27.44)	0.9520	0.3292
Family history of T2DM	17 (9.19)	280 (6.25)*	2.2129	0.1369
Alcohol intake	26 (11.45)	630 (11.92)	0.0457	0.8308
Physical activity level				
Low	128 (56.39)	3107 (58.80)	2.1202	0.3464
Moderate	40 (17.62)	1017 (19.25)		
High	59 (25.99)	1160 (21.95)		
BMI (kg/m^2)	25.73 (23.04-28.12)	23.89 (21.69-26.22)	7.0489	<0.0001
WC (cm)	88.80 (80.15-95.00)	81.50 (75.00-88.50)	8.4016	<0.0001
SBP (mm Hg)	130.33 (118.33-141.33)	121.00 (110.67-134.33)	6.4633	<0.0001
DBP (mm Hg)	82.00 (74.67-89.00)	77.00 (70.67-85.33)	5.8533	<0.0001
FPG (mmol/L)	5.51 (5.13-5.84)	5.19 (4.88-5.49)	8.4145	<0.0001
TC (mmol/L)	4.45 (3.93-5.07)	4.28 (3.75-4.90)	2.8161	0.0049
TG (mmol/L)	1.65 (1.13-2.36)	1.32 (0.95-1.89)	5.9001	<0.0001
HDL-C (mmol/L)	1.10 (0.95-1.25)	1.14 (0.99-1.32)	-2.6806	0.0073
LDL-C (mmol/L)	2.50 (2.05-2.90)	2.40 (2.00-2.90)	0.7814	0.4346

Note. *Family history of T2DM in 804 participants was unknown. Data are median (interquartile range) or percentage (%). BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 2. Clinical and Biochemical Characteristics of SNP rs7903146 Genotypes in New-onset Diabetes and Non-T2DM Participants

Variable	T2DM (*n*=227)		*Z* Value	*P* Value*	Non-T2DM (*n*=5284)		*Z* Value	*P* Value*
	CC+CT	TT			CC+CT	TT		
FPG (mmol/L)	7.51 (6.24-8.87)	7.93 (7.59-14.85)	1.2207	0.2222	5.20 (4.88-5.56)	5.41 (5.06-5.50)	0.5777	0.5634
FPI (mIU/L)	12.87 (8.61-20.33)	25.70 (20.29-27.83)	1.9950	0.0460	9.81 (7.00-13.41)	10.66 (9.67-15.07)	1.1168	0.2641
HOMA-β	71.61 (46.43-113.67)	99.22 (45.29-125.62)	0.4286	0.6682	118.74 (81.30-167.84)	110.57 (102.09-192.25)	0.3586	0.7199
HOMA-IR	4.45 (2.72-7.21)	9.81 (6.84-16.96)	2.0919	0.0364	2.25 (1.60-3.13)	2.56 (2.30-3.39)	1.2542	0.2098
TC (mmol/L)	4.79 (4.20-5.47)	5.89 (4.28-6.50)	1.1730	0.2408	4.42 (3.85-5.09)	4.25 (3.85-4.75)	-0.6989	0.4846
TG (mmol/L)	1.86 (1.33-2.68)	2.73 (2.46-3.08)	1.5284	0.1264	1.42 (1.02-2.03)	1.66 (1.30-2.61)	1.6279	0.1036
HDL-C (mmol/L)	1.08 (0.92-1.27)	1.07 (1.03-1.15)	0.0467	0.9627	1.10 (0.95-1.27)	1.03 (0.90-1.22)	-1.0284	0.3038
LDL-C (mmol/L)	2.74 (2.26-3.22)	3.74 (1.89-4.03)	0.8940	0.3713	2.56 (2.11-3.09)	2.23 (1.94-2.74)	-1.3607	0.1736

Note. Data are median (interquartile range). *Mann-Whitney Wilcoxon test. FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β-cell function; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 3. Obesity Indexes of SNP rs290487 and rs12104705 Genotypes in New-onset Diabetes and Non-T2DM Participants

Variable	T2DM (*n*=227)		*Z* Value	*P* Value*	Non-T2DM (*n*=5284)		*Z* Value	*P* Value*
rs290487	CC+CT	TT			TT+CT	CC		
BMI (kg/m^2)	25.77 (22.95-28.26)	26.27 (24.17-28.79)	1.3368	0.1813	24.66 (22.32-27.02)	24.55 (22.33-27.06)	0.1603	0.8726
WC (cm)	88.25 (81.00-94.50)	90.88 (85.25-96.13)	1.9997	0.0455	84.00 (77.25-90.50)	83.25 (77.00-90.25)	-0.7640	0.4449
WHtR	0.54 (0.51-0.60)	0.57 (0.54-0.62)	2.8769	0.0040	0.53 (0.48-0.57)	0.53 (0.48-0.57)	-0.7321	0.4641
rs12104705	TT+CT	CC			TT+CT	CC		
BMI (kg/m^2)	86.25 (81.13-90.50)	90.00 (83.25-96.50)	-2.3336	0.0196	24.77 (22.47-27.04)	24.61 (22.28-27.03)	0.9758	0.3292
WC (cm)	24.37 (22.17-27.03)	26.25 (23.61-28.79)	-2.3557	0.0185	0.53 (0.49-0.57)	0.53 (0.48-0.57)	0.5923	0.5536
WHtR	0.52 (0.50-0.56)	0.56 (0.53-0.61)	-3.2230	0.0013	84.20 (77.25-90.25)	83.75 (77.00-90.50)	0.4093	0.6823

Note. Data are median (interquartile range). *Mann-Whitney Wilcoxon test. BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio.

Genetic elements are involved in the pathogenesis of T2DM, but lifestyle seems to trigger pathogenic factors. We found no interaction between the *TCF7L2-GCG* gene and gene environment associated with the risk of T2DM, but our results suggest that *TCF7L2* and *GCG* genetic variants may be associated with fasting insulin level, insulin resistance, and obesity in new-onset diabetes.

Epidemiological evidence indicates that obesity, and particularly abdominal obesity, is an independent risk factor for T2DM. Visceral fat affects insulin metabolism by releasing free fatty acids, which may reduce hepatic clearance of insulin, leading to insulin resistance and hyperinsulinemia. In addition, fat cells secrete a series of cytokines including leptin, adiponectin, interleukin-6 (IL-6), and tumor necrosis factor (TNF-α), which are related to insulin resistance[7-8]. Our results support these findings; moreover, we found that SNP rs290487 in *TCF7L2* and rs12104705 in *GCG* were associated with both general obesity and abdominal obesity in new-onset diabetes.

Furthermore, we found that rs7903146 was associated with fasting insulin level and HOMA-IR in new-onset diabetes. Le Bacquer et al. found that the *TCF7L2* variant rs7903146 risk allele is associated with impaired insulin secretion, reduction of total islet number, and quantitative as well as qualitative morphological changes in human islets[9]. Zhou et al. have also identified a *TCF7L2*-regulated transcriptional network responsible for its effect on insulin secretion in human pancreatic islets. The risk T-allele of rs7903146 was associated with increased *TCF7L2* expression, as well as decreased insulin content and secretion[10]. We found a compensatory increase in fasting insulin levels and HOMA-IR in carriers of risk T-allele in new-onset diabetes. Therefore, *TCF7L2* not only regulates synthesis of proinsulin, but also processing and possibly clearance of proinsulin and insulin[10]. Although a cohort design was employed in this study, several limitations should be considered when interpreting the findings. First, an oral glucose tolerance test (OGTT) was not consistently performed, which might lead to underestimation of the incidence of T2DM and induce misclassification bias. Second, the number of cases was small, and there was no significant interaction between the *TCF7L2* and *GCG* genes in the incidence of diabetes. Third, a baseline fasting insulin level was not obtained, and dynamic changes in fasting insulin, HOMA-IR, and HOMA-β were not analyzed.

Our study provided important evidence for the association of *TCF7L2* and *GCG* genetic variants with insulin resistance and obesity in new-onset diabetes.

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Table S1. Genotypic and Allelic Distributions of Single Nucleotide Polymorphisms of *TCF7L2* and *GCG* Gene

Gene	SNPs	Genotype/allele	T2DM	Non-T2DM	χ^2 value	*P* value
TCF7L2	rs7903146	TT	3 (1.32)	16 (0.30)	7.8235	0.0200
		CT	24 (10.57)	701 (13.27)		
		CC	200 (88.11)	4567 (86.43)		
		T	30 (6.61)	733 (6.94)	0.0062	0.9372
		C	424 (93.39)	9835 (93.06)		
	rs290487	TT	99 (43.61)	2231 (42.22)	2.1621	0.3392
		CT	108 (47.58)	2417 (45.74)		
		CC	20 (8.81)	636 (12.04)		
		T	306 (67.40)	6879 (65.09)	0.4281	0.5129
		C	148 (32.60)	3689 (34.91)		
	rs11196218*	GG	128 (57.40)	2814 (54.13)	1.8806	0.3905
		AG	77 (34.53)	2024 (38.93)		
		AA	18 (8.07)	361 (6.94)		
		A	113 (25.34)	2746 (26.41)	0.5642	0.4526
		G	333 (74.66)	7652 (73.59)		
GCG	rs12104705*	TT	0	32 (0.62)	2.7105	0.2579
		CT	36 (16.14)	695 (13.37)		
		CC	187 (83.86)	4472 (86.02)		
		T	36 (8.07)	759 (7.30)	0.0150	0.9026
		C	410 (91.93)	9639 (92.70)		

***Note*.** Data were number (%). *The genotypes of 89 participants of SNP rs11196218 and rs12104705 deletion respectively.

Table S2. Association of *TCF7L2* and *GCG* SNPs with Risk of Incident T2DM during Follow-up

SNP	Genotype	Crude HR (95% *CI*)	*P* value	Adjusted HR (95% *CI*)*	*P* value*
rs7903146	CC	Reference		Reference	
	CT	0.815 (0.533-1.246)	0.3452	1.028 (0.647-1.633)	0.9070
	TT	3.799 (1.213-11.893)	0.0219	4.068 (1.270-13.026)	0.0181
	TT+CT *vs.* CC	0.893 (0.597-1.336)	0.5829	1.133 (0.731-1.755)	0.5771
	TT *vs.* CT+CC	3.893 (1.244-12.175)	0.0195	4.051 (1.268-12.946)	0.0183
rs290487	TT	Reference		Reference	
	CT	1.027 (0.781-1.351)	0.8473	1.033 (0.745-1.433)	0.8466
	CC	0.792 (0.489-1.281)	0.3417	0.827 (0.478-1.430)	0.4958
	CC+CT *vs.* TT	0.981 (0.754-1.277)	0.8877	0.991 (0.724-1.356)	0.9526
	CC *vs.* CT+TT	0.781 (0.493-1.237)	0.2919	0.813 (0.483-1.367)	0.4343

Continued

SNP	Genotype	Crude HR (95% *CI*)	*P* value	Adjusted HR (95% *CI*)*	*P* value*
rs11196218	AA	Reference		Reference	
	AG	0.676 (0.404-1.129)	0.1348	0.928 (0.486-1.772)	0.8206
	GG	0.818 (0.499-1.343)	0.4257	0.974 (0.517-1.834)	0.9351
	GG+AG *vs.* AA	0.757 (0.467-1.227)	0.2580	0.954 (0.515-1.770)	0.8818
	GG *vs.* AG+AA	1.137 (0.870-1.485)	0.3464	1.038 (0.757-1.425)	0.8164
rs12104705	CC	Reference		Reference	
	CT	1.285 (0.898-1.838)	0.1696	1.236 (0.803-1.903)	0.3354
	TT	NA	0.9755	NA	0.9765
	TT+CT *vs.* CC	1.239 (0.866-1.772)	0.2407	1.174 (0.762-1.807)	0.4666
	TT *vs.* CT+CC	NA	0.9754	NA	0.9763

***Note*.** The analysis involved 5284 normoglycemic individuals and 227 new-onset diabetes patients during follow-up. *Adjusted for baseline age, sex, family history of T2DM, smoking, alcohol intake, physical activity level, BMI, waist circumference, fasting plasma glucose, total cholesterol, triglyceride, HDL-C and LDL-C. NA not available.